2024 | volume 13:s1

ISSN: 2239-7132

Italian Journal of Food Safety

Editor in Chief:
Gerardo Manfreda

pagepress

Italian Journal of Food Safety

pISSN 1974-7071 | eISSN 2239-6268

Publisher
PAGEPress Publications
via A. Cavagna Sangiuliani 5
27100 Pavia, Italy
Tel. 39.0382.1549020
info@pagepress.org – www.pagepress.org

2023 Impact factor: 1.8 ©JCR Clarivate Analytics

EAVLD 2024

7th Congress of the
European Association of Veterinary
Laboratory Diagnosticians

21st-23rd October 2024

ITALY - PADUA

Italian Journal of Food Safety

2024 volume 13 s1

Table of Contents

pagepress

KEYNOTE SPEAKERS

THE MANY HISTORIES OF ONE HEALTH AND THE SHIFT OF ANIMAL WELFARE FROM TOOL TO AIM

Bernardino Fantini

Geneva University, Switzerland

Extended Summary

The history of the relationships between human populations and the natural world is characterized by profound permanences and a few major discontinuities. In prehistory this relationship was based exclusively on the predator-prey relationship with animals and edible-poisonous for plants and a small part of minerals. With the origin of agriculture and the domestication of a small number of animal and vegetal species, animals and plants started to play a relevant role in human societies, at the level of agricultural and then industrial production, but also as part of cultural, social and religious life.

Animals have always accompanied human populations, in various forms, mainly as tools, useful for their productive value, their use in military campaigns, for hunting and as pets. Animal welfare was a goal to be achieved, to safeguard the instrumental value of animals. Horses had to be kept in good health, ready for the battle or for the military transports.

A new sensitivity towards animals and the natural world emerged at the end of the eighteenth century, particularly with the Romantic movement. Until the eighteenth century, animal health was entrusted to blacksmiths and marshals for army animals and to farmers themselves or to itinerant quacks for farm animals. In the same period emerged the professionalization of the veterinary physicians and surgeons, with the establishment of veterinary schools and colleges. The first veterinary schools were created, equipped with clinics and laboratories, and many famous doctors, such as Rudolf Virchow and William Osler, taught at the same time in medical and veterinary schools. However, the health of animals remained in large part instrumental to the conservation of their productive value as tools.

Only in the last decades a new conscience and the health of animals, and more generally of the environment, has become the aim of environmental policies and health strategies. Plants, animals and minerals are not resources to be exploited without limits or useful tools for various human activities, but components of a complex ecosystem to be protected, safeguarded and developed. There is an emerging awareness that the environment does not belong to us, but human populations belong to the environment and the health of populations depends to an increasing extent on the health of other species and the natural environment. In this context animal welfare is not a way to preserve important tools but becomes an aim in itself.

As a result of this new awareness, a new strategy, called One Health, has been promoted. The One Health principle is an integrated and unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems. This principle recognizes that the health of humans, domestic and wild animals, plants and the environment in general, including ecosystems, are closely linked and interdependent. These proposals aim to mobilize multiple sectors, appealing not only to medical and health disciplines, but also to all disciplines and communities that interact with the environment at different levels of society, in order to work together to address threats to health and ecosystems and promote the well-being of all forms of life and the environment.

The historical analysis allows us to isolate, albeit schematically, five main roots of One Health strategies. The first is the result of the shock caused by the emergence of new zoonoses, the latest of which is the Covid19 pandemic, with the consequent need to prevent new pandemics and limit zoonotic diseases. The concept of OH has in fact emerged internationally, with a series of publications, scientific conferences and the establishment of working groups, mainly to address animal-human interactions that cause emerging and re-emerging zoonotic diseases.

A second push for the development of the One Health approach came, starting from the end of the nineteenth century, from veterinarians, who pushed for a de facto unification between the medical and veterinary professions, with the slogan ‘One Medicine’. Supporters of ‘One Medicine ’have strongly argued that in fact human medicine is greatly indebted to animals, from which knowledge of anatomy and physiology often derives, as well as vaccines and the testing of new drugs. Health and disease in humans and animals therefore differ only in detail and not in their nature. In recent years, the wide range of benefits that humans derive from their relationships with animals and from the role that animals play in everyday life and society has been recognized.

A third root of One Health must then be found in the development of ecological movements. If in the nineteenth century, with Alexandre von Humboldt, Goethe and the Romantic poets, nature and its importance for well-being were discovered, in recent decades environmental crises have pushed for the development of new strategies for the protection of ecosystems.

A fourth root of One Health is given by the recognition of the importance of the social and environmental determinants of health, as put in evidence by the WHO Commission on Social Determinants of Health.

Finally, the fifth root is the awareness that human beings with their activities and territorial, structural and climatic changes have a strong impact on geological and climatic processes, which has led to the introduction of a new term to indicate a new geological era, the Anthropocene, which forcefully raises the question: How can we live in a world where there is no nature without people?. The slogan “One Health/One World” is therefore affirmed, a holistic concept that encompasses all of nature in a single integrated vision: human health, animal health and the sustainability of ecosystems constitute a single objective, because achieving all three at the same time is the only way to achieve each of them.

HIGH PATHOGENICITY AVIAN INFLUENZA (HPAI): EXPECT THE UNEXPECTED

I. Monne

Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), viale dell'Università, Legnaro (Padua), Italy

The epidemiological and ecological dynamics of highly pathogenic avian influenza (HPAI) viruses have changed dramatically in recent years, with devastating consequences for the health of domestic and wild birds as well as for biodiversity and livelihoods. This worrisome trend is mainly the consequence of the spread of the HPAI viruses of the H5 subtype descendent of the H5N1 virus A/goose/Guangdong/1/1996 (Gs/GD), which was first detected in China in 1996. Since their emergence, these viruses have dramatically expanded their geographical distribution having been found on all continents, except Oceania, and remaining well entrenched in a number of countries. Over the years, they have acquired a broader host range with a constantly expanding list of free-living wild bird species, which can potentially be affected by the virus. The replication of these viruses in multiple hosts and in different poultry production systems has favored the emergence of a multitude of virus strains through drift in individual genes and genetic reassortment. The dynamic interplay between viruses and their hosts has resulted in a wide spectrum of presentation of the disease, ranging from subclinical infections to mass mortality events. Recently, the perceived threat to human health from H5 has increased due to the greater number of spillover events in mammalian species and, in particular, to the occurrence of outbreaks in mammals as well as of mammal to human transmission events.
In a constantly evolving epidemiological landscape, early detection and characterization of HPAI viruses play a critical role in guiding prevention and control measures and in counteracting the threat of a new pandemic. Faced with a virus that is constantly changing the rules of the game, the use of a multidisciplinary approach that combines different diagnostic methods and perspectives is a strategic goal for the scientific community to reduce the impact of the disease on animal health and to mitigate the risk of AIV adaptation to mammals and humans.

PHYLOGENETIC DIVERSITY AND CURRENT TAXONOMY OF THE GENUS *MYCOBACTERIUM*: CONSEQUENCES FOR THE DIAGNOSIS OF TUBERCULOSIS

Antonio Martínez-Murcia

Genetic PCR Solutions™ and Universidad Miguel Hernández, Orihuela, Alicante, Spain

Till last decade, the taxonomy of the genus *Mycobacterium* has been very complex and comprised a huge number of distantly related species. Recent phylogenomic and comparative genomic studies showed evidence of a polyphyletic structure and the division of this group of mycobacteria into an emended genus *Mycobacterium* and four novel genera, was proposed and validated by the International Committee on Systematics of Prokayotes (ICSP). Some authors reacted to this proposal by arguing that, because these taxa include several relevant pathogens, the newly validated five- genera may cause confusion in clinical treatment. Another relevant taxonomic issue was related to the pathogenic species *Mycobacterium tuberculosis*, still today considered a complex of several species, including *M. africanum, M. bovis, M. caprae, M. microti,* and *M. pinnipedii*, each infecting specific animals. Literature review indicates that this scenario had led to a considerable number of new proposals, i.e., emended, new taxa combinations. In our experience, this gives rise to confusion with consequences at the diagnostic level. Nevertheless, we count with phylogenetic evidence indicating that *Mycobacterium tuberculosis* complex is rather a single species comprising ecotypes developing unique capacities to colonize specific host species. As suggested by members of the ICSP, the species concept currently in use is considered to be pragmatic, operational, universally applicable, and successfully used for identification processes.
Molecular clocks are hypothetical makers of phylogenetic relationships in bacteria. In the 1980s, Carl Richard Woese 1928-2012 revolutionized bacterial systematics by considering that mutations in the ribosomal RNA sequence are in rhythm with evolutionary time. Although rRNA has shown a very good chronometric capacity resolving phylogenetic relationships at the genus level and above, it often fails to split species and below. We latter have learnt that housekeeping protein-coding genes are more precise clocks to split species, also resolving at the intra-species level, because they are subjected to a degenerative code (different evolution mode) and accept more changes during evolution (fast clocks). The strategy named multi-locus phylogenetic analysis (MLPA) is based on a hypothetically synchronized mode of evolution of genomes, i.e., genes evolve in concert and, consequently, concatenated multigene phylogeny may be 'the mirror' of the overall relationships of the entire genomic content. Apart from the genomic results referenced above, a comprehensive systematic study comparing both types of clocks was not found in the literature. Our work confirms current taxa validations, demonstrating that *Mycobacterium tuberculosis* complex is a single species comprising ecotypes with capacities to colonize different host animal species.
Acknowledgements. I am grateful to my team at GPS™

CHALLENGES AND NEW INITIATIVES IN VETERINARY CLINICAL MICROBIOLOGY

Dorina Timofte

University of Liverpool, UK

Antimicrobial resistance (AMR) is one of the most important public health threats, endangering our ability to treat bacterial infections in both humans and animals. The emergence of AMR has highlighted the key role that clinical microbiology laboratories play in driving antimicrobial stewardship and appropriate antimicrobial use. Despite the increased recognition of its importance, there are several areas of improvement which need to be addressed so that veterinary microbiologists could fulfil their role in preventing further development and spread of AMR. Bacterial culture, identification and antimicrobial susceptibility testing (C&ID and AST) are key tools for antimicrobial therapy guidance; however, the lack of specific guidelines for laboratory processing of companion animal clinical specimens poses great difficulties for laboratory staff. Similarly, the lack of specific guidelines for interpretation and reporting of AST for some veterinary pathogens, as well as guidance for detection of AMR mechanisms in clinical isolates from companion animals, hinders accurate detection and identification of resistant organisms, which is paramount for infection control and preventing zoonotic transmission.

The adoption and implementation of new clinical breakpoints (CBPs) used for interpretation of antimicrobial susceptibility testing are important (but also a challenge) for both human and veterinary clinical laboratories. CBPs are routinely updated by both the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) to recognise and adapt to the evolving resistance mechanisms and thus, to reflect the current clinical outcomes. In particular, the changes seen in the last few years with CLSI introducing the susceptible dose-dependent (SDD) category and EUCAST redefining the "Intermediate" category as "Susceptible, increased exposure", seem to be difficult to adopt and implement by both human and veterinary laboratories. A recent survey undertaken in the U.S. laboratories identified that up to 70% of College of American Pathologists (CAP)-accredited and 45% of CAP-accredited laboratories outside the U.S. use several obsolete clinical breakpoints to interpret AST results to guide patient care[1].

Each of the aspects highlighted above have implications for the diagnosis and management of infections, and impact overall on antimicrobial use and stewardship, highlighting the need for harmonization of diagnostic procedures across veterinary microbiology diagnostic laboratories. As more resistance emerges, laboratories need to be better equipped both in technologies and increased laboratory expertise to deal with these threats. Therefore, there is also a need to train more veterinary clinical microbiology specialists to deal with these changes, keeping pace with the emergence of new molecular technologies which require genomic and bioinformatics skills for high- resolution typing of pathogens and detection of genes associated with antimicrobial resistance or pathogenicity[2].

In this talk, I will cover several recent developments which address some of these challenges. For instance in 2019, a COST Action project entitled "European Network for Optimization of Veterinary Antimicrobial Treatment (ENOVAT)" (https://cost.eu/actions/CA18217/ and https://www.enovat.eu/) was established with a primary aim to optimize veterinary antimicrobial use, with a focus on the development of new antimicrobial treatment guidelines and harmonization of microbiological diagnostic procedures. The urgent need for laboratory harmonization through standardization of bacterial culture and antimicrobial susceptibility testing was outlined in a ENOVAT position paper[3]. Work undertaken by ENOVAT included surveying diagnostic methodologies in veterinary laboratories from 34 European countries, which identified a broad variety of methodologies in regular use; for instance, only 48% and 46% of veterinary diagnostic laboratories routinely screened isolates for methicillin resistance and ESBL production, respectively (manuscript under review)[4]. Furthermore, a combination of EUCAST and CLSI clinical breakpoints (CBPs) was the most used approach for interpretation of AST. The use of multiple standards for AST is a major limitation when comparing susceptibility data between laboratories and/or countries, thereby compromising global AMR surveillance in animal pathogens[5]. Consequently, the survey identified areas of improvement and led to an ENOVAT follow-up initiative for creating an Archive of Protocols for Veterinary Microbiology Investigations (companion animal clinical specimens); this archive is currently under development and will be hosted on the European College of Veterinary Microbiology (ECVM website https://ecvmicro.org/) which will provide a repository of veterinary microbiology diagnostic consensus protocols, to be freely accessible online by laboratory users.

Another development is represented by the establishment of the European Antimicrobial Resistance Surveillance Network in Veterinary Medicine (EARS-Vet)[6] which was recently set up to fill an important gap on surveillance of AMR in pathogens from animal clinical infections. Although several EU countries have a national AMR surveillance system for bacterial pathogens of animals, these systems are not harmonised between countries for monitoring AMR in the same animal species, bacterial species and antimicrobials, and often use different testing methodologies and interpretative criteria. The establishment of EARS-VET is an especially positive development; firstly, as it is complementing the ECDC and EFSA monitoring systems through the food chain, in truly One- Health strategy for surveillance of AMR, and secondly by aiming to take a harmonised approach to AMR surveillance in bacterial pathogens from animals across Europe.

All of these developments are stepping stones towards greater consistency in the performance and reporting of bacteriological diagnostic procedures across veterinary microbiology laboratories in Europe and beyond.

References

1. Simner PJ, Rauch CA, Martin IW, Sullivan KV, Rhoads D, Rolf R, She R, Souers RJ, Wojewoda C, Humphries RM. 2022. Raising the bar: improving antimicrobial resistance detection by clinical laboratories by ensuring use of current breakpoints. Open Forum Infect Dis 9. https://doi.org/10.1093/ofid/ofac007
2. Rossen JWA, Friedrich AW, Moran-Gilad J, ESCMID Study Group for Genomic and Molecular Diagnostics (ESGMD). 2018. Practical issues in implementing whole-genome-sequencing in routine diagnostic microbiology. Clin Microbiol Infect 24:355–360. Crossref. PubMed. ISI.
3. Timofte, D., Broens, E. M., Guardabassi, L., Pomba, C., Allerton, F., Ikonomopoulos, J., . . . European Network for Optimisation of Veterinary Antimicrobial Treatment (ENOVAT); ESCMID Study Group for Veterinary Microbiology (ESGVM); European College of Veterinary Microbiology (ECVM) and European Association of Veterinary Laboratory Diagnosticians (EAVLD) (2021). Driving laboratory standardisation of bacterial culture and antimicrobial susceptibility testing in veterinary clinical microbiology in Europe and

beyond. Journal of Clinical Microbiology. 59, doi:10.1128/jcm.02572-20
4. Tom Koritnik, Iskra Cvetkovikj, Flavia Zendri, Shlomo Eduardo Blum, Serafeim Christos Chaintoutis, Peter A. Kopp, Cassia Hare, Zrinka Štritof, Sonja Kittl, José Gonçalves, Irena Zdovc, Erik Paulshus, Andrea Laconi, David Singleton, Fergus Allerton, Els M. Broens, Peter Damborg, Dorina Timofte. Towards harmonised laboratory methodologies in veterinary clinical bacteriologyoutcomes of a European survey. Frontiers in Microbiology, under review (Manuscript ID: 1443755)
5. Marques C, Gama LT, Belas A, Bergström K, Beurlet S, Briend-Marchal A, Broens EM, Costa M, Criel D, Damborg P, van Dijk MAM, van Dongen AM, Dorsch R, Espada CM, Gerber B, Kritsepi- Konstantinou M, Loncaric I, Mion D, Misic D, Movilla R, Overesch G, Perreten V, Roura X, Steenbergen J, Timofte D, Wolf G, Zanoni RG, Schmitt S, Guardabassi L, Pomba C. 2016. European multicenter study on antimicrobial resistance in bacteria isolated from companion animal urinary tract infections. BMC Vet Res 12:213. doi: 10.1186/s12917-016-0840-
6. Mader R, Damborg P, Amat JP, Bengtsson B, Bourély C, Broens EM, Busani L, Crespo-Robledo P, Filippitzi ME, Fitzgerald W, Kaspar H, Madero CM, Norström M, Nykäsenoja S, Pedersen K, Pokludova L, Urdahl AM, Vatopoulos A, Zafeiridis C, Madec JY; EU-JAMRAI. Building the European Antimicrobial Resistance Surveillance network in veterinary medicine (EARS-Vet). Euro Surveill. 2021 Jan;26(4):2001359. doi: 10.2807/1560-7917.ES.2021.26.4.2001359. PMID: 33509339; PMCID: PMC7848785.

LATENT CLASS MODELS: VALIDATION OF DIAGNOSTICS WITHOUT A REFERENCE TEST

Polychronis Kostoulas

Laboratory of Epidemiology, Artificial Intelligence & Biostatistics, Faculty of Public & One Health, University of Thessaly, Greece

Latent class models (LCMs) offer an intuitive and robust statistical framework for validating diagnostic tests in the absence of an affordable, reliable, noninvasive perfect reference test (gold standard).[1] In veterinary diagnostics, LCMs have been increasingly popular for estimating the sensitivity (Se) and specificity (Sp) of diagnostic tests and the World Organization for Animal Health (OIE) has endorsed their use for this purpose.[2] Further, Bayesian approaches to LCMs have the flexibility of incorporating prior knowledge about disease prevalence, Se and Sp as well as the ability to accommodate non-identifiable models3. In the latter case the specification of informative priors enables these methods to overcome the inherent limitations of traditional maximum likelihood estimation methods. Classical (B)LCMs are based on the analysis of cross-classified test outcomes from multiple imperfect tests.[4] The flexibility of BLCMs extends to various test combinations, allowing for the incorporation of conditional dependencies between tests that detect similar biological responses,[5] a key advancement from earlier methods reliant on the assumption of test independence. Recently, BLCMs have also been used for the validation of continuous test results and novel methodological advancements include their expansion across a range of diagnostic settings. For instance, hierarchical LCMs have been introduced to manage complex data structures, as in the common case of clustering (*e.g.*, animals within herds or farms).[6] These models allow for more granular inferences by estimating disease prevalence at different hierarchical levels, providing insights that are critical for disease control strategies.

References

1. Cheung, A., et al. (2021). “Bayesian Latent Class Analysis when the Reference Test is Imperfect.” Rev. Sci. Tech. Off. Int. Epiz. 40(1): 271-286.
2. (OIE (World Organisation for Animal Health), 2016. Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. Chapter 1.1.6 – Principles and Methods of Validation of Diagnostic Assays for Infectious Diseases, Available at http://www.oie.int/en/international-standard-setting/terrestrial- manual/access-online
3. Branscum, A.J., Gardner, I.A., & Johnson, W.O. (2005). “Estimation of Diagnostic Test Sensitivity and Specificity through Bayesian Modeling.” Preventive Veterinary Medicine 68(2-4): 145-163.
4. Hui, S.L., & Walter, S.D. (1980). “Estimating the Error Rates of Diagnostic Tests.” Biometrics 36(1): 167-171.
5. Dendukuri, N., et al. (2009). “Modeling Conditional Dependence Between Diagnostic Tests: A Multiple Latent Variable Model.” Statistical Methods in Medical Research 18(2): 275-293.
6. Hanson, T.E., et al. (2003). “Hierarchical Latent Class Models for Clustered Binary and Continuous Responses.” Journal of the American Statistical Association 98(463): 726-736.

ONE HEALTH, MANY LIVES: PRACTICAL IMPLEMENTATION CASES IN THE GLOBAL SOUTH

Giorgia Angeloni

President of Vétérinaires Sans Frontières International; Vicepresident of Veterinari Senza Frontiere Italia, Researcher at Research and International Cooperation, Unit of IZSVe, Italy

In recent years, the concept of One Health has gained significant recognition as a vital framework for addressing the interconnectedness of human, animal, and environmental health. This approach is particularly relevant in the Global South, where complex socio-economic challenges and environmental changes exacerbate health risks and impact livelihoods. For over 30 years, members of Vétérinaires Sans Frontières International (VSF International) have played a crucial role in promoting One Health principles through grassroots initiatives and collaborative projects aimed at improving health outcomes for vulnerable populations. In 2023 alone, the network reached over 1.49 million families and treated more than 27.3 million animals across 55 countries in Africa, Asia, the Americas, and Europe.

The slogan "One World, One Health" highlights the strong interconnections among countries and shared global challenges; however, it is essential to tailor One Health activities to local contexts. Each community possesses unique socio-economic conditions, cultural practices, and environmental factors that must be considered to enhance the effectiveness and sustainability of health interventions and policies.

The implementation of One Health initiatives in the Global South has yielded numerous practical experiences illustrating the effectiveness of this approach. One prominent case is the VSF-Suisse HEAL project (One Health Units for Humans, Environment, Animals, and Livelihoods), which aims to enhance the well-being of vulnerable pastoralist and agro-pastoralist communities in Ethiopia, Somalia, and Kenya by establishing One Health Units (OHUs). These units facilitate coordination among governmental departments (through the creation/support to One Health Taskforces at institutional level), private providers, and communities to strengthen health services and develop sustainable strategies for adapting to climate change. Given the mobility of pastoralist communities, the HEAL project incorporates both mobile and static OHUs to address health needs across borders, ensuring access to services for communities on the move. By implementing integrated veterinary services, the project enhances livestock health and promotes food security and nutrition. Training local veterinarians and community animal health workers (CAHWs) empowers communities to manage livestock diseases more effectively, resulting in reduced mortality rates and improved productivity. The project also adopts a participatory approach, engaging communities at every phase to ensure their needs are met, particularly through Multi-Stakeholder Innovation Platforms (MSIPs) that empower women in livestock management. With strong partnerships among experts, HEAL is creating impactful and efficient solutions that transcend geographical boundaries.

Figure 1. One Health Unit (Photo Credits: Micol Fascendin).

Another practical One Health project by VSF Italy focuses on strengthening the control system for food of animal origin in the Sahrawi refugee camps in southern Algeria, where over 170,000 people have relied on humanitarian aid since 1974. The food provided by WFP mainly consists of storable items, leading to widespread malnutrition due to the lack of fresh produce. For over 20 years, VSF Italy has collaborated with the Directorate of Veterinary Affairs to enhance livestock health and productivity, ensuring the safety of local animal products through systematic health inspections and supporting an industrial poultry farm that provides fresh protein. The project emphasizes training staff on inspection techniques, establishing a *Salmonella* detection system, and updating biosecurity measures in the poultry farm. Given the high rates of malnutrition, it is crucial to prevent the waste of eggs and meat for sanitary reasons, while traditional cooking habits among the Sahrawi people help mitigate health risks.

Figure 2A and 2B. Activities carried out in the veterinary laboratory in Saharawi refugees camps (Photo Credits: Sara Di Lello, VSF Italy).

It is also important to recognize that One Health does not solely pertain to the control of zoonotic diseases. In fact, Peste des Petits Ruminants (PPR) and African Swine Fever (ASF) viruses do not infect humans, yet their impact on livestock health directly affects food security and income for pastoralists and farmers. By implementing vaccination campaigns and training local veterinarians and CAHWs, VSF International members enhances disease prevention and management strategies. These efforts not only protect livestock but also ensure that communities can support their families, local incomes and economies, maintain traditional breeding and products transformation practices, and avoiding biodiversity lose. In essence, controlling PPR and ASF is crucial for fostering resilience in communities, enabling them to thrive despite environmental and economic challenges.

Figure 3A and 3B. PPR vaccination in Niger (Photo Credits: Tim Dirven, VSF Belgium.) and a breeder of East Balkan Swine, the last indigenous pig breed of Bulgaria now threatened by ASF (Photo Credits, Alessandro Cristalli, VSF Italy)

Expanding the One Health concept beyond infectious diseases is important to capture its multifaceted dimensions. Since 2020, many VSF members have actively supported Ukrainian refugees by providing care for their pets, recognizing the vital role animals play in mental well- being and emotional support during crises. Activities include offering veterinary services, vaccinations, and health check-ups to ensure pets remain healthy and can safely accompany their owners. This initiative alleviates stress for refugees and fosters a sense of normalcy amid upheaval. By addressing the health of companion animals, VSF promotes a holistic One Health approach that acknowledges the interconnections between human health, animal health, and the environment. Furthermore, ensuring that pets are disease-free helps prevent the spread of zoonotic diseases, thereby protecting public health.

Figure 4A and 4B. Mobile Pet Clinics in Moldova, close to the Ukrainian border (photo credit, Pierangeloni Casale, VSF Italy)

In conclusion, the experiences of VSF International members in implementing One Health initiatives in the Global South highlight the potential of this integrated approach to address complex health challenges. They also underscore the necessity of adapting actions and policies to the diverse realities and the many lives of the communities we aim to support. This summarising the essence of "Multiple Worlds, One Health".

Acknowledgements: Margherita Gomarasca, Daniela Rana, Sara Di Lello and all the staff of theVSF International members for their inspiring work.

DIAGNOSTIC LIMITATIONS IN CONTROLLING THE RECURRENCE OF BOVINE TUBERCULOSIS IN EUROPEAN COUNTRIES

B. Romero[1,2], L. de Juan[1,2], J. Álvarez[1,2], F.J. Lozano[1], V. Lorente-Leal[1], P. Pozo[1], L. Domínguez[1,2], J.Bezos[1,2]

[1]*VISAVET Health Surveillance Centre, Complutense University of Madrid, Madrid, Spain*
[2]*Facultad de Veterinaria, Departamento de Sanidad Animal, Universidad Complutense de Madrid, Madrid, Spain*

Bovine tuberculosis (bTB) is an infectious disease to be eradicated and controlled in the European Union (EU) based on the Regulation (EU) 2016/429. This legislation provides the disease-specific rules for the prevention and control of several animal diseases, and includes the diagnostic methods for the granting and maintenance of disease-free status, being for bTB the tuberculin skin tests (single and comparative) and the gamma interferon assay. However, other diagnostic techniques together with complementary surveillance measures (such as postmortem examination at slaughterhouse or controlling the disease in other animal species) can be implemented to detect and remove the infection by the *Mycobacterium tuberculosis* complex.

bTB situation in the EU is heterogeneous and has slightly improved in recent years. From 2013 to 2022, there was a decrease in the annual number of infected cattle herds (−24.4% and 46.3%) in non-disease-free zones and countries with an approved eradication programme, respectively; whereas prevalence has remained stable or increased by 14.2%, respectively (1). Its eradication seems difficult to achieve due to some reasons such as the limited performance of the current diagnostic tests, the complex epidemiology (including domestic and wildlife reservoirs), the limited knowledge of the complex immunological response against the infection, the absence of a vaccine, or the societal aspects.

The persistence of bTB in herds, either herd recurrence or prolonged periods of restriction could be related to i) local reinfection due to infected wildlife, contaminated environment, or farm-to-farm contacts with infected neighboring herds; or to ii) ongoing transmission due to residual (persistent but undetected) infection and/or the entry of undetected infected animals. Therefore, it is essential to consider the complexity of this disease and not only the diagnostic limitations in order to control its spread.

In order to control the recurrence of bTB it is crucial to know the limitations in the sensitivity of available diagnostic tests. These can be associated to the performance of the *in vivo* test itself, the subjectivity of test interpretation (skin test), the potency of the tuberculin used in the programmes, the immunology status of the animal, and the chronic nature of the disease, among others. All these questions will be addressed during the presentation.

References

1. European Food Safety Authority (EFSA) | European Centre for Disease Prevention and Control(ECDC). The European Union One Health 2022 Zoonoses Report. EFSA Journal. 2023;21:e8442. https://doi.org/10.2903/j.efsa.2023.8442.

FROM ONE HEALTH TO PLANETARY HEALTH: TOWARDS AN ECOSYSTEM-BASED APPROACH TO FOOD PRODUCTION AND PUBLIC HEALTH WITH SYNECOCULTURE

Masa Funabashi

Research Director and Senior Researcher at Sony Computer Science Laboratories, Inc. Tokyo, Japan

In this talk, we explore the transition from a One Health framework, which emphasizes the interconnectedness of human, animal, and environmental health, to the broader concept of Planetary Health. This new paradigm recognizes the intricate interdependencies of global ecological systems and human well-being. Synecoculture, an advanced form of regenerative agriculture, is highlighted as a viable strategy to operationalize this shift. By fostering biodiversity and enhancing ecosystem services through the principle of augmented ecosystems, Synecoculture aims to create resilient agricultural systems that can sustainably produce food while restoring environmental health. The talk delves into the principles and practices of Synecoculture, emphasizing its potential to mitigate climate change, enhance soil fertility, and support diverse plant and animal species. Furthermore, it discusses the health benefits of such an ecosystem-based approach, including improved nutrition and reduced exposure to agricultural pollutants. The integration of Synecoculture within the Planetary Health framework illustrates a comprehensive pathway to address the pressing challenges of food security, environmental degradation, and public health. This holistic approach promotes a sustainable future where human health and the health of our planet are inextricably linked and mutually reinforcing.

EARLY CAREER RESEARCHERS

ECR-OP1

Antimicrobial resistance / susceptibility testing

EPIDEMIOLOGY OF ANTIMICROBIAL RESISTANCE IN THE MONOPHASIC VARIANT OF *SALMONELLA TYPHIMURIUM*

P. Carrizo[1], M. Ugarte-Ruiz[2], V. Rodríguez-Paredes[4], S. Pino-Rosa[4], Á. Aguarón[6], S. Collado[5], J.L. Sáez[5], C. De Frutos[3], S. Herrera-León[4], J. Alvarez[1]

[1]Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense de Madrid, Spain / Departamento de Sanidad Animal, Facultad de Veterinaria, Universidad Complutense de Madrid, Spain
[2]Centro de Vigilancia Sanitaria Veterinaria (VISAVET), Universidad Complutense de Madrid, Spain.
[3]Laboratorio Central de Veterinaria (LCV Algete), Ministerio de Agricultura, Pesca y Alimentación, Madrid, Spain [4]Laboratorio de Referencia de Enfermedades Transmitidas por Agua y Alimentos, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain
[5]Subdirección General de Sanidad e Higiene Animal y Trazabilidad, Dirección General de la Producción Agraria, Ministerio de Agricultura, Pesca y Alimentación, Madrid, Spain
[6]Syva S.A, León, Spain

Introduction
Despite control and prevention measures implemented throughout the food chain, non-typhoidal *Salmonella* infections remain one leading causes of foodborne disease worldwide. The monophasic variant of *Salmonella Typhimurium* is the third most prevalent serovar in humans in the EU and the most resistant one, being its main source
pig-derived products[1]. The presence of multi-resistant strains is a challenge for public health but also for animal health since this serovar can also cause disease in swine; therefore, identifying the transmission dynamics of monophasic
Salmonella along the food chain is crucial to optimize control measures[2].

Materials and Methods
Phenotypic data of antimicrobial resistance (AMR) in isolates retrieved between 2001 and 2023 from finishing pigs sampled at the abattoir, diseased pigs and humans were compared to evaluate the diversity and richness of resistance phenotypes and identify changes in profiles and factors associated with clinical conditions. Isolates from finishing pigs were retrieved from the AMR surveillance program (n=282), diseased pig isolates from veterinary diagnostic laboratories (n=68), and human isolates from the CNM-ISCIII (n=2800).

Results
Resistance to ampicillin, sulfamethoxazole and tetracycline was present in >80% of isolates regardless of origin, as well as with other antimicrobials, with higher levels in diseased and finishing pigs. There was greater diversity in resistance profiles in isolates from diseased pigs, while the diversity in finishing pigs and humans was similar. Despite different sample sizes, a higher richness of resistotypes was found in pigs compared to humans.

Discussion and Conclusion
This suggests different strain subpopulations may be causing disease in swine versus those reaching the final stages in the production chain and leading to disease in humans. Increased resistance in clinical animal strains may suggest a link between resistance genes and virulence factors, although whole genome sequencing would be needed to determine the mechanisms and factors contributing to the dissemination of resistance in this serovar.

References
1. EFSA, ECDC (2023). The European Union One Health 2022 Zoonoses Report. EFSA Journal, 21(12).
2. Soliani, L et al. (2023) *Salmonella* Infection in Pigs: Disease, Prevalence, and a Link between Swine and Human Health. Pathogens, 12.

ECR-OP2

Genomics

POULTRY SUPPLY CHAIN IMPACT ON CAMPYLOBACTERIOSIS SURVEILLANCE IN ITALY OVER ONE YEAR

F. Marotta[4], R. Di Romualdo[4], F. Di Timoteo[4], E. Di Domenico[4], A. Abass[4], G. Foschi[4], M. Ancora[3], L. Villa[2], M. Napoleoni[1], A. Garcia-Fernandez[2], C. Villani[4], G. Garofolo[4]

[1]*Centro di Riferimento Regionale Patogeni Enterici, CRRPE, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "T. Rosati", Perugia, Italy*
[2]*Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy*
[3]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy*
[4]*National Reference Laboratory for Campylobacter, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy*

Introduction

Campylobacter is the main cause of bacterial gastroenteritis in EU, but outbreaks are rarely reported. Poultry acts as the main reservoir for transmission, as shown by many source attribution studies [1,2].

Materials and Methods

In this study we perform cluster analysis using whole-genome sequencing (WGS) on 193 *C. jejuni* and 66 *C. coli* strains isolated in 2023, including 150 human cases and 109 poultry carcasses sampled at slaughterhouses for process hygiene criteria (Reg. 2073). CgMLST typing, with a cluster distance threshold of 10 alleles was used to detect clusters of clinical isolates and match them to isolates from poultry meat.

Results

The cgMLST analysis revealed the presence of 38 small outbreak-like clusters including 90 poultry and 59 clinical isolates, with about 30% matching those from poultry. ST2116, ST2863 and ST50 for *C. jejuni* and ST10304, ST8195 and ST830 for *C. coli* emerged as the predominant STs associated with poultry and humans. One large cluster persisted all year representing 3% of all *C. jejuni* human cases. This cluster type was detected in 17 chicken samples and was traced back to 4 slaughterhouses. A smaller cluster was detected for 1 *C. coli* human case matching with 4 poultry isolates traced back to one slaughterhouse. Moreover, almost a 30% of clinical isolates clustered with other clinical isolates without a source match suggesting the existence of hidden outbreaks likely attributed to the limited number of sequenced isolates (Figures 1, 2).

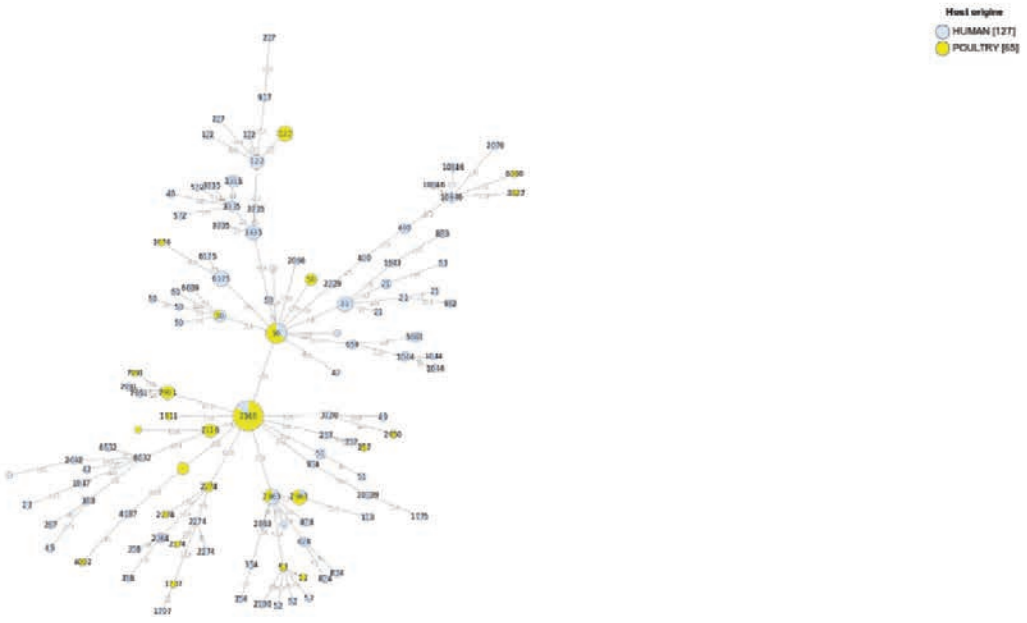


Figure 1. Minimum spanning tree (MST) generated for *C. jejuni* isolates based on cgMLST profiles. The distance labels correspond to the number of discriminating alleles. The yellow nodes correspond to poultry isolates and the blue nodes to human isolates.

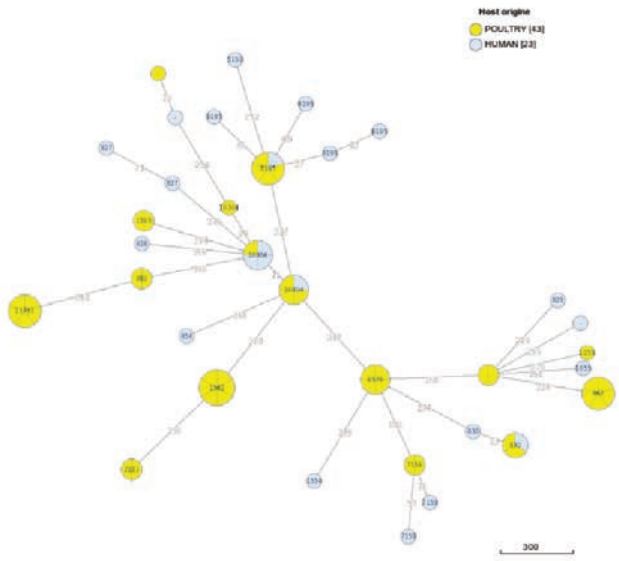


Figure 2. Minimum spanning tree (MST) generated for *C. coli* isolates based on cgMLST profiles. The distance labels correspond to the number of discriminating alleles. The yellow nodes correspond to poultry isolates and the blue nodes to human isolates.

Discussion and Conclusion

A comprehensive approach is necessary to reduce *Campylobacter* spp. in the poultry meat chain. This includes primary interventions at the farm level, control measures during slaughter and consumer awareness campaigns. WGS-based surveillance can enhance our comprehension of the dynamics of *Campylobacter* strain occurrence in chicken meat and its correlation with clusters of human cases indicating possible prompt interventions to control outbreaks

References

1. Garcia-Fernandez A. et al. "Antibiotic resistance, plasmids, and virulence-associated markers in human strains of *Campylobacter* jejuni and *Campylobacter* coli isolated in Italy." Frontiers in Microbiology 14 (2024): 1293666.
2. Marotta F. et al. "Genomic and antimicrobial surveillance of *Campylobacter* population in Italian poultry." Foods 12.15 (2023): 2919.

ECR-OP3

Animal health

SUBCLINICAL MASTITIS IN LACAUNE AND CROSSBREED SHEEP FROM FARMS IN CENTRAL GREECE. ETIOLOGIC AGENTS, EFFECT ON MILK CHARACTERISTICS, AND EVALUATION OF INFRARED THERMOGRAPHY AS A DIAGNOSTIC TOOL

M. Lysitsas[3], C.N. Tsokana[4], S. Termatzidou[2], P. Kazana[1], V. Spyrou[2], C. Billinis[3], G. Valiakos[3]

[1]*Department of Agriculture, University of Ioannina, Arta, Greece*
[2]*Department of Animal Science, University of Thessaly, Larissa, Greece*
[3]*Faculty of Veterinary Science, University of Thessaly, Karditsa, Greece*
[4]*School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*

Introduction

Subclinical mastitis (SCM) is a major problem affecting dairy sheep. It is regularly underdiagnosed, causing economic losses through a downfall in milk yield, as well as animal welfare issues. The objective of this study was to identify the incidence, etiologic agents, antimicrobial resistance, milk characteristics, and predisposing factors for SCM in farms in central Greece. Moreover, to evaluate the potential of infrared thermography for an early, non-invasive diagnosis of relevant cases.

Materials and Methods

A total of 700 milk samples were obtained from phenotypically healthy Lacaune or Lacaune crossbreed sheep between November 23 and May 24. At the same time, more than 2000 thermal images were captured using a FLIR E96 camera. Aerobic culture, susceptibility testing, total mesophilic count (TMC), somatic cell count (SCC), and milk chemical analyses were performed. Infrared images were analyzed using FLIR Research Studio (2024.03.1) software.

Results

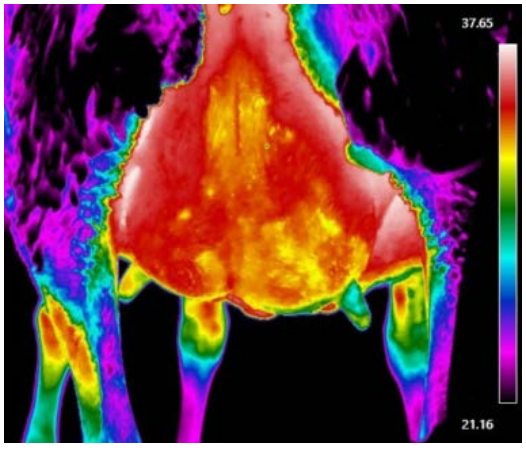
A high incidence of SCM was identified, with 184 pathogens obtained from 127 ewes (36.29%). Staphylococci were isolated in the majority (149/184, 80.97%) of these cases. The prevalent species were *S. epidermidis* (49), *S. chromogenes* (21), S. hyicus (17), and *S. simulans* (17). Considerable resistance rates were detected for tetracycline (29.67%), ampicillin (28.57%), and sulfamethoxazole–trimethoprim (23.26%). SCM was correlated with a significant increase in both TMC and SCC. Examination of the obtained images through evaluation of the mean temperature of specific udder regions highlighted the effectiveness of infrared thermography as a diagnostic tool. In particular, in 60 cases of unilateral SCM, detectable temperature variations were observed in the bilateral images.

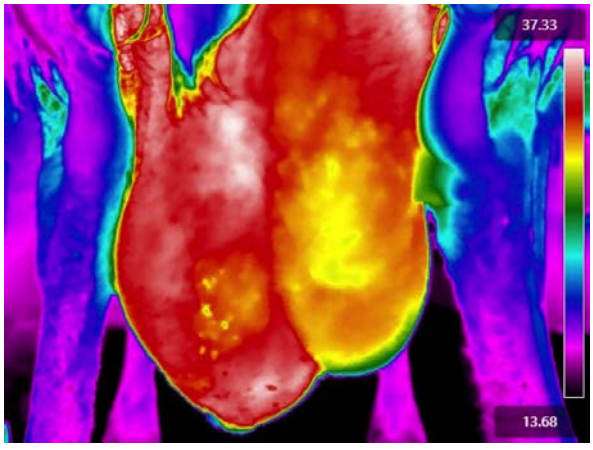
Discussion and Conclusion

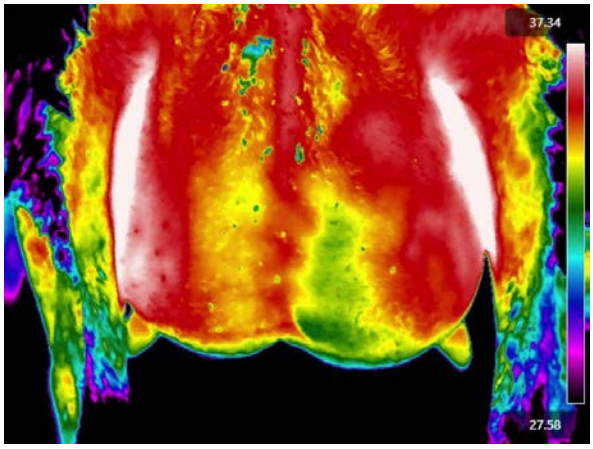
These results demonstrated the prevalence of SCM in dairy sheep in Greece and the potential of infrared thermography in its diagnosis. The implementation of targeted sampling after screening by the thermographic camera, using the proposed measurements, would confirm and strengthen this potential.

References

1. N.D. Giadinis et al. "Milk-drop syndrome of ewes": Investigation of the causes in dairy sheep in Greece, Small Ruminant Research, 2012, 106, 33-35.
2. I.A. Fragkou et al. Diagnosis of clinical or subclinical mastitis in ewes, Small Ruminant Research, 2014, 118, 86-92







ECR-OP4

Antimicrobial resistance / susceptibility testing

CIRCULAR ECONOMY AND ANTIMICROBIAL RESISTANCE: ASSESSMENT OF THE IMPACT ON THE AGRI-FOOD SYSTEMS

G. Barbieri[2], F. Gigliucci[2], P. Chiani[2], M. Carollo[1], M. Marra[1], A. Knijn[2], G. Brambilla[2], S. Morabito[2]

[1]*Core Facilities Technical Scientific Service Team (FAST), Istituto Superiore di Sanità, Rome, Italy*
[2]*Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy*

Introduction

The recycling of biowaste and water is crucial for sustainable agri-food supply chains. However, their regular and intense use as topsoil improvers (TSIs) is debatable, as they can lead to the diffusion of emerging hazards, such as agents of zoonoses and antimicrobial resistance genes (AMRs), in food production environments.

Materials and Methods

Thirty-one TSI samples of anthropogenic, animal and green waste origin and fifteen irrigation water samples from rivers, canals and civil wastewater treatment plants (cWWTPs) were analysed using shotgun metagenomic sequencing to detect AMR determinants and cultivated to assess whether the molecular signals observed were derived from live bacterial organisms.

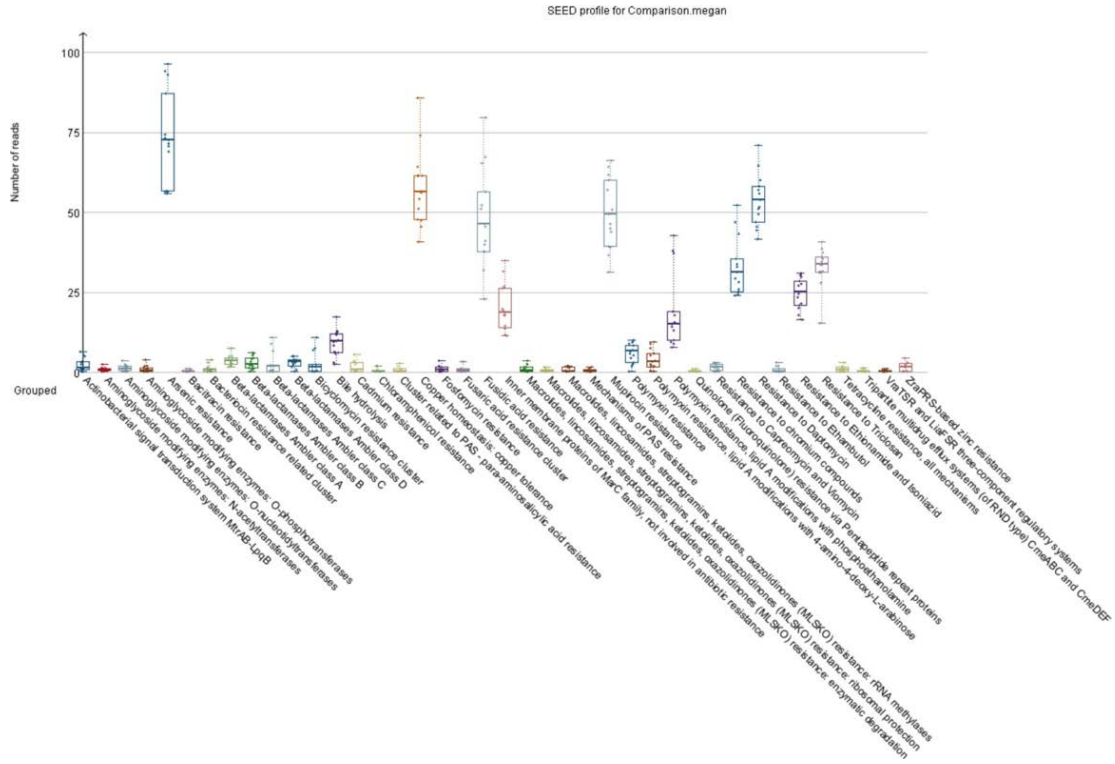


Figure 1. Antimicrobial resistance determinants in top-soil improvers.

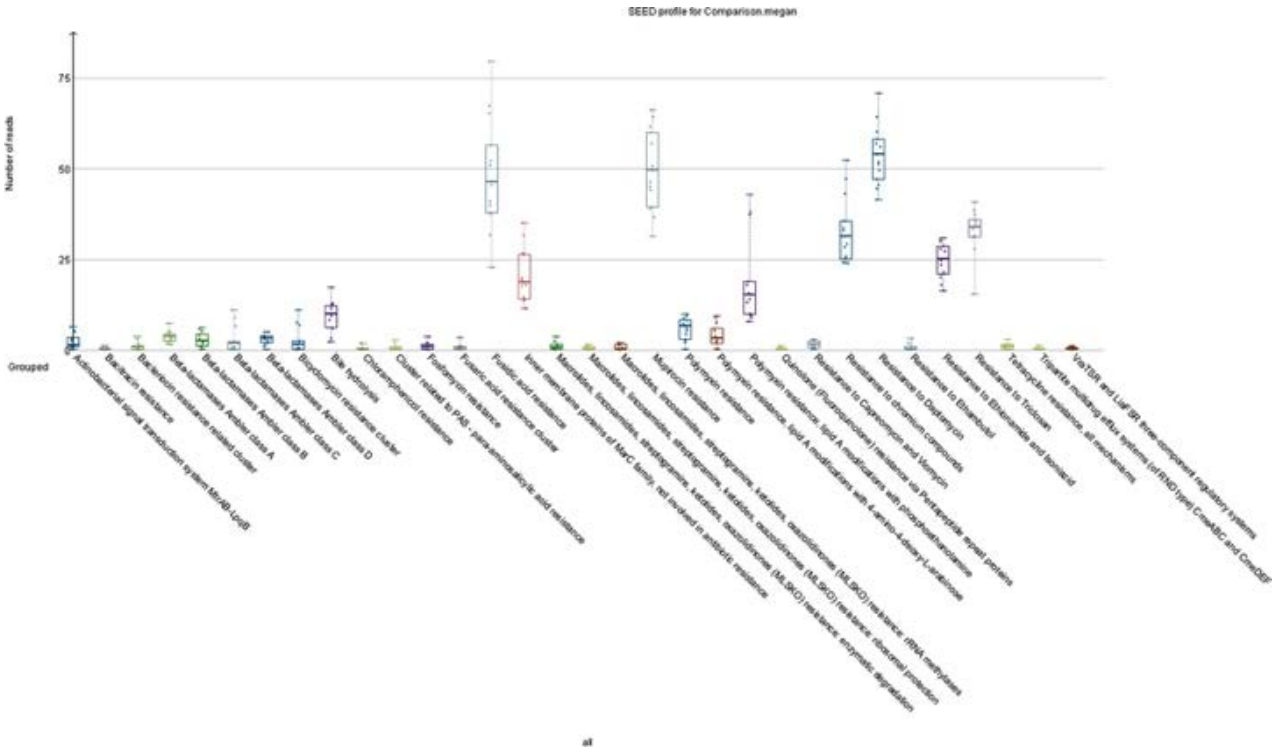


Figure 2. Antimicrobial resistance determinants in irrigation water samples.

Figure 3. Co-occurrence of antibiotic and heavy metal resistance genes in TSI and irrigation water.

Results
All the samples showed the presence of antibiotic and heavy metal resistance genes. The genes conferring resistance to mupirocin and fusidic acid were the most represented both in biosolids and irrigation water. Traits associated with resistance to molecules used in human and veterinary practice, such as tetracycline, fosfomycin, macrolides, quinolones and extended-spectrum β-lactamases, were identified. Interestingly, we detected genes related to antibiotics relevant for human clinical practice, such as ethionamide-isoniazide and daptomycin. The most represented metal resistance genes were related to arsenic and copper, in co-occurrence with AMR determinants. We could isolate from the samples live microorganisms belonging to multiple species such as *Bacillus*, *Clostridium*, *Lysinibacillus* and *Paenibacillus* spp., that were phenotypically resistant to the molecules identified with the metagenomic screening and possessed the expected multi-drug resistance gene cassettes.

Discussion and Conclusion
The use of TSI and irrigation water may contribute to the maintenance of an environmental pool of antibiotic resistance genes (ARGs) that can be spread in food-transmitted bacteria. Such ARG reservoir can be stabilized through the selective pressure exerted by heavy metal pollution in the soil, resulting in the selection of bacteria that are resistant to both compounds.

References
Tozzoli R. et al., 2017. https://doi.org/10.1111/jam.13308
Gigliucci F., et al., 2017. https://doi.org/10.1016/j.envres.2017.02.004 Barbieri G. et al., 2023. https://doi.org/10.1021/envhealth.3c00070
Yu, Z. et al., 2017. https://doi.org/10.1016/j.fm.2016.12.009

ECR-OP5

Proficiency Testing – participant and organizer perspective

SALMONELLA PROFICIENCY TESTS, THE EXPERIENCE OF THE ITALIAN NATIONAL REFERENCE LABORATORY

G. Cento[1], K. Antonello[1], S. Friso[1], E. Marafin[1], P. Zavagnin[1], S. Canali[1], C. Saccardin[1], M. Mancin[1], L. Barco[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

Introduction
The PT AQUA SA has been established as an institutional activity of the Salmonella Italian Reference Laboratory (NRL) at the Istituto Zooprofilattico Sperimentale delle Venezie. Since 2007, annually the NRL provides two schemes of the PT, regarding *Salmonella* isolation in the context of primary production and serotyping, which involve both public and private laboratories. Since 2015, the participation at the isolation scheme by private laboratories became a ministerial requirement to perform analyses in the context of the National Salmonellosis Plan in poultry primary productions. Furthermore, in 2022 NRL started to produce internally the Reference Material (RM). In regards to the serotyping scheme, it consists of the identification of 20 *Salmonella* serovars. Moreover, from 2014, it has been introduced an additional scheme specifically aimed to the identification of relevant serovars for broilers (10 isolates), to meet laboratories' need to perform analysis on samples from poultry farms and fresh chicken meat. Finally, in 2022, both for isolation and serotyping schemes NRL received the accreditation according to UNI CEI EN ISO/IEC 17043:2010[1].

Materials and Methods
The matrix of isolation of *Salmonella* spp. scheme consists of poultry feces, tested negative for *Salmonella* ISO 6579-1:2017/Amd.1:2020[2] and verified for Enterobacteriaceae and CMT. Participating laboratories have to assemble negative feces with RM, which is *Salmonella*-positive or -negative lyophilus. Instead, the matrix of the serotyping scheme consists of *Salmonella* strains, annually selected by the NRL based on their antigenic variability and epidemiological characteristics.

Results
The number of participants has been increasing over the years, with about 60 participants in the isolation scheme and 20 in the serotyping scheme per year. In case of incorrect performance, NRL provides a training for the involved laboratories, in order to attend a follow-up scheme.

Discussion and Conclusion
PT AQUA SA aims both to ensure quality control of the results of participating laboratories and to compare the obtained results, in order to evaluate their performance over time, especially for those laboratories involved in national control programmes in poultry.

References
1. UNI CEI EN ISO/IEC 17043:2010
2. ISO 6579-1:2017/Amd.1:2020

ECR-OP6

Immunology and vaccines

PREDICTING LUNG VIRAL LOAD IN VACCINATED PIGS EXPOSED TO INFLUENZA A VIRUS USING CYTOKINE EXPRESSION ANALYSIS OF WHOLE BLOOD STIMULATION

G. Fantoni[2], A. Müllebner[2], N. Palmai[3], F. Deutskens[1], J.C. Duvigneau[2], I. Kiss[3]

[1]*Ceva Animal Health (Riems) GmbH, An der Wiek 7, D-17493 Greifswald, Germany*
[2]*Department for Biological Sciences and Pathobiology, Veterinary University Vienna, Veterinärplatz 1, A-1210 Vienna, Austria*
[3]*Scientific Support and Investigation Unit, Ceva-Phylaxia Campus in H-1107 Szállás u. 5. Budapest, Hungary*

Introduction
The swine species is critical in influenza virus ecology serving as a mixing vessel for viruses of different origin (swine, human, avian). Emerging reassortant viruses may pose zoonotic and public health risks. For testing new vaccines, knowledge on cytokine changes upon vaccination and/or challenge with swine influenza virus (SIV) would be useful, but is still limited. Aiming to identify indicators of an effective vaccine response, we questioned whether cytokine expression in ex-vivo stimulated whole blood (WB) is predictive for viral load outcomes in pigs.

Materials and Methods
We studied the correlation between cytokine (IL2, IFNg and IL4) gene expression levels and SIV load in the lungs of pigs vaccinated against, and challenged with SIV. Pigs (Danbred) 8 weeks of age were vaccinated with either of two different constructs (day 0), followed by booster (day 21) and challenge with SIV (strain H1avN1, 3 ml per nostrils, of a titer 108,3 TCID50/ml Bad Griesbach; day 42). Heparinized blood was taken at day 43 and 47, added to RPMI (4+1) and stimulated for 6h with SIV (same titer as for challenge). Cytokine mRNA levels were quantified by RTqPCR. SIV load was determined by qPCR within lung tissue over all lobes at sacrifice (day 47).

Results
Low viral load in lungs correlated well with a high capacity to express IL2 in SIV-stimulated WB at day 43. However, at day 47 the capacity to express IL4 in SIV-stimulated WB was higher in animals with low virus load. Thus, the switch of the IL4/IL2 ratio in SIV-stimulated WB from lower to higher values during the days after challenge predicts low virus levels in lungs.

Discussion and Conclusion
Our data suggest that a strong Th1 response against SIV in WB one day after challenge indicates a rapid activation of memory cells, enhanced virus recognition and clearance. In conjunction with the subsequent shift towards Th2, the kinetics of the IL4/IL2 expression ratio elicited by SIV in WB is a useful marker for immune protection against SIV. Our study demonstrates the suitability of cytokine expression analysis in ex-vivo stimulated WB as a predictive tool for vaccine efficacy.

ECR-OP7

Animal health

EVALUATION OF FIVE ELISA KITS FOR THE SEROLOGICAL DIAGNOSTICS OF ENZOOTIC BOVINE LEUKOSIS

Z. Zurovac Sapundžić[1], D. Glišić[1], S. Šolaja[1], L. Veljović[1], N. Jezdimirović[1], V. Milićević[1]

[1]*Institute of Veterinary Medicine of Serbia, Janisa Janulisa 14, Serbia*

Introduction
Enzootic bovine leukosis (EBL) is the most important neoplastic disease in cattle, presented worldwide. It is caused by bovine leukemia virus (BLV), closely related to human T-cell leukaemia virus. Although the disease is usually asymptomatic, persistent lymphocytosis present in 30% of infected leads to various clinical outcomes (Aida et al., 2013). EBL is commonly diagnosed using serological tests. Discrepancies between different tests araise concerns, specifically in a term of false negative results. As National Reference Laboratory for EBL in Serbia we aimed to compare performances of five commercially available ELISA kits.

Materials and Methods
A total of 138 cattle sera submitted for the confirmatory testings together with 100 sera from BLV-negative herds were tested using five ELISA kits. The considered ELISAs were: Leukosis Serum X2 Ab Test, IDEXX (test A), ID Screen® BLV Competition, IDvet (test B), Bovine Leukemia Virus Antibody Test Kit, ELISA, VMRD (test C), SVANOVIR® BLV gp51-Ab, Svanova (test D) and INgezim BLV Compac 2.0, Ingenasa (test E).

Results

Perfect agreements of 100% and Cohen's k=1 were obtained for the tests B, D and E. Almost perfect agreement (93%, Cohen's k=0.67) was obtained when comparing these to the test C. Slight agreement of 92% and Cohen's k=0.14 was acquired between tests A and B, D and E. Between test A and C, a slight agreement of 88.4% (Cohen's k=0.099) was observed. Diagnostic sensitivity (Dse) and specificity (Dsp) of the used tests are presented in the picture.

Discussion and Conclusion

EBL causes significant economic losses in cattle industry. Elimination of EBL is an ultimate goal, achievable through reliable and accurate diagnostic. Results of the study showed different Dse and Dsp, but also different agreements between the used tests. Diagnostic performances of the used tests in practice can differ from manufacturers' reported ones. Choice of the used test should depend on its diagnostic performances, that could assure precise results and synergistically support the applied eradication programs.

References

Aida Y, Murakami H, Takahashi M, et al., 2013. Mechanisms of pathogenesis induced by bovine leukemia virus as a model for human T-cell leukemia virus. Front Microbiol 4:1–11.

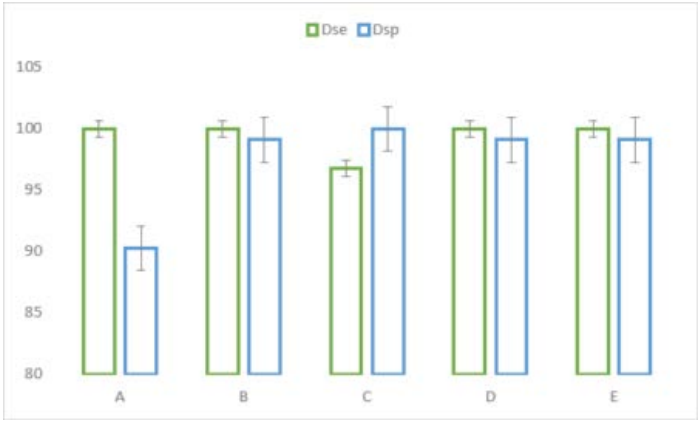


Figure 1.

ECR-OP8

Emerging and re-emerging diseases

ADVANCING ASF DIAGNOSIS: INSIGHTS FROM NON-HAD GENOTYPE II AFRICAN SWINE FEVER VIRUSES IN THE EU (2017-2022)

N. Casado[2], A. Soler[2], E. Madueño[2], R. Nieto[2], C. Pérez[2], A. Simón[2], C. Gómez[2], I. Nurmoja[1], L. Krivko[3], M. Frant[5], S. Pileviciene[4], M. Arias[2], C. Gallardo[2]

[1]Estonian NRL: National Centre for Laboratory Research and Risk Assessment (LABRIS), Tartu, Estonia
[2]European Union Reference Laboratory for African Swine Fever (EURL), Centro de Investigación en Sanidad Animal, INIA-CISA, Valdeolmos, Madrid, Spain
[3]Latvia NRL: Laboratory of Microbiology and Pathology, Institute of Food Safety, Animal Health and Enviroment, BIOR, Riga, Latvia
[4]Lithuania NRL: National Food and Veterinary Risk Assessment Institute (NFVRAI), Vilnius, Lithuania
[5]Poland NRL: National Veterinary Research Institute, Puławy, Poland

Introduction

African swine fever (ASF) is a highly virulent hemorrhagic disease affecting domestic pigs and wild suids, caused by the DNA-ASF virus (ASFV), the sole member of the Asfarviridae family. ASF affects only members of the Suidae family and has no zoonotic potential. ASFVs are classified into 24 genotypes, with genotype II responsible for the current epizootic in Europe, Asia and the Pacific, and the Americas. In 2017, the first non-haemadsorbing (HAD) and attenuated genotype II ASFV was isolated in the EU, characterized by a mutation in the EP402R gene [1]. Since then, 13 additional non-HAD ASFVs have isolated from hunted wild boar in Lithuania, Latvia, Estonia and Poland. The objective of this study was to understand the molecular mechanisms behind these non-HAD ASFVs and their impact on ASFV transmission and virulence.

Materials and Methods
The full EP402R gene of the 13 non-HAD ASFV was sequenced and 8 of them were used for in vivo experiments to assess its pathogenicity. 3- months' old domestic pigs were divided into different groups, with 5-6 pigs per group. Two pigs per group were intramuscularly inoculated with 10 TCDI50 of each of the non-HAD ASFVs selected. The remaining pigs act as in contact pigs. Clinical signs, lesions, viremia, excretion, and viral loads in organs and tissues were evaluated.

Results
Sequencing of the EP402R gene from 13 non-HAD ASFV isolates revealed seven different mutations in various domains of the CD2v protein. Biological characterization of eight non-HAD ASFVs showed that four isolates were attenuated, two were virulent, and two were moderately virulent. The virulent strains, after in vivo passages, recovered the HAD phenotype while maintaining the genetic characteristics of the mutated EP402R gene.

Discussion and Conclusion
This study demonstrates that non-HAD ASFV can induce various clinical forms of ASF, ranging from acute to subclinical infections, and can revert to the HAD phenotype. These findings are crucial for surveillance and control from a diagnostic perspective, highlighting the importance of identifying and understanding the dynamics of different ASFV strains circulating to improve diagnostic accuracy and disease management.
This study was supported by the EURL for ASF (grant nº UE- LR PPA).

References
1. Gallardo C, et al 2019. Transbound Emerg Dis. 2019;66(3):1399-1404. doi:10.1111/tbed.13132

ECR-OP9

New Diagnostic Testing Technologies

EVALUATION OF ALTERNATIVE SAMPLES AND PROTOCOLS FOR SEROLOGICAL MONITORING OF AVIAN INFLUENZA VACCINATION IN DUCK FLOCKS

M. Walch[1], C. Castille[1], B. Casalinho[1], M. Gaimard[2], C. Redal[2], G. Croville[1], S. Lesceu[2], J. Guerin[1]

[1]*IHAP, Université de Toulouse, INRAE, ENVT, Toulouse, France*
[2]*Innovative Diagnostics, Grabels, France*

Introduction
Specific lineages of H5 highly pathogenicity Avian Influenza viruses (AIV) cause large-scale epizootics, substantial economic losses and zoonotic threats. Beside biosecurity and culling of infected poultry flocks, vaccination is a supplementary option and is implemented in ducks in France since October, 2023. Such vaccination program requires intensive surveillance, including PCR and serological testing. Regular blood sampling must be performed by trained staff, which is costly and time-consuming for large-scale monitoring programs. In this perspective, we evaluated alternative sample matrices, associated with deposit of samples on paper cards, for the serological monitoring of ducks.

Materials and Methods
Individual serological monitoring was performed on mule duck flocks vaccinated with H5 registered vaccines. H5 antibody were titrated using a commercial H5 Duck indirect ELISA (IDvet). For each bird, blood and feather samples were collected at different time-points after H5 vaccination: blood samples were taken using (1) regular blood sampling or alternatively, non-invasive bleeding and deposition of a blood droplet onto transport cards. Immature feather sampling was performed using three different methods: (1) residual blood from immature feathers, (2) interstitial fluid from feather pulps, and (3) feather pulp. For each bird, sample type and time point, ELISA titers were determined and compared at the individual and flock levels.

Results
A total of 200 mule ducks was sampled for this study. ELISA assays showed that the different blood and feather samples and protocols resulted in similar ELISA titers at the flock level, with correlation coefficients ranging from 0.80 to 0.85. Non-invasive bleeding reveals particularly interesting in very young ducklings, for which regular bleeding is hazardous.

Discussion and Conclusion
Preliminary results suggesting the potential of feather-based methods as reliable and less invasive alternatives for serological monitoring of HPAI antibody levels in duck flocks. The integration of transport cards optimizes sample collection, transport, and storage, thereby improving the feasibility of serological monitoring.

ECR-OP10

Emerging and re-emerging diseases

ACUTE INFECTION DYNAMICS OF DOMESTIC CAT *HEPADNAVIRUS* (DCH)

F. Pellegrini[1], M. Burgio[1], V. Cicirelli[1], G. Diakoudi[1], G. Elia[1], A. Salvaggiulo[1], B. Di Martino[2], F. Di Profio[2], N. Decaro[1], M. Camero[1], G. Lanave[1], V. Martella[1]

[1]*Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy*
[2]*Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy*

Introduction

The Hepadnaviridae family comprises circular DNA viruses, including human hepatitis B virus (HBV). Domestic cat hepadnavirus (DCH) was discovered in 2018 and it is associated with chronic liver disease in cats [1]. The dynamics of acute DCH infection in feline populations remain unclear. In this study we investigated the prevalence and characteristics of acute DCH infection in juvenile cats (6-12 months old), assuming that animals positive at this age are likely in the acute phase of the infection.

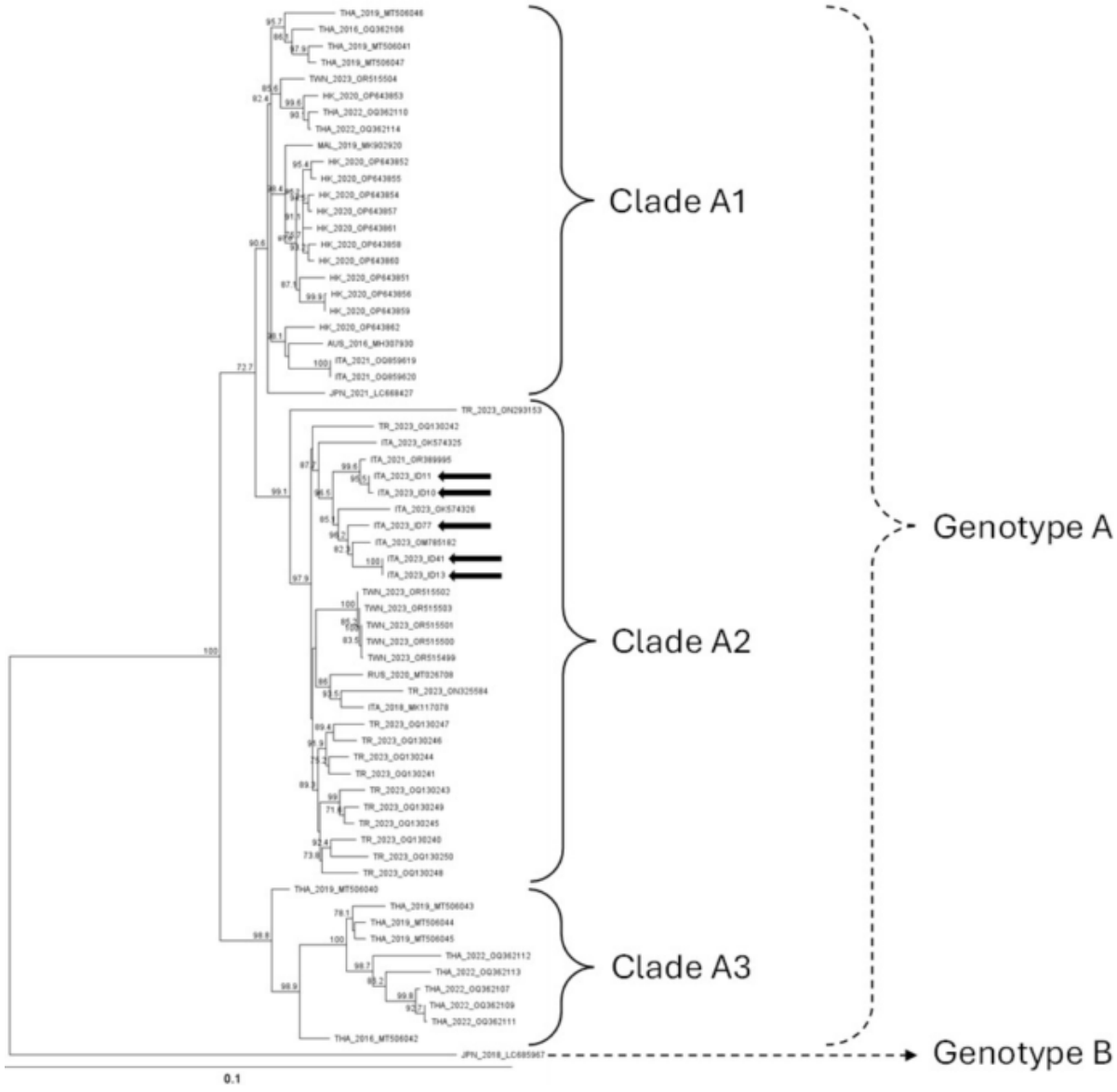


Figure 1. Neighbor-joining phylogenetic tree based on the complete genomes of domestic cat hepadnavirus (DCH). The tree was elaborated using the alignment the full-length nucleotide sequence of DCH strains generated in this study and the cognate sequences of DCH strains retrieved from GenBank database.

Materials and Methods

During spaying surgery, serum, mucosal swabs (oral, nasal, rectal, vaginal), and ovarian tissue were collected from 77 female cats. Samples were screened for DCH by a quantitative (q) PCR [2]. The full genome was obtained using an Artic- like strategy. The PCR products were pooled and used as the template for library preparation with SQK-LSK110 kit and sequencing on Oxford Nanopore Technology (ONTTM) platform.

Results

Five cats tested positive in the serum (15.7-27.8 Ct). DCH DNA was also detected in the mucosal swabs (26.2-42.1 Ct) of the five cats and in the ovarian tissues of a single animal. All the animals remained positive in the sera (17.9-24.5 Ct) and swabs (32.9-41.1 Ct) when re-screened after 3 months. On sequence and phylogenetic analysis, the DCH strains fell into two different sub-clusters within clade AII, which comprises other strains found in Europe.

Discussion and Conclusion

The prolonged duration of acute infection in cats is similar to the patterns observed in HBV-infected human patients. Likewise, the detection of DCH in genital swabs suggests that the virus may be shed via the genital route for long periods, implying possible sexual and perinatal transmission, as observed for HBV [3]. Screening of juvenile cats before reproduction could be useful for effective prevention and control of DCH in cats [4].

References

1. Capozza P., et al., 2023. 10.3390/vetsci10120668
2. Lanave G., et al., 2019. 10.1038/s41598-019-47175-8
3. Zhao X., et al., 2022. 10.2147/IDR.S359113
4. Noubiap J.J., et al., 2022. 10.1016/s2214-109x(22)00046-8

ORAL PRESENTATION

OP1

Epidemiology / Risk analysis and communication

BUSINESS INTELLIGENCE TOOLS TO ENHANCE ANTIMICROBIAL STEWARDSHIP: THE ROLE OF DIAGNOSTIC LABORATORIES IN PRODUCING USEFUL AND COMPARABLE DATA.

A. Maisano[1], G. Santucci[1], G. Bontempi[1], N. Formenti[1], M. Bottinelli[2], A. Barberio[2], L. Gagliazzo[2], L. Alborali[1], A. Ricci[2], G. Varisco[1], L. Candela[3], A. Maggio[3], G. Filippini[3], U. Della Marta[3], S. Catania[2]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna, Brecia, Italy*
[2]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*
3Italian Ministry of Health, Rome, Italy

Introduction
Farm-level monitoring of antimicrobial usage (AMU) is a priority (1). By using business intelligence tools, it is possible to manage, analyze, and summarize high-density data related to veterinary medical products and their usage. Reducing AMU and selecting the proper drug are crucial for maintaining antimicrobial efficacy, which should be based on antimicrobial susceptibility (AMS) testing. The ClassyFarm system is Italy's main strategy for promoting prudent antimicrobial use and serves as the national surveillance system for AMU and AMS.

Materials and Methods
The Italian public health institutes (Istituti Zooprofilattici Sperimentali - IIZZSS) have established standards for selecting, executing, and interpreting minimum inhibitory concentration (MIC) tests for significant bacterial pathogens. All AMS data generated by IIZZSS are gathered and processed in an organized manner. Consequently, the importance of laboratory tests in monitoring antimicrobial resistance on a national level becomes fundamental for the whole process.

Results
In fact, starting from 2021, more than 70,000 MIC test records have been loaded into the database, and authorized users can easily consult all AMU and AMS information via interactive dashboards generated by Microsoft Power BI. ClassyFarm users (authorities, veterinarians, farmers, and industry stakeholders) can quickly consult AMU and AMS data at different levels, from national to individual farms. Users can analyze detailed data (e.g., AI, administration route, age group, trends) and compare it with national and local averages. Authorities use ClassyFarm to plan pharmacosurveillance controls through risk-based farm selection (e.g., high AMU).

Discussion and Conclusion
ClassyFarm is a significant public health application of business intelligence, essential for planning controls, verifying AMU reduction policies, and monitoring antimicrobial resistance. For private stakeholders, it provides a benchmarking system for AMU and a tool for selecting appropriate antimicrobial therapy.

References
1. Munk P, Yang D, Röder T, Maier L, Petersen TN et al. The European livestock resistome. mSystems. 2024 Apr 16;9(4):e0132823. doi: 10.1128/msystems.01328-23.

OP2

Epidemiology / Risk analysis and communication

AFRICAN SWINE FEVER IN WILD BOARS: EVALUATION OF THE SPEED OF ADVANCEMENT OF THE INFECTION FRONT IN AREAS WITH DIFFERENT ECOLOGICAL FEATURES OF THE PROVINCE OF PAVIA

V. Cappa[1], M. Tironi[1], A. Scaburri[1], S. Salvato[1], V. Maggiano[1], S. Bellini[1]

[1]*Lombardy Epidemiological Surveillance, Istituto Zooprofilattico Sperimentale Lombardia ed Emilia Romagna (IZSLER), Brescia, Italy*

Introduction
In June 2023, the first case of African Swine Fever (ASF) was detected in wild boars in the province of Pavia. This first occurrence was

identified on the border with Piedmont, where the disease was introduced in 2022 (1). Since its introduction, ASF has spread in wild boars in Pavia and, to date, in the province there are two main clusters of infection, one to north and the other to south of Po River (Figure 1); the two clusters are not directly connected. The two areas have different ecological characteristics: the first is located in contiguity with Piedmont, an area with an agricultural vocation; the second is in Ticino Park, an ecological corridor where wild boar has reached high densities. Considering the ecological characteristics of the two clusters, in this study we compared speed of advancement of the infection front in the two areas.

Materials and Methods

Data of ASF positive wild boar were extracted from the Animal Disease Information System (ADIS). Kernel density was employed to identify clusters while Kriging method was used to estimate the speed of advancement of the infection fronts. For each cluster, the speed is expressed as a mean value of days that elapse from the first case to each of the following ones (2). The differences between the infection fronts were evaluated using the Kruskal-Wallis test. Maps were produced using ArcGISPro 3.0.3 and statistical analysis was performed using R 4.3.1.

Results

From ADIS were extracted 209 positive cases: 136 belonging to the southern cluster and 73 to the northern one. The speed of advancement was 255.4 days in the south and 126.0 days in the north. Therefore, it resulted faster in the northern cluster than in south ($p<0.0001$) (Table 1). In the map, municipalities with the highest speed were reported in red (Figure 2).

Discussion and Conclusion

In a previous study, the risk of ASF transmission at the interface between wild and domestic pigs in the municipalities of the second cluster resulted higher than in municipalities of the first cluster (3). In this study, it was observed that in the municipalities of the second cluster the speed of advancement of the infection was also faster. This information may be relevant for disease control purpose and to establish priority of intervention.

References

1. doi:10.3390/ani14091295
2. doi:10.4236/oalib.1102663
3. doi:10.3390/pathogens12121462

	The first cluster in south	The second cluster in north
Number of positive cases	136	73
Speed, mean (*st. dev.*)	255.4 (53.5)	126.0 (52.9)
Date of **first** positive case in cluster	19/06/2023	04/10/2023
Date of **last** positive case in cluster	17/05/2024	20/05/2024
Number of days passed from first to the last positive case	334 days	229 days

Table 1. Number of positive wild boar and number of days from the first case of each cluster in Pavia

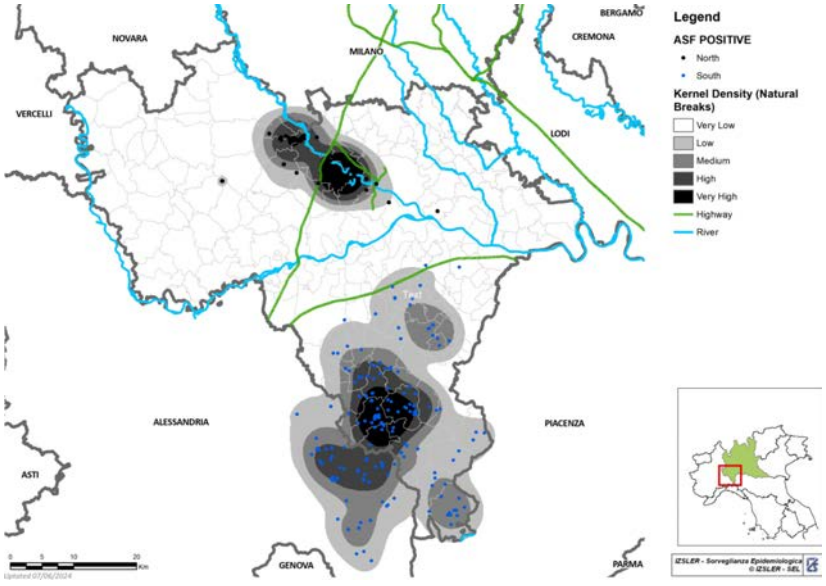


Figure 1. Kernel density map of positive wild boar in Pavia

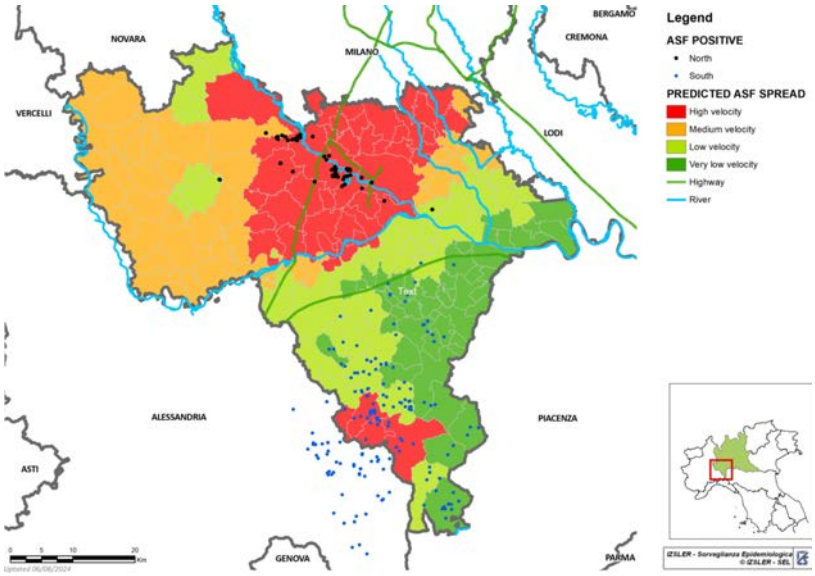


Figure 2. Map representing the speed of ASF transmission at municipality level (Kriging method)

OP3

Epidemiology / Risk analysis and communication

COMPARING APPLES TO ORANGES: USING SOCIAL RESEARCH TO HARMONIZE VETERINARY CLINIC DIAGNOSTIC PRACTICES

M. Zago[1], G. Mascarello[1], A. Pinto[1], M. Bernardini[2], E. Mazzotta[1], P. De Benedictis[1], S. Crovato[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*
[2]*Università degli studi di Padova, Italy*

Introduction
Veterinary diagnostic practices for rare diseases vary and often rely on practitioners' individual decisions based on their knowledge, experiences, and attitudes. While most European territories are free from rabies virus, lyssaviruses circulate in bat reservoirs and can occasionally infect cats, causing fatal encephalitis indistinguishable from rabies. The Connetti-Cat project aims to improve and harmonize syndromic surveillance for rabies/lyssavirus in cats. Social research methods were used to gather information on current practices to develop a standardized diagnostic protocol whose objective was to harmonize clinical diagnostic procedures.

Materials and Methods
Veterinary practitioners from 12 Italian neurological clinics were involved, using two social research techniques. The Delphi questionnaire, with eight open-ended questions about lyssaviruses, collected information to define the diagnostic protocol proposal. The Consensus Conference, a formal and structured meeting among experts, validated this proposal and defined the final diagnostic protocol.

Results
Experts agreed on a clinical diagnostic protocol. Figure 1 summarizes crucial steps for including or excluding lyssavirus infection in differential diagnosis. The protocol was implemented through an online open-source flowchart guiding veterinarians towards case definition and further actions to rule out the hypothesis.

Discussion and Conclusion
Participatory social research techniques enabled the harmonization of the clinical flowchart, improving syndromic surveillance for lyssavirus in cats. The project outputs will be available to veterinarians in clinics to facilitate a rapid and effective detection of lyssavirus infections in cats, providing accurate information to stakeholders.

References
Powell M. and Kleinman D.L. (2008). Building citizen capacities for participation in techno-scientific decision making: The democratic virtues of the consensus conference model. Public Understanding of Science, 17, 329-348.

WOAH Terrestrial Manual 2023. Chapter 3 . 1 . 1 8 . Rabies (infection with rabies virus and other lyssaviruses). Available online at https://www.woah.org/fileadmin/Home/eng/Health_standards/tahm/3.01.18_RABIES.pdf

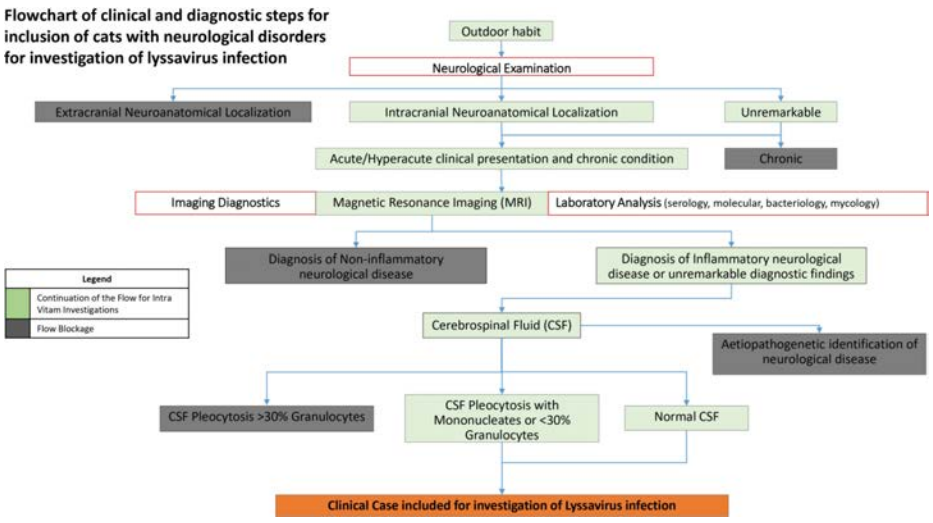


Figure 1.

OP4

Miscellaneous

A BAYESIAN APPROACH TO INTER-LABORATORY DIAGNOSTIC TEST EVALUATION FOR SEROLOGICAL ASSAYS

V. Ahlberg[3], M. Denwood[4], E. De Freitas Costa[5], M. Counotte[5], A. Dekker[5], M. Arnold[2], A. Asfor[2], A. Dastjerdi[2], G. Koukam[1], S. Valas[1], A. Comin[3]

[1]*Agency for Food, Environmental and Occupational Health & Safety (ANSES), France*
[2]*Animal Plant and Health Agency (APHA), the United Kingdom*
[3]*Swedish Veterinary Agency (SVA), Sweden*
[4]*University of Copenhagen (UCPH), Denmark*
[5]*Wageningen Bioveterinary Research (WBVR), the Netherlands*

Introduction

Inter-laboratory comparisons such as proficiency-testing programmes improve the validation of diagnostic methods but focus only on the analytical accuracy. The evaluation of diagnostic accuracy is often left to individual labs and is frequently overlooked, especially when lacking characterized or positive samples. This poses challenges when inferring disease prevalence or substantiating disease freedom in populations. Bayesian latent class models (BLCMs) are the recommended framework for test evaluation when samples come from animals of unknown infectious status [1] and are widely used in veterinary diagnostics [2]. The aim of the study was to develop a proof-of-concept for inter- laboratory evaluation of serological tests using BLCMs, with Bovine Viral Diarrhoea (BVD) as a case study.

Materials and Methods

The study encompassed 485 bovine serum samples sourced from four European countries. Samples were collected from outbreak farms, surveillance activities or biobanks and chosen to design populations with either high or low/absent BVD seroprevalences. Samples were aliquoted, sent to each of the other partner institutes (Figure 1), and analysed in parallel using available routine diagnostic tests. A BLCM was formulated to determine the sensitivity and specificity of each test, considering presence of antibodies as the latent status.

Results

All tests showed a high degree of agreement, with 437/485 (90.1%) samples having the same outcome for all tests. Sensitivity (Se) was high for all tests, ranging from 95.1% to 99.1%. Specificity (Sp) was also high (97.3% to 99.7%) for all tests except SVANOVIR (90.4%) which also varied considerably between populations (Table 1). The BLMC further allowed the creation of ROC curves as guidance for cut-off optimization, as illustrated in Figure 2.

Discussion and Conclusion

This proof-of-concept study displays the potential for applying BLCM in an inter-laboratory setting for cost-effective test evaluation of routine diagnostics. Using samples from multiple countries helps to ensure populations with varying prevalences, allowing for robust estimates despite unknown sample status. This is especially useful for countries that lack positive sample material due to the disease being absent.

References

1. WOAH Terrestrial manual (2023) chapter 1.1.6.
2. Cheung et al. (2021) DOI: 10.20506/rst.40.1.3224

Table 1. Diagnostic sensitivity and specificity estimates for the BVD ELISA tests included in the study, as provided by the BLCM.

Laboratory	Country	ELISA test	Sensitivity	Specificity
ANSES	France	Bio-X Monoscreen Ab ELISA BVDV (EO)	95.1%	99.7%
		ID Screen BVD p80 Antibody Competition – short protocol	98.8%	99.1%
		ID Screen BVD p80 Antibody Competition – long protocol	98.0%	99.4%
		IDEXX total Ab X3 – long protocol	97.8%	99.7%
APHA	United Kingdom	IDEXX BVDV total Ab test	98.3%	97.7%
SVA	Sweden	SVANOVIR BVDV-Ab ELISA	98.8%	90.3%
		ID Screen BVD p80 Antibody Competition – short protocol	98.8%	99.3%
WBVR	Netherlands	PrioCHECK BVDV Antibody ELISA	98.9%	99.2%

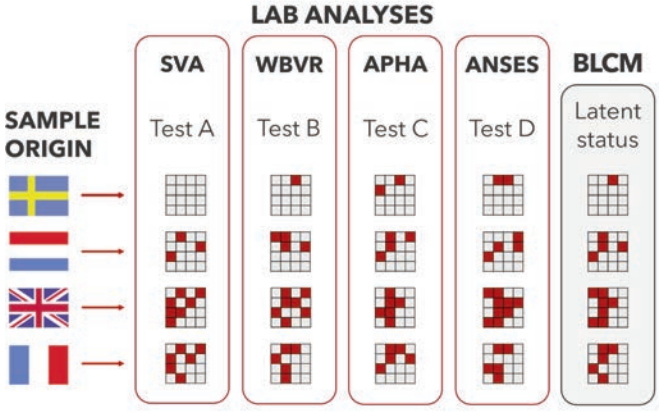


Figure 1. Schematic view of the study design of the inter-laboratory diagnostic test evaluation and the role of the Bayesian latent class model (BLCM) to determine the latent status (the unknown true disease status) of each sample.

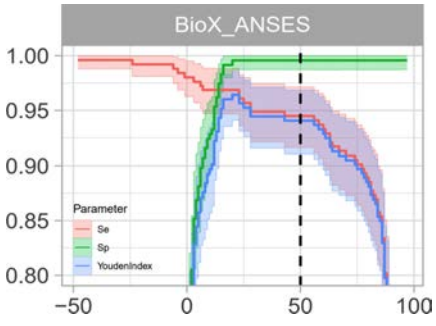


Figure 2. Receiver operating characteristic (ROC) curve of the Bio-X Monoscreen Ab ELISA BVDV test as performed by ANSES, indicating sensitivity (red), specificity (green) and YoudenIndex (blue). The dashed vertical line indicates the cut-off used.

OP5

Zoonoses / vector borne diseases

GENETIC DIVERSITY OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* FROM FREE-RANGING RED DEER

S. Lauzi[3], C. Luzzago[3], P. Chiani[2], M. Nava[3], V. Michelacci[2], S. Giacomelli[1], G. Scavia[2], S. Morabito[2], R. Tozzoli[2]

[1]*ATS della Montagna, Sondrio, Italy*
[2]*Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità Rome, Italy*
[3]*Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Milan, Italy*

Introduction
Red deer (*Cervus elaphus*) are known to play a role as carriers for Shiga toxin-producing *Escherichia coli* (STEC), with potential zoonotic impact (1-3). The aim of this study was to characterize STEC isolates in free-ranging red deer in Central Italian Alps by whole genome sequence (WGS) analysis.

Materials and Methods
Faeces were collected from hunted animals in Valle Camonica, Northern Italy during September-December 2022. Following enrichment, DNA was extracted and the presence of *stx* genes was detected by qPCR. Positive samples were streaked onto MacConkey agar and single colonies tested by PCR for *stx* and *eae* genes. STEC isolates were subjected to WGS.

Results
stx genes were detected by qPCR in 68/92 (73.9%) faecal samples and STEC were isolated from 11 (11.9%) of the positive samples. Subtyping of *stx* identified *stx2b* (n = 9), *stx2a* (n = 1), *stx1c* (n = 2) and *stx1a* (n = 1). Eight different serotypes and 8 sequence types were identified, with O27:H30 ST753 detected in more than one isolate (n = 3). Interestingly, the cgMLST analysis showed no correlation among these strains (AD ranging from 44 to 98). Additional virulence factors were detected in all strains, with gad (n = 11), terC (n = 10), mch (n = 9), subAB (n = 9), traT (n = 9), espI (n = 9), iha (n = 8), tia (n = 8) and iss (n = 8) predominating. Five STEC strains harbored the *ehx* gene, a hallmark of a large STEC virulence plasmid. Notably, only one isolate, the one harboring stx2a, possessed the *eaeA* gene and belonged to serotype O26:H11.

Discussion and Conclusion
Our results showed a wide range of strain types circulating in a red deer population in the Central Italian Alps, confirming that red deer are carriers for STEC strains (1, 2). In particular, we could isolate one STEC strain carrying *eae* gene as well as *stx2a*, an *stx* subtype often associated with STEC from severe disease in humans. Altogether, our findings highlight the need of STEC surveillance in wild ruminants.

References
1. Dias D. et al., 2022. The Science of the total environment, 812, 152324.
2. Lauzi S. et al., 2022. Transboundary and emerging diseases, 69(4), 1902–1911
3. Szczerba-Turek A. et al., 2023. Animals, 13(5), 901

OP6

Zoonoses / vector borne diseases

FIRST DETECTION OF *LEPTOSPIRA INTERROGANS* SEROVAR CANICOLA IN *CARACAL CARACAL*: A THREAT TO ZOO ANIMALS AND WORKERS?

G. Sgroi[1], L. Younes[2], S. Pasquale[3], G. Amalia[1], S. Rea[1], B. Degli Uberti[1], M. Dimatteo[1], M. D'Amore[1], P. Khademi[4], M.G. Lucibelli[1], N. D'Alessio[1]

[1]*Experimental Zooprophylactic Institute of southern Italy, Portici (Naples), Italy*
[2]*MEPHI - Microbes, Evolution, Phylogénie et Infections, Marseille (Paris) France*
[3]*The Naples Zoo, Naples, Italy*
[4]*University of Urmia, West Azerbaijan, Iran*

Introduction
Zoonotic leptospirae are a threat for wildlife conservation and zoo workers (Webb *et al.,* 2020). The aim of this study was to report the first case of fatal leptospirosis in a caracal (*Caracal caracal*).

Materials and Methods
An 8-years old neutered male caracal of the Naples zoo (southern Italy), died after one week displaying sialorrhoea, lingual ptosis and

neurological changes, was delivered to the Unit of Wildlife Diseases of the Experimental Zooprophylactic Institute of southern Italy (Portici, Naples), for necroscopic, histopathological and molecular investigations.

Results

Necropsy revealed severe lesions, with massive jaudince, epatomegaly, splenomegaly, abundant peri-renal fat and kidney alterations. Histopathology showed diffuse renal tubular degeneration and kidney, spleen and liver samples tested positive for *Leptospira interrogans* serovar Canicola by the combined real-time/endpoint PCR-sequencing approach. Phylogenetic analysis of the *LipL32* gene indicated a close relationship of the sequence type herein found with those of humans from China, clusterizing with a bootstrap value >90%.

Discussion and Conclusion

Necroscopic and histopathological lesions indicated the severe clinical picture of *L. interrogans* sv. Canicola in caracals, indicating risk of infection for zoo workers (ECDC, 2023). Improving preventative measures, such as protective clothes, synanthropic population control and proper water/ feed storage, is advocated for mitigating the transmission of this bacterium in zoos (Dossou *et al.*, 2022). The close relationship of *L. interrogans* sv. Canicola herein found with those of humans requires further pathogenic investigations, as well as assessing prevalence and transmission routes of this zoonotic agent in zoos.

References

1. Dossou HJ, Le Guyader M, Gauthier P, et al. Fine-scale prevalence and genetic diversity of urban small mammal- borne pathogenic Leptospira in Africa: A spatiotemporal survey within Cotonou, Benin. Zoonoses Public Health. 2022;69(6):643-654.
2. ECDC. Leptospirosis. Annual Epidemiological Report for 2021. Stockholm; 2023.
3. Webb JK, Keller KA, Sander SJ, et al. Clinical disease and treatment of Leptospira kirschneri sv Grippotyphosa in a Sumatran tiger (Panthera tigris sumatrae). J Am Vet Med Assoc. 2022;260(11):1-6.

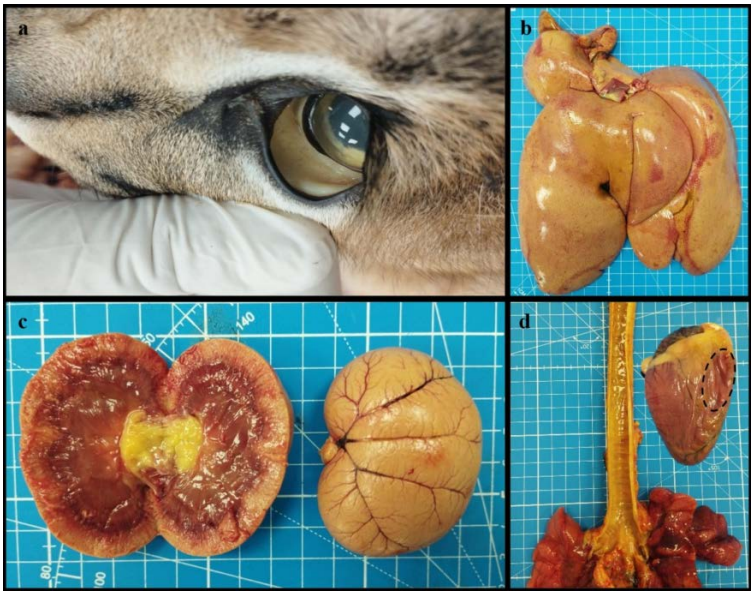


Figure 1. Macroscopical findings at the necropsy examination of the caracal. (a) Jaundiced ocular mucosa. (b) Jaundiced and enlarged liver. (c) Jaundiced sectioned and intact kidney with hypervascularization. (d) Jaundiced trachea and heart with left atrioventricular deflection (black target)

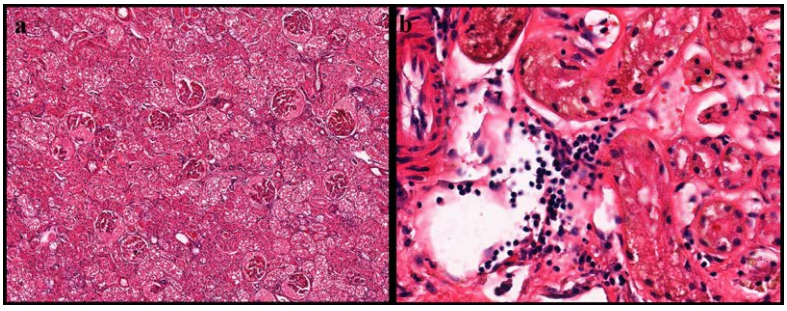


Figure 2. Macroscopical findings at the necropsy examination of the caracal. (a) Jaundiced ocular mucosa. (b) Jaundiced and enlarged liver. (c) Jaundiced sectioned and intact kidney with hypervascularization. (d) Jaundiced trachea and heart with left atrioventricular deflection (black target).

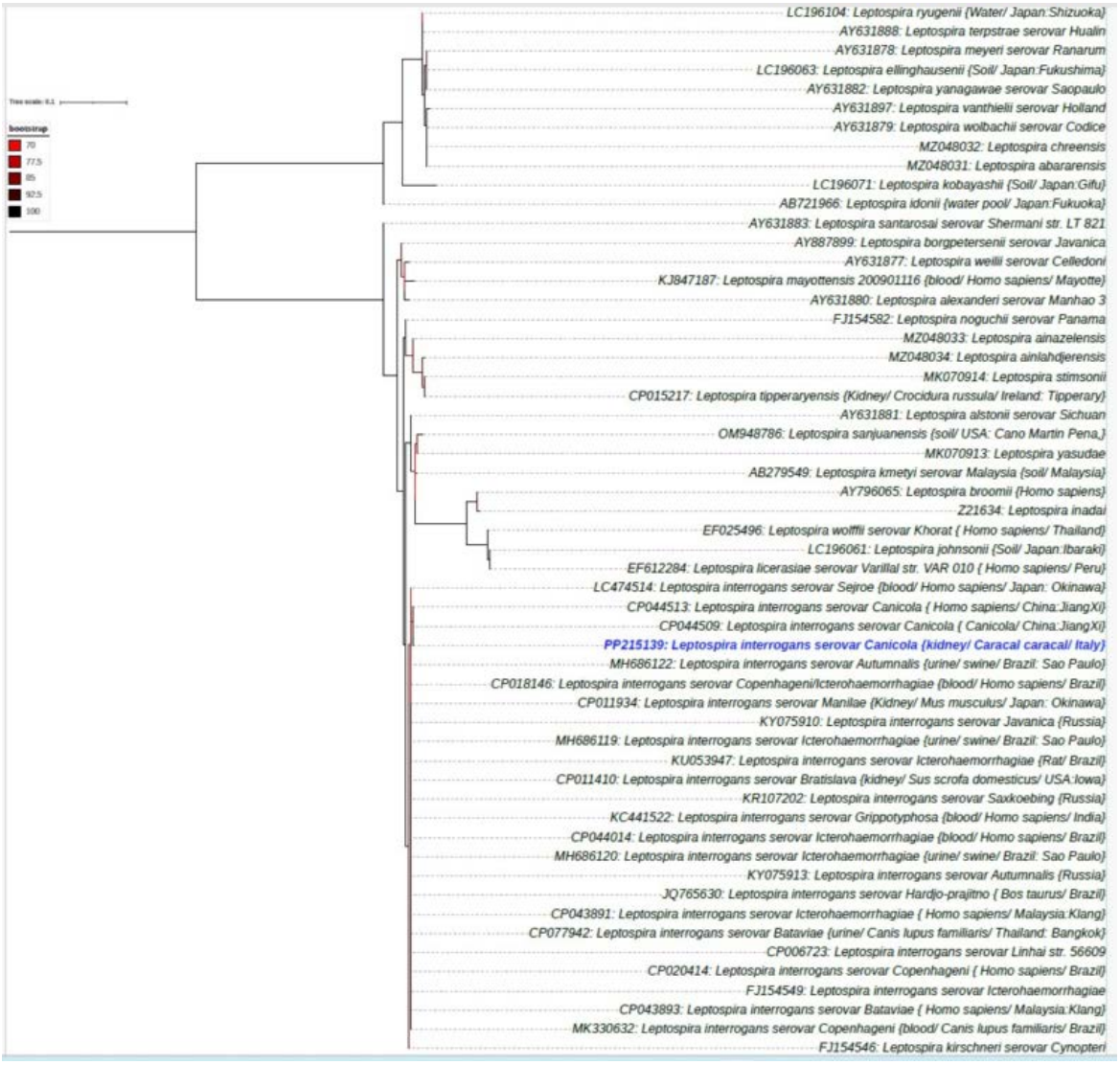


Figure 3. Phylogenetic relationship of *Leptospira interrogans* serovar Canicola in *Caracal caracal* of this study with those from different hosts and countries. The phylogenetic analysis was based on 307bp *LipL32* gene sequences of *Leptospira* spp. The maximum likelihood phylogeny was run under 100,000 bootstrap replications, best fit model was selected using Iqtree software and final tree was edited by Itools. Bold blue indicates the sequence type amplified in this study.

OP7

Biosecurity in animal health

COULD CALLIPHORIDAE SERVE AS A POSSIBLE BOVINE VIRAL DIARRHEA VIRUS (BVDV) MECHANICAL VECTOR AND RESERVOIR? PRELIMINARY RESULTS OF AN INSECT COMPETENCE STUDY.

F. Crescenti[1], F. Montarsi[1], G. Piegari[3], S. Carlin[1], G. Chiarello[1], V. Russo[3], M. Mion[1], M. Giammarioli[2], L. Ceglie[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy*
[2]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy* [3]*Università degli Studi di Napoli Federico II, Napoli, Italy*

Introduction

The single-stranded RNA bovine viral diarrhea virus (BVDV) and the notorious classical swine fever virus (CSFV), belonging to the genus of *Pestivirus*, can infect both domestic and wild animals. Pathogens affecting animals at the time of their death can be investigated using

molecular methods and immune-histochemical (IHC), depending on the post-mortem cadaveric status. However, in case of liquefaction and decomposition of tissues, viral proteins or genomes are often degraded and no more detectable. Blowflies, exhibiting necrophagous behavior, are attracted and feed on fresh death animals (1) and may play a relevant role as potential vectors of these pestiviruses. In this study, we used molecular methods and IHC to examine *Calliphora vomitoria* (Linnaeus, 1758) fed on BVDV-infected lung samples to verify whether these insects can be an alternative source to detect pathogens in case of advanced decomposition status of the animal (2).

Day of exposure	ID sample	Group A Ct average	Group B Ct average
Day 0	Larva	27,40	25,59
	Wash 1	29,92	29,54
	Wash 5	NEG	33,23
	Wash 10	NEG	NEG
Day 1	Larva	27,13	23,26
	Wash 1	32,80	29,73
	Wash 5	34,65	NEG
	Wash 10	NEG	NEG
Day 2	Larva	19,85	19,73
	Wash 1	29,24	27,14
	Wash 5	31,28	NEG
	Wash 10	32,60	35,67
Day 3	Larva	21,11	29,71
	Wash 1	28,21	NEG
	Wash 5	NEG	NEG
	Wash 10	NEG	NEG
Day 4	Larva	18,95	NEG
	Wash 1	28,46	NEG
	Wash 5	33,66	NEG
	Wash 10	34,30	NEG
Day 5	Larva	25,40	NEG
	Wash 1	28,15	NEG
	Wash 5	34,62	NEG
	Wash 10	NEG	NEG
Day 6	Larva	21,11	NEG
	Wash 1	30,80	NEG
	Wash 5	34,45	NEG
	Wash 10	NEG	NEG
Pupa	Pupa	33,69	NEG
	Wash 1	27,81	NEG
	Wash 5	NEG	NEG
	Wash 10	NEG	NEG
Adult	Adult	NEG	NEG
	Wash 1	NEG	NEG
	Wash 5	NEG	NEG
	Wash 10	NEG	NEG

Table 1. Ct (Threshold cycle) average obtained by rRT-PCR analysis of both A and B groups. In the table information on time of exposure (D0 until D6, pupa and adult stages) groups of insects, Ct values' average detected in molecular analyses on insects after washing and relative washes (1, 5, 10) are presented.

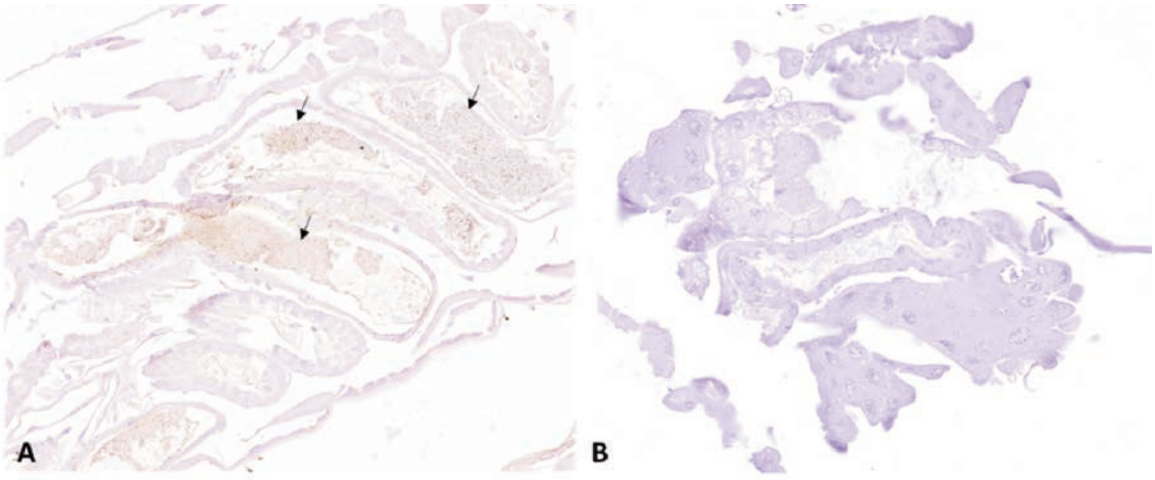


Figure 1. A: larvae fed on BVDV-infected lung tissue samples showed a moderate amount of anti-BVD immunopositive amorphous material in the alimentary tract (arrows). Figure 1B: larvae fed on BVDV-free tissue sample (group C) showing no reactivity to anti-BVD antibody.

Materials and Methods
In this study, 422 *C. vomitoria* newly hatched larvae were used and divided in 3 groups. Group A contained 196 larvae bred on BVDV-infected lung tissues; group B composed by 196 larvae bred for 48 hours on BVDV-infected lung sample and afterwards on BVDV-free splenic tissue; group C included 30 control larvae fed on virus-free splenic tissue during all the experimental phases. Insects were daily collected from the larva stages, including pupal and adult ones. Five insects underwent washing 10 times before RNA extraction. Isolated RNAs from insects and washes 1, 5 and 10 tested for BVDV by rRT-PCR, while 5 formalin-fixed insects underwent IHC analysis with Anti-BVDV Monoclonal antibody (BIO 295) (Bio-X Diagnostics).

Results
Molecular investigations showed positive results, as reported in Table 1. IHC results allowed us to detect a mild to moderate amount of immunopositive amorphous material in the alimentary tract of insects (Picture 1A) until 5 days of exposure. Control samples tested negative in all evaluated cases (Picture 1B).

Discussion and Conclusion
Our results suggest a potential use of necrophagous larvae to detect pestiviruses by molecular and IHC methods in cadavers as well as an indirect potential surveillance tool in farms.

References
1. Forth JH et al. 2018 doi.org/10.1111/tbed.12688
2. Carlson JM et al. 2020 doi.org/10.1177/1040638719898688

OP8

New Diagnostic Testing Technologies

SIMULTANEOUS DETECTION OF ANTIGEN AND ANTIBODIES OF AFRICAN SWINE FEVER IN A NOVEL COMBO LATERAL FLOW ASSAY

C. Aira[3], G. González-García[3], J. Martínez-Cano[3], N. De La Roja[3], C. Galán[3], M. Giammarioli[5], F. Feliziani[5], Ž. Šteingolde[4], J. Buitkuviene[6], P. Václavek[1], D. Glišic[7], C. Gallardo[2], P. Sastre[3], A. Fresco-Taboada[3], M. García-Durán[3], P. Rueda[3]

[1]*Department of Virology, State Veterinary Institute Jihlava, Jihlava, Czech Republic*
[2]*European Union Reference Laboratory for African Swine Fever, Centro de Investigación en Sanidad Animal (CISA-INIA/CSIC), Madrid, Spain*
[3]*Gold Standard Diagnostics Madrid S.A. (GSD Madrid), Madrid, Spain*
[4]*Institute of Food Safety, Animal Health and Environment "BIOR", Riga, Latvia*
[5]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" (IZSUM), Perugia, Italy*
[6]*National Food and Veterinary Risk Assessment Institute, Vilnius, Lithuania*
[7]*Virology Department, Institute of Veterinary Medicine of Serbia, Belgrade, Serbia*

Introduction
African Swine Fever (ASF) is an infectious disease of swine, caused by an enveloped double-stranded DNA virus, the ASF virus (ASFV). Infection with ASFV correlates with a wide range of clinical syndromes from almost unapparent disease to haemorrhagic fever with high fatality rates. To date, the active transmission of the ASFV across the globe, and the lack of licenced vaccines available worldwide left early diagnosis as the main available tool for control. To properly identify infected animals, point-of-care (POC) diagnosis is crucial. Lateral flow assays offer advantages that make them suitable for this POC application, what allows the early diagnosis of ASF. In this work, we validated a combo test for the combined antigen and antibody detection in field.

Materials and Methods
The new combo assay was composed by the combination of a strip for antigen detection (as in INgezim® ASFV CROM Ag 2.0) and a strip for antibody detection (as in INgezim® ASFV CROM Ab 2.0) within a single combo cassette. To evaluate the performance of the combined detection, a total of 332 positive and 193 negative blood samples were evaluated. Samples were collected from field during different surveillance campaigns performed in Latvia, Lithuania, Czech Republic, and Serbia. These samples were previously characterized by PCR and serology (ELISA and/or IPT) and separated in groups according to their Ct value.

Results
The combined antigen and antibody detection improved the percentage of positive results in all the PCR-positive groups tested. Notably, when no viral load was detected by PCR, this combined detection allowed the identification of 93 antibody-positive animals. Higher improvement was observed for samples collected from wild boar than from domestic pigs.

Discussion and Conclusion
The new combo assay (INgezim® ASFV Combo CROM Ag/Ab) was shown to be a valuable tool for ASF surveillance. Our results support the idea that combined antigen/antibody detection give valuable information for an improved control of ASF, allowing the identification

of more infected animals. Biggest improvement was found in wild boar due to the different surveillance approach (carcases/haunting vs signs control).

References

doi: 10.3390/vaccines12030307

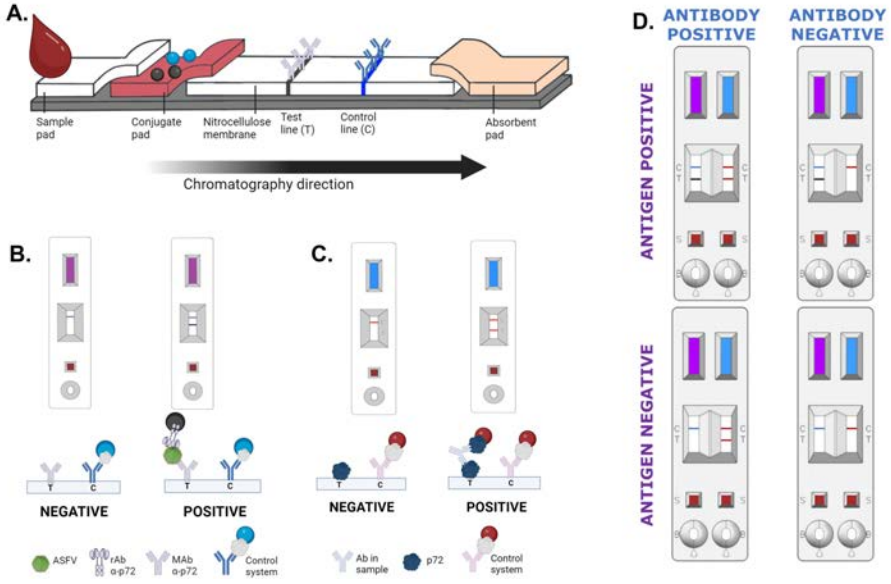


Figure 1. (A) General scheme of the lateral flow strip. (B) Result interpretation for the INgezim® ASFV CROM Ag 2.0, purple strip. (C) Result interpretation for INgezim® ASFV CROM Ab 2.0, blue strip. (D) Result interpretation for INgezim® ASFV Combo CROM Ag/Ab (GSD Madrid, Madrid, Spain). Created with Biorender.com.

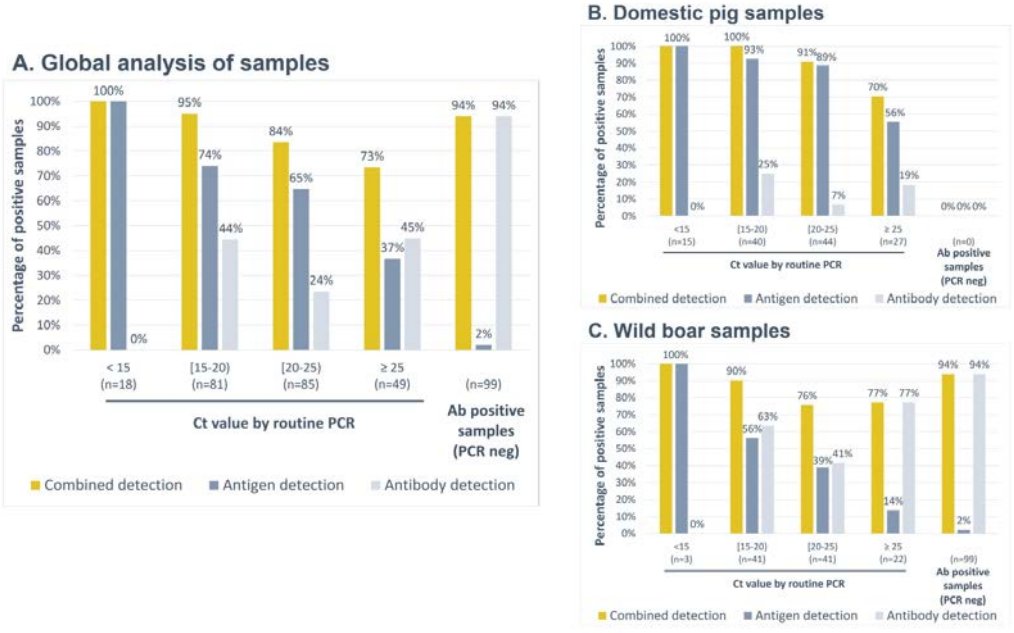


Figure 2. (A) Analysis of field blood samples with the INgezim® ASFV Combo CROM Ag/Ab assay. (B) Result obtained only for domestic pig samples. (C) Result obtained only for wild boar samples. Combined detection results show the percentage of positive samples by any of the strips included in the cassette, antigen, or antibody. Antigen detection shows percentages of positive results obtained only with the antigen detection strip. Antibody detection shows percentages of positive results obtained only with the antibody detection strip. The X-axis shows the groups of samples divided according to their viral load by PCR (Cq). The Y-axis shows the percentage of positive samples in each group.

OP9

New Diagnostic Testing Technologies

CREATING A DATASET AND ENHANCING BOVINE HERPESVIRUS 1 CPE DETECTION THROUGH ARTIFICIAL INTELLIGENCE

Z. Akkutay-Yoldar[1], B. Akkaş[3], T. Yoldar[3], S. Şurak[2], F. Garip[2], O. Turan[3], A. Özkul[1], B. Ünver[3]

[1]Department of Virology, Faculty of Veterinary Medicine, Ankara University, Ankara Türkiye [2]Graduate School of Health Sciences, Ankara University, Ankara, Türkiye
[3]TURK AI Artificial Intelligence Information and Software Systems, Bilkent Cyberpark, Ankara, Türkiye

Introduction

Bovine Herpesvirus 1 (BoHV-1) is responsible for respiratory and reproductive disorders in cattle, leading to significant financial losses[1]. Virus isolation is important for diagnosing and understanding the virulence and pathogenicity of BoHV-1, as well as for the development of vaccines and antivirals. Conventional methods for detecting CPE are time- consuming and subjective. Machine learning provides a promising option to automate and enhance the accuracy of CPE detection. The objective of this study is to create a dataset and apply machine learning to detect CPE caused by BoHV-1.

Materials and Methods

The BoHV-1 Cooper strain was propagated in MDBK cells, and the viral titer was determined using the Tissue Culture Infective Dose 50% ($TCID_{50}$) assay. The cells were infected with various multiplicities of infection (MOI), ranging from 1 to 10^{-4}. CPEs were observed at magnifications of 5X, 10X, 20X, 40X, and 60X; then images were taken at 8, 24, 28, 32, 48, 56, and 72 hours after infection. The dataset was used to train ResNet18 and ResNet50 deep learning models. The model's performance was validated by using a distinct test set and evaluated based on accuracy, precision, and recall.

Results

The propagation of BoHV-1 in MDBK cell lines was carried out successfully, and the $TCID_{50}$ was determined. CPE was detected at all multiplicities of infection (MOIs) within the time frame of 8-24 hours, except for the 10^{-4} MOI which exhibited a delayed onset. A total of 2823 images of BoHV-1 CPEs were labeled. Following data augmentation, preprocessing, and hyperparameter optimization, the ResNet18 and ResNet50 models demonstrated accuracies of 90.84% and 96.94%, respectively (Table 1).

Model	Accuracy (%)	Precision (%)	Recall (%)	F1 (%)	Loss/Test
ResNet18	90.84	91.54	91.14	91.34	0.26
ResNet50	96.94	97.00	97.07	97.03	0.10

Table 1. Performance metrics of AI models for BoHV-1 CPE detection.

Discussion and Conclusion

The dataset and models provide a reliable and efficient tool for fast and accurate detection of BoHV-1. This method decreases the amount of time and laborious work to diagnose and reduces the occurrence of mistakes made by humans. Future studies should broaden the dataset and enhance models to include additional bovine viruses. Applying this technology into diagnostic processes could improve the control and eradication of BoHV-1 infections in the livestock industry.

References

1. Nettleton, P., & Russell, G. (2017). Update on infectious bovine rhinotracheitis. In Practice, 39(6), 255-272.

OP10

New Diagnostic Testing Technologies

MULTI-PLATFORM INVESTIGATION OF BOVINE RESPIRATORY DISEASE-RELATED MICROBIAL COMMUNITIES AMONG SOUTHERN ITALY FARMS AND SLAUGHTERHOUSES

S. Castellana[1], L. Capozzi[1], A. Bianco[1], L. Del Sambro[1], G. Schino[1], D. Pugliese[1], A. Milano[1], L. Addante[1], C. Bulzacchelli[1], V. Manzulli[1], V. Rondinone[1], A. Petrella[1], M. Caruso[1], A. Parisi[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*

Introduction

Bovine respiratory disease ("BRD") represents one of the most common diseases for cattles: it is characterized by heterogeneous aetiology, morbidity and mortality rates and also influenced by farming conditions and interaction among microorganisms. The main viral agents responsible for BRD are BPIV-3, BRSV, BCoV, BoHV-1, BAdV, BVDV. Concerning Bacteria, common pathogens are: *M. bovis*, *P. multocida*, *M. haemolitica* and *H. somni* [1]. In this research project, we aimed at implementing a multi-platform approach for microbiome investigation and pathogen detection from cattles collected from Southern Italy farms and slaughterhouses.

Materials and Methods

A total of 236 samples, prevalently nasal swabs and pulmonary biopsies, were collected during 2020-2022. Sample aliquots were processed for pathogen-specific bacteriological exams. Then, molecular methods were implemented: 16S rDNA amplicon sequencing for species assignment on positive bacterial cultures; real time PCR for the detection of a viral/bacterial pathogen panel on DNA/RNA extracts; Whole Genome Sequencing for a subset of isolated strain (followed by assembly, genotypization and anti-microbial resistance 'AMR' gene prediction); Third Generation Sequencing ('TGS') shotgun metagenomics for microbiome investigation and pathogen detection for positively tested samples.

Results

Real Time PCR allowed the detection of at least one known BRD-associated organism in the 169 out of 236 tested samples (71%). From the 89 bacterial cultures, 21 strains were of interest and predicted with at least one *AMR* gene. For 5 samples, found positive to multiple pathogens, TGS analyses revealed traces (tens to hundreds read counts) of such pathogens (*e.g.*, Figure 1, sample positive to *P. multocida*, *M. haemolitica*), while more than 95% of reads were attributed to commensal bacteria or contaminants (*e.g.*, human DNA).

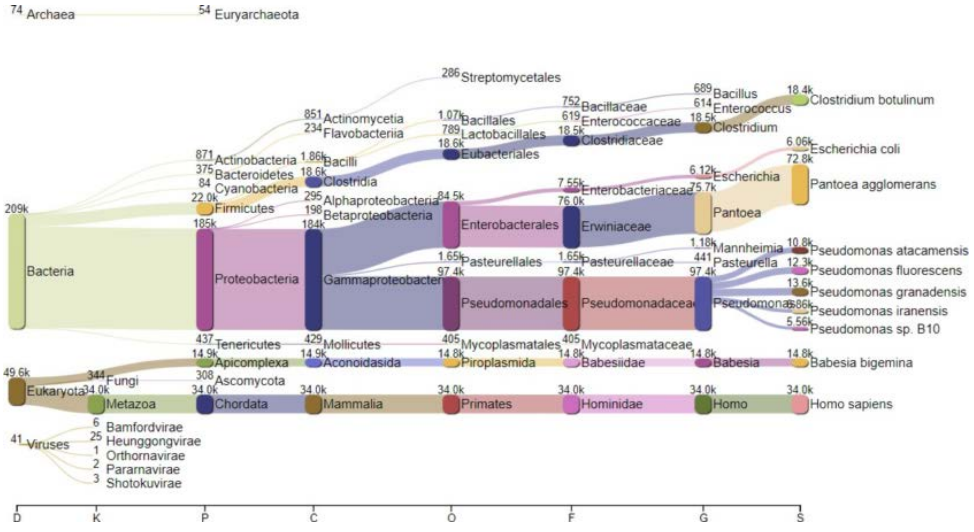


Figure 1. Sankey map for classified long-reads from Sample BRD_236. Vertical bars: read counts for each taxon (indicated in parenthesis); horizontal line: taxonomic levels (domain, kingdom, phylum, class, order, family, genus, species).

Discussion and Conclusion

We will extend TGS to a further subset of positive samples, with the purpose of: improving TGS protocol; better defining tissue-specific microbiomes and contaminant levels; evaluating congruency between pathogen detection strategies; correlating pathogen relative abundances to Real Time outcome and, whenever possible, to bovine clinical data.

References

1. https://doi.org/10.1186/s13567-022-01086-1

OP11

New Diagnostic Testing Technologies

TB OR NOT TB – THAT´S THE QUESTION / IMPROVED INTRA-VITAM DIAGNOSTICS IN A BOVINE TB OUTBREAK HERD

C. Schroeder[2], A. Ahrens[1], S. Horner[2]

[1]*Thuringia Animal Health Service, Germany*
[2]*Thuringian State Authority for Food Safety and Consumer Protection, Veterinary Division, Germany*

Introduction
Germany is recognized as free of bovine tuberculosis since 1997. However, there are sporadic outbreaks, mostly affecting small herds. In 2022, an outbreak was diagnosed in Thuringia's largest cattle herd. The experience gained in the eradication efforts, using Comparative Intradermal Tuberculin Test (SCIT) and a new and a modified interferon- gamma release assay (IGRA) will be presented.

Materials and Methods
The infected herd counts 1800 dairy cows, 400 heifers plus ~1000 calves annually. In Germany, the SCIT using bovine tuberculin and poultry tuberculin of the company WdT and the "ID Screen® Bovine Tuberculosis IFN-g Test" (ID-Vet) IGRA are officially approved. For comparison a modified QuantiFERON-TB (Qiagen) IGRA based on peptide antigens was used. Interferon-gamma was detected using ID-Screen Ruminant IFN-g ELISA (ID-Vet). The ID-Vet-IGRA was evaluated according to the manufacturer's instructions and also based on alternative assessments. The QFT-IGRA was evaluated as a corrected OD value based on a preliminary cutoff. The aim of this study was to generate data on the feasibility, robustness and reliability of the above-mentioned IGRAs in a cattle herd with proven *M. bovis* infection.

Results
After establishing logistics (transportation time and temperature), all samples reacted in the stimulation control of the IGRAs and could therefore be evaluated. Groups of dairy cattle and calves with positive, questionable and negative skin test results were tested in both IGRAs. The SCIT positive animals showed a high level of agreement with both IGRAs.In the SCIT weakly reactive, suspect and negative animals, the modified QFT-IGRA showed more reactors. Some of these also showed clear reactions in both PPBb and PPDa in the ID-Vet IGRA. Since paratuberculosis is also present in the herd, specific PPDb reactions could be masked by simultaneously occurring PPBa reactions.

Discussion and Conclusion
A testing scheme that considers reactions for PPDa, PPDb and NIL was established. In case of questionable results, the modified QFT-IGRA is used. So far ~ 1200 ID-Vet IGRAs and ~ 350 mod. QFT IGRAs where performed. The ID-Vet IGRA has proven to be robust and reliable. In herds with concurrent Mycobacterium avium-complex infections, we recommend the modified QFT-ELISA due to its high specificity for *Mycobacterium tuberculosis* complex infections.

OP12

New Diagnostic Testing Technologies

IMPROVING THE ANTE-MORTEM DIAGNOSIS OF CAPRINE TUBERCULOSIS THROUGH THE USE OF SPECIFIC ANTIGENS FOR *IN VITRO* DIAGNOSTIC PLATFORMS

C. Velasco[8], Á. Roy[7], A. Gómez-Buendía[8], J. Álvarez[8], M.B. Boniotti[2], M. Domínguez[6], E. Gormley[4], G. Jones[5], A. Martuccielo[1], P. Mazzone[3], I. Moreno[6], L. De Juan[8], L. Domínguez[8], B. Romero[8], J. Bezos[8]

[1]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Salerno, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy*
[3]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy*
[4]*School of Veterinary Medicine, University College Dublin, Dublin, Ireland*
[5]*TB Immunology and Vaccinology, Department of Bacteriology, Animal and Plant Health Agency, New Haw, Addlestone, Surrey, UK*
[6]*Unidad de Inmunología Microbiana, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain*
[7]*VISAVET Health Surveillance Centre, Complutense University of Madrid, Madrid, Spain*
[8]*VISAVET Health Surveillance Centre, Complutense University of Madrid, Madrid, Spain; Facultad de Veterinaria, Departamento de Sanidad Animal, Universidad Complutense de Madrid, Madrid, Spain*

Introduction
Caprine tuberculosis (TB) poses a threat to public and animal health. TB ante-mortem diagnosis primarily utilizes cell- based techniques

such as the skin test and the interferon-gamma release assay (IGRA). However, the sensitivity and specificity of both techniques is limited due to, among other factors, the use of Purified Protein Derivatives (PPDs) as reagents. PPDs are poorly characterized and share antigens with non-tuberculous mycobacteria, limiting the specificity of TB diagnostic techniques. In this sense, the aim of the present study was to evaluate more specific reagents (DST-F fusion protein and the P22 protein complex) using cell-based and humoral TB diagnostic techniques in a TB infected setting under field conditions.

Materials and Methods

We randomly selected 167 goats from two dairy caprine herds (around 15% of total census) with a high prevalence of reactors to the comparative intradermal tuberculin test (>20%). Blood samples were collected from the selected animals to perform the IGRA and the P22 ELISA. Interferon-gamma production was evaluated using the Bovigam commercial kit (Thermo Fisher Scientific, USA) after blood stimulation with bovine PPD, DST-F and P22 applying two different cut-off points (0.1 and 0.05). The antibody response was assessed by the experimental indirect P22 ELISA using a cut-off of 100% ELISA percentage.

Results

The highest ratio of reactors was observed in the IGRA-P22 (17.9% and 29.8% when applying the 0.1 and 0.05 cut-off, respectively), significantly higher than the IGRA-PPD (20.8%; $p<0.01$) when using the cut-off of 0.05 (Figure 1). Also, the percentage of reactors to the IGRA-DST-F was slightly higher (23.9%), although not statistically significant, than that of the IGRA-PPD when using the more stringent cut-off. Regarding the antibody response, we observed a significantly higher ($p<0.001$) number of goats reacting to the P22-ELISA (25.7%) with regard to the IGRA-PPD (13.7%) and IGRA- DST-F (11.9%) when applying 0.1 as cut-off.

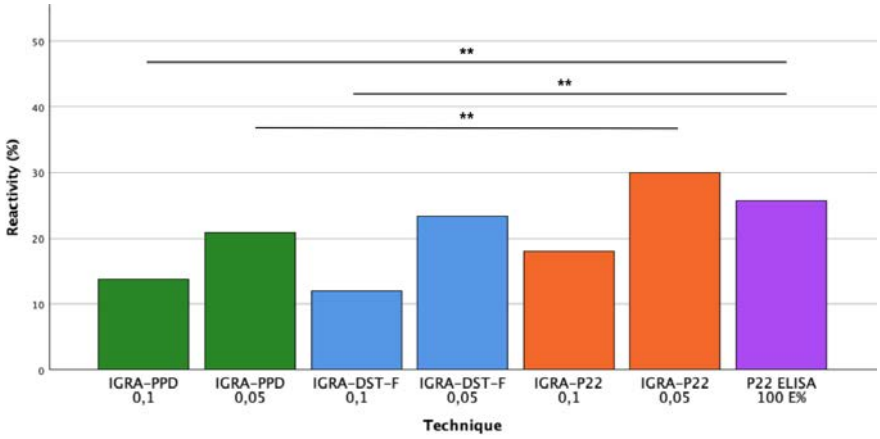


Figure 1. Percentage of reactors (%) observed in the IGRA-PPD (green), IGRA-DST-F (blue), IGRA-P22 (orange) and the indirect P22 ELISA (purple) techniques in the present study. ** $p<0.01$; Cochram´s Q test.

Discussion and Conclusion

The results suggest that when specific antigens are used in the IGRA, the sensitivity could be similar to that achieved using bovine PPD. The P22 ELISA results suggest a high sensitivity of this technique. Nevertheless, additional studies in TB-free herds should be performed to assess the impact on the specificity.

OP13

Animal health

USING PCR FOR ANTIGEN RECEPTOR REARRANGEMENTS (PARR) FOR FAST CYTOLOGICAL AND HISTOPATHOLOGICAL DIAGNOSIS OF CANINE LYMPHOMA

A.S. Bolas[1], C. Rodrigues[1], S. Andrade[1], C. Pomba[1]

[1]*Molecular Veterinary Diagnostic Laboratory-Genevet, Carnaxide, Portugal*

Introduction

PCR for antigen receptor rearrangements (PARR) detects monoclonal genetic loci at the rearrangements of the V(D)J regions of the T-cell and B-cell receptor genes (TRG [T-cell receptor gamma gene] and immunoglobulin heavy chain gene [IGH]). Thus, PARR is based on the identification of monoclonal lymphomas versus benign or reactive polyclonal cells. The aim of this study is applying the molecular diagnosis by PARR in cases in which histopathology is suggestive or inconclusive for the diagnose of canine lymphoma.

Materials and Methods
Thirty-six samples: histopathological and cytological (n=29) or other samples (n=7) suggestive or inconclusive of lymphoma were analyzed by PARR for immunogenotyping. The samples were sent from various veterinary hospitals and clinics in Portugal and were submitted to the GeneVet laboratory. DNA was extracted from histopathological and cytological slides, paraffin blocks or other samples (effusion, peripheral blood EDTA, direct fine needle lymph nodes aspirates) using a commercial extraction kit. The PRRA reactions were carried out according to previously optimized protocols.

Results
Immunogenotyping by PARR allowed the classification of 13 clonal type B lymphoma, with rearrangement in the variable region of the immunoglobulin heavy chain gene, 13 clonal T-type lymphoid neoplasm, with the presence of a rearrangement for the TCRγ region and negative for type B lymphoma. Nine samples were not Lymphoma and one was inconclusive.

Discussion and Conclusion
Since canine PARR is the only genomic test available to determine clonality in cytologically and histologically ambiguous cases of canine lymphoma, it may be used as a first-line test to confirm a faster diagnosis of lymphoid neoplasia. Furthermore, the determination of the type of lymphoma is highly relevant for a correct and fast treatment choice allowing better prognosis and more successful outcomes.

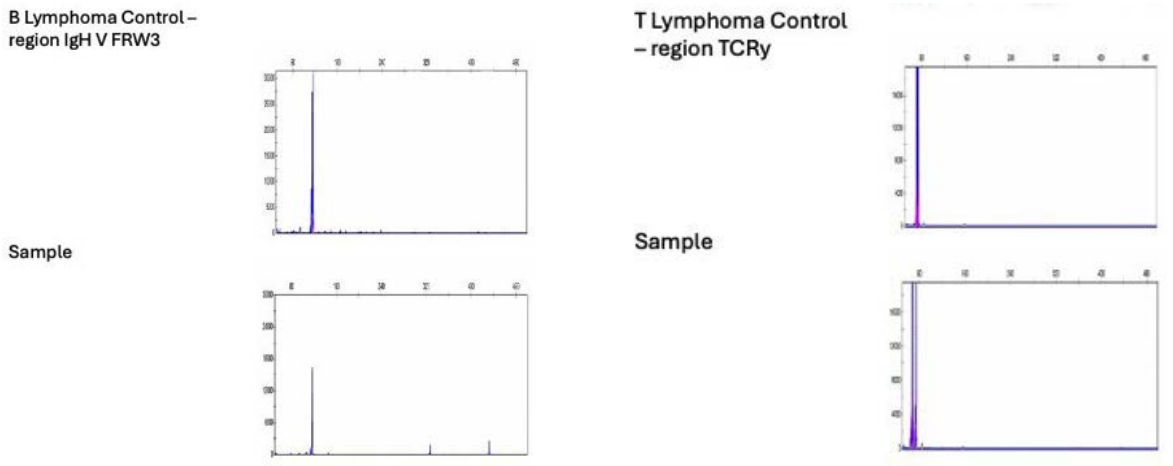


Figure 1.

OP14

Animal health

FIELD COMPARISON OF ASFV TARGET TISSUES AND NON-INVASIVE SAMPLES IN WILD BOARS DURING THE GENOTYPE II ITALIAN EPIDEMIC

E. Carella[1], B. Moroni[1], A. Fulmini[4], F. Rossi[3], P.L. Bassi[2], I. Giorgi[2], F. Garrone[2], A. Dondo[1], S. Zoppi[1], F. Feliziani[4], M.S. Beato [4]

[11]. Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy
[2]Azienda Sanitaria Locale (ASL) di Alessandria (ASL AL), Alessandria AL, Italy
[3]Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Italy
[4]National Reference Laboratory for Swine Fevers, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy

Introduction
African swine fever (ASF) is a viral disease that affects domestic and wild pigs with high lethality, caused by a dsDNA virus belonging to the Asfarviridae family, Asfivirus genus. ASF poses a significant threat to the global swine industry, with devastating economic impacts. Virus detection targets spleen, kidneys, lungs, tonsils, lymph nodes, and bone marrow from dead animals. However, field collection of such tissues and organs may be complex and result in environmental contamination of the carcass surrounding area. Recent studies (1,2,3,4,5,6) have focused on the use of non-invasive samples to facilitate samples field collection for a faster diagnosis, investigating the ASFV in faeces, oral, nasal and/or rectal swabs. However, all studies were based on experimental infection of swine and collecting different samples impairing a proper study comparison.

Materials and Methods
The present study aimed to compare the ASFV presence in target organs and in non-invasive samples during passive surveillance activities in wild boars from an endemic area in Piedmont between 2023 and 2024. Faeces, nasal, oral and blood swabs were collected along with spleen and kidney from each dead wild boar. Samples were processed by Real- Time PCR for ASFV using the ID Gene™ African Swine Fever Duplex kit. One hundred wild boars were collected for a total of 400 samples.

Results
Twenty six wild boars resulted ASFV positive when testing the spleen. All but one (n=25) presented at least one non- invasive sample and two of them had no non-invasive samples positive. Twenty-four wild boars presented at least one non-invasive sample with Ct values below 30.

Discussion and Conclusion
This is the first field comparison of non-invasive samples such as faeces, oro-nasal and blood swabs and target organs such as spleen from negative and positive ASFV wild boars, suggesting their potential use, although further field validation on additional wild boar population is necessary.

References
1. Niederwerder et al. 2021
2. Lee et al. 2021
3. Flannery et al. 2020
4. Davies et al. 2015
5. Guinat et al. 2014
6. de Carvalho Ferreira et al. 2014

OP15

Animal health

IDENTIFICATION AND CHARACTERIZATION OF PROTOPARVOVIRUS CARNIVORAN1 STRAINS CIRCULATING IN IBERIAN LYNXES (*LYNX PARDINUS*)

G. Diakoudi[2], S. Castro-Scholten[1], J. Caballero-Gómez[1], C. Desario[2], F. Pellegrini[2], G. Lanave[2], N. Decaro[2], I. García-Bocanegra[1], V. Martella[2]

[1]*Department of Animal Health, University of Córdoba, Córdoba, Spain*
[2]*Department of Veterinary Medicine, University of Bari Aldo Moro, Bari, Italy*

Introduction
Protoparvovirus carnivoran1 species includes the feline panleukopenia virus (FPV) and the closely related canine parvovirus (CPV-2), two highly contagious pathogens that cause acute and often fatal diseases in both domestic and wild carnivores. Interestingly, some animal species can be infected by both FPV and CPV-2 variants, namely 2a, 2b, and 2c (1). The aim of the present study was to investigate the parvovirus strains circulating in the Iberian lynx (*Lynx pardinus*), a vulnerable species.

Materials and Methods
A total of 245 spleen samples were collected from Iberian lynxes throughout the Iberian Peninsula. DNA extracts were screened for CPV/FPV by quantitative PCR. Viral genome sequences were generated from positive samples with high viral titer using two multiplex PCR protocols amplifying fifteen PCR-tiling amplicons and sequencing using MinION Mk1C platform (ONT™, Oxford, UK).

Results
Overall, 49/245 (20.0%) samples tested positive for CPV/FPV DNA. For 5 of the positive samples, the complete genome sequences were generated and were further characterized as FPV (n=2), CPV-2a (n=2), and CPV-2c (n=1). Sequence analysis of the complete VP2-genomic region (ORF2) revealed one amino acid (aa) mutation in both FPV and CPV-2c strains, and two aa mutations in both CPV-2a strains. Sequence analysis of the NS1-genomic region showed several aa mutations in the FPV strains. On phylogenetic analysis of the complete ORF2, the two CPV-2a strains formed a separate cluster, while the two FPV strains were segregated apart (Figure 1).

Discussion and Conclusion
Overall, this study provided evidence for a wide circulation of parvoviruses in the Iberian lynx population, with a prevalence higher than that observed in other studies (2). Future studies are needed to assess the impact of parvoviruses on the health status of the Iberian lynx to ensure animal welfare and conservation.

References
1. Ndiana LA, et al. Circulation of diverse protoparvoviruses in wild carnivores, Italy. Transbound Emerg Dis. 2021;68(4).
2. Nájera F, et al. Disease Surveillance during the Reintroduction of the Iberian Lynx (*Lynx pardinus*) in Southwestern Spain. Animals (Basel). 2021 Feb 19;11(2):547.

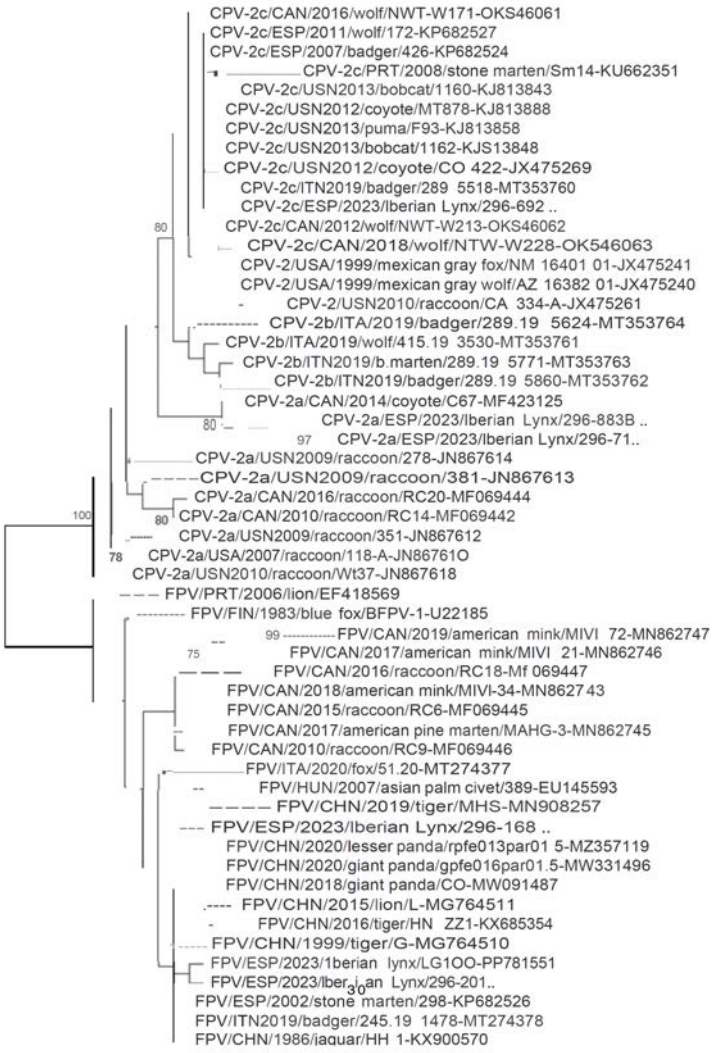


Figure 1.

OP16

Animal health

A MULTIDISCIPLINARY APPROACH TO CHARACTERIZE A *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS UNUSUAL OUTBREAK IN FENCED IBERIAN IBEX (*CAPRA PYRENAICA*)

M. Perez-Sancho[3], C. Herranz[3], A. Perello[3], T. García-Seco[3], A. Casamayor[3], L. Torre-Fuentes[3], J. Alvarez[3], M. Ugarte-Ruiz[3], J.A. Ortiz[2], L. Dominguez[3], C. Gortazar[1]

[1]*Instituto de Investigación en Recursos Cinegéticos, Spain*
[2]*Medianilla, Spain*
[3]*Universidad Complutense de Madrid, Spain*

Introduction

Salmonella is the second most frequent foodborne gastrointestinal agent in the European Union, with *Enteritidis* being the most common serotype involved (1). More limited information exists regarding *Salmonella* occurrence in wildlife, although susceptibility to infection (2) and even fatal cases (3), have been described. Here, we describe an unusual outbreak of clinical salmonellosis in fenced Iberian ibex (*Capra pyrenaica*) that demonstrates the potential of *S. Enteritidis* as primary pathogen in other hosts.

Materials and Methods
In the context of the project PLEC2021-008113 (funded by MCIN/AEI/10.13039/ 501100011033/ and the European Union NextGeneration EU/PRTRA), an outbreak of abortions, neonatal deaths and sporadic septicaemic infection in adults was detected on an Iberian ibex open-air fenced farm. Ibex shared space with a red-legged partridge (*Alectoris rufa*) farm, and birds, rodents, lagomorphs and small carnivores could eventually access the setting. A combination of (i) bacteriology (culture, MALDI-TOF identification and serotyping), (ii) pathology (necropsy, macroscopic examination and samplings), (iii) environmental DNA detection (sampling using sponged prehydrated and PCR for *Salmonella* detection on animal and environmental surfaces) and (iv) whole genome sequencing (WGS) was implemented to establish an etiologic diagnosis and characterizing the outbreak.

Results
S. enteritidis was identified on samples from adults, fetus and stillbirth. *Salmonella* DNA was detected on feeder, drinker and ibex surfaces while samples from red-legged partridge and other birds were negative, suggesting they were not the source of contamination. WGS revealed a single ST11 strain related with the so-called Atlantic clade was present.

Discussion and Conclusion
Although the susceptibility of domestic goats to *Salmonella* seems to be low (4), our study demonstrated *S. enteritidis* could cause an unusual outbreak of clinical disease with relevant consequences for farm viability. Environmental DNA sampling can be a useful tool to identify *Salmonella* hotspots in multi-host settings. Further studies focused on the epidemiology of *Salmonella* on these species are necessary considering the risk of zooanthroponosis.

References
1. 10.2903/j.efsa.2023.8442
2. 10.2478/acve-2022-0015
3. 10.1111/tbed.12037
4. 10.1016/ j.prevetmed.2022.105766

OP17

Animal health

EVALUATION OF *IN VITRO* ANTIVIRAL ACTIVITY OF FUNGAL SECONDARY METABOLITES AGAINST BOVINE CORONAVIRUS

F. Fiorito[5], P. Capozza[7], L. Del Sorbo[5], R. Giugliano[5], M.M. Salvatore[3], A. Staropoli[1], R. Nicoletti[2], C. Acconcia[4], G. Caputo[4], G. Fusco[8], V. Vasinioti[6], A. Andolfi[3], L. Russo[4], F. Vinale[5], R. Iacovino[4], A. Pratelli[6]

[1]*Department of Agricultural Sciences, University of Naples Federico II, Portici, Naples, Italy; Council for Agricultural Research and Economics, Research Centre for Olive, Fruit and Citrus Crops, Caserta, Italy*
[2]*Department of Agricultural Sciences, University of Naples Federico II, Portici, Naples, Italy; Council for Agricultural Research and Economics, Research Centre for Olive, Fruit and Citrus Crops, Caserta, Italy*
[3]*Department of Chemical Science, University of Naples Federico II, Naples, Italy*
[4]*Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Caserta, Italy*
[5]*Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy*
[6]*Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy*
[7]*Department of Veterinary Medicine, University of Bari, Valenzano, Bari, Italy 8Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Naples, Italy*

Introduction
Bovine coronavirus (BCoV) is a Betacoronavirus belonging to the Coronaviridae family, which includes, among others, the viruses associated with the epidemic severe acute respiratory syndrome coronavirus type 1 (SARS-CoV-1), Middle East respiratory syndrome coronavirus (MERS-CoV), as well as the recent pandemic SARS-CoV-2. BCoV is distributed worldwide and mainly causes enteric disorders in calves, winter dysentery in adult, and Bovine Respiratory Disease (BRD) in cattle of all ages. Natural compounds, such as fungal secondary metabolites (SMs), due to their wide range of diversity in chemical structure and their bioactivity, can represent a major source for the development of drug against various diseases. Recently, SMs such as 6-pentyl-α-pyrone (6PP), vermistatin (VER) and penisimplicissin (PS) have been shown to be able to counteract canine coronaviruses (CCoV) infection *in vitro*. Hence, the first objective of this study was the evaluation of the antiviral activity of these SMs against BCoV, aimed at a translational study on SARS-CoV-2.

Materials and Methods
Chromatographic techniques, bioscreen *in vitro* in bovine kidney cells (MDBK), virus yield analyses, immunofluorescence assay, molecular modelling, molecular docking.

Results
Following BCoV infection, non-cytotoxic doses of 6PP, VER and PS resulted in increased cell viability and in a significant reduction in viral spike protein expression. These results were accompanied by a modulation in the expression of aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor that regulates the host immune response to viral infections, activated by BCoV, but downregulated by SMs. It is fascinating that a high sequence identity of the obtained 3D structural models for the two domains (PASB and TAD) of human and bovine AhRs was revealed by bioinformatics analysis.

Discussion and Conclusion
Overall, our preliminary results highlight that SMs could have antivirals activity against BCoV. In subsequent steps, inclusion complexes with β-cyclodextrin will be prepared and characterized to enhance the pharmacokinetics of selected SMs to be tested in vivo (cattle).

OP18

Animal health

PREVALENCE OF *MYCOPLASMA EQUIGENITALIUM* IN MARTINA FRANCA (ITALY) AND ANDALUSIAN (SPAIN) DONKEY POPULATIONS

M.S.M. Yusuf[1], M. Burgio[1], G.G. Aiudi[1], J.V. Delgado Bermajo[2], C. Rivas López[2], M.S. Lucente[1], G. Lanave[1], M. Losito[1], M. Corrente[1], M. Tempesta[1], G. Greco[1]

[1]*Department of Veterinary Medicine - University of Bari Aldo Moro, Valenzano, Bari, Italy*
[2]*University of Córdoba, Faculty of Veterinary Sciences,Department of Genetics, Córdoba, Spain*

Introduction
Mycoplasma equigenitalium has been associated with infertility, endometritis in mares and with reduce fertility and balanoposthitis in stallions (1). Furthermore, *M. equigenitalium* was isolated from the genital tract of donkeys in Argentina (2).
Currently, there are no studies describing the presence and impact of *M. equigenitalium* on the reproductive sphere of donkeys in Italy and Spain. This study aimed at investigating the occurrence and risk factors for *Mycoplasma* spp. infection in genital tract of Martina Franca (Italy) and Andalusian (Spain) donkey populations.

Materials and Methods
From March 2023 to June 2024, a cross-sectional study was carried out on 120 healthy donkeys, with the majority being jennies (106, 88.3%), mainly reared under semi-extensive lifestyle in Italy (60.8%) and Spain (39.2%). Genital swab samples were collected, cultured in modified Hayflick media. The identification of the isolates was performed using different PCR assays targeting 16S, ITS (3) and rpoB (4) regions associated with sequence analysis.

Results
An overall prevalence of 25.83% (31/120, 95% CI: 18.00-33.67) of *M. equigenitalium* was recorded, with one donkey (0.833, 95% CI: 0.000-2.460) exhibiting a mixed infection of *M. equigenitalium* and *M. subdolum*. Biosecurity measures significantly influenced the infection frequence, with farm respecting good practice of biosecurity having significantly lower infection rates (p=0.0031, OR=0.173). Furthermore, no significant risk was detected for country, age, gender, or breed.

Discussion and Conclusion
This study represents the first report of *M. equigenitalium* and *M. subdolum* infections in healthy donkeys in Italy and Spain, and Europe in general, with low adoption of biosecurity measures identified as risk factors. However, more studies need to be performed to address the possible link to reproductive performances of donkeys.

References
1. Spergser J, Aurich C, Aurich JE et al. (2002). Vet. Microbiol., 87: 119-129.
2. Tamiozzo P, Sticotti E, Bragulat AF et al. (2022). Intern J Equ Sci, 1, 33-35.
3. Volokhov DV, Batac F, Gao Y et al. (2019). Intern J System and Evol Mictobiol, 69, 363-370.
4. Nehra K, Rana R, Viswas KN et al. (2015). Iran J Vet Res, 16, 176.

OP19

Food Safety

IDENTIFICATION OF FOOD ADULTERATION BY THE EXAMINATION OF SPECIES-SPECIFIC DNA IN COMMERCIAL MEAT PRODUCTS, BY MULTIPLEX REAL-TIME PCR

D. Johnston[1], Q.H. Sun[1], M. Gutierrez[1], D. Prendergast[1]

[1]*Food Microbiology, Department of Agriculture, Food and the Marine (DAFM), Ireland*

Introduction

Regulatory food authorities strive to promote honest and informative labelling to help consumers make informed choices. EU legislation (Regulation (EU) No 1169/2011) requires meat to be labelled with the animal species of origin, and to quantify the ingredients. Our procedure was used to assess regulation compliance in commercial meats by the simultaneous detection of horse, cattle, sheep, pork, goat, chicken, and turkey species, using two multiplex real-time qualitative PCRs.

Materials and Methods

Commercial meat samples (n=359, 5±1g) submitted to DAFM laboratories as part of different official control programmes consisting of both non-EU, EU and domestically produced raw, cooked, processed, and meat alternatives (intact cuts and minced meat) were tested. Samples were homogenised in a paint-shaker, followed by DNA extraction and real-time PCR (Figure 1). The first multiplex PCR simultaneously identified horse, cattle and sheep DNA along with an internal amplification control, the mammalian myostatin gene (MSTN) present in mammals and poultry. The second multiplex simultaneously detected pork, goat, chicken and turkey DNA.

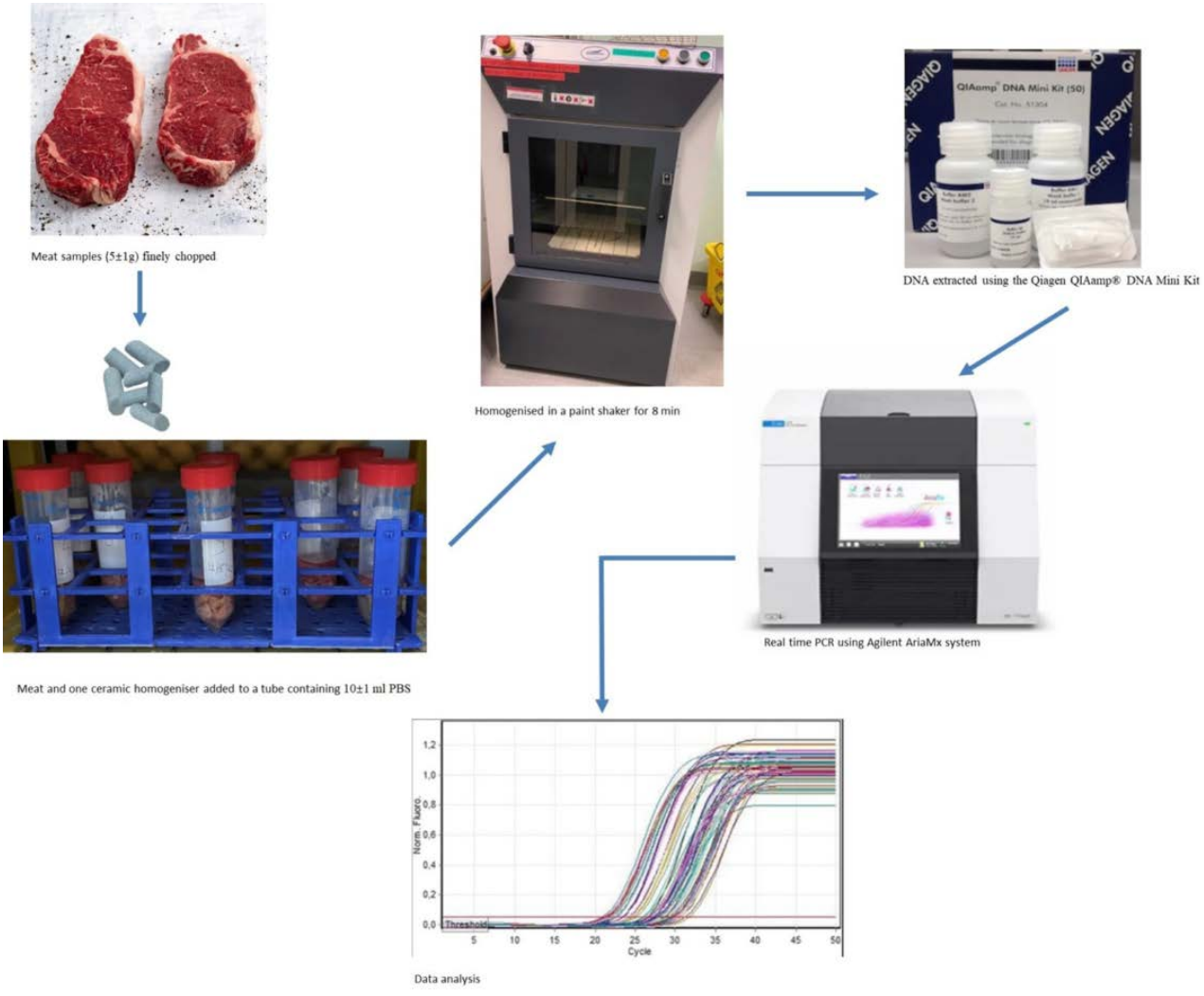


Figure 1. The workflow of meat homogenisation, DNA extraction and real time PCR.

Results
Amongst the range of meat products (fresh, cooked, sausages, cold cuts, ground meat, plant-based meat alternatives, meat pastry pies etc.) that were analysed, two samples were found to contain a species not listed on the product label. Bovine and porcine DNA were detected in one beef burger which originated from an establishment producing both bovine and porcine minced meat products. A mushroom and chicken vol au vent contained beef DNA, but it likely originated from the milk used in the sauce. No animal DNA was detected by PCR in any commercial plant-based and or meat alternative product.

Discussion and Conclusion
Incidents of food adulteration reduce the quality and integrity of food products, damage the industry reputation, may violate consumers' religious practices, and potentially threaten human health. The present study detected a high level of compliance in commercial meat products, both EU and non-EU, with species labelling legislation. These results show a high level of compliance with EU regulations and should reassure EU meat consumers.

References
Regulation (EU) No 1169/2011 of the European Parliament and of the Council

OP20

Food Safety

MALDI-TOF AND MACHINE LEARNING: PRELIMINARY RESULTS OF *CAMPYLOBACTER JEJUNI* SUB-TYPING

G. Garofolo[3], E. Felicioni[3], T. Romualdi[3], L. Prandini[2], V. Indio[2], F. Savini[2], G. Federica[1]

[1]*Dipartimento di Medicina Animale, Produzioni e Salute, Università di Padova, Italy*
[2]*Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Italy*
[3]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy*

Introduction
MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization - Time of Flight) mass spectrometry is the gold standard for bacterial identification(1). Hitherto, relatively little interest and few studies on sub-species typing using MALDI-TOF MS exist in the literature and its routine use for bacterial typing remains a challenge. The use of Artificial Intelligence techniques could allow the detection of specific biomarkers in the foodborne pathogens *Campylobacter jejuni* for sub-typing (2). This study aims to assess *C. jejuni* MALDI-TOF mass spectra by machine learning algorithm for rapidly distinguish the main clonal complexes (CC) circulating in Italy.

Materials and Methods
A mass spectra database was constructed using 114 strains of *C. jejuni* isolated from humans, animals, food of animal origin and water. Four clonal complexes (CC) were determined using WGS, specifically CC21 (n=32), CC353 (n=45), CC354 (n=20) and CC45 (n=17). A minimum of 12 spectra including analytical and biological replicates were collected for each strain. Spectra were normalized in several m/z wide windows by summing the intensities within each of them. Such cleaned spectra were randomly splitted in 4-fold cross validation sets used to iteratively train and test a machine learning algorithm based on random forest and the performances were evaluated.

Results
The algorithm was able to correctly classify 91 out 114 strains showing an overall accuracy and sensitivity up to 85% for all the four different CCs, with a specificity up to 69% and correlation coefficient up to 62% (Mattew-index).

Discussion and Conclusion
MALDI-TOF MS seems capable of rapidly identify *C. jejuni* CCs in agreement with a preliminary study(2), thus enabling fast typing. Ideally, the same MALDI-TOF data used for bacterial identification could be used for subtyping. Further studies are needed to evaluate and validate this promising application of MALDI-TOF.

References
1. Calderaro et al. 2024. MALDI-TOF MS: a reliable tool in the real life of the clinical microbiology laboratory. Microorganisms 12: 322.
2. Feucherolles et al. 2022. Combination of MALDI-TOF Mass Spectrometry and machine learning for rapid antimicrobial resistance screening: the case of Campylobacter spp. Frontiers in Microbiology 12: 804484.

OP21

Food Safety

FIRST REPORT OF STX2K-PRODUCING *E. COLI* STRAINS FROM FOOD IN EUROPE, 2015

G. Nobili[2], G. La Bella[2], V. Michelacci[1], R. Tozzoli[1], A.M. Damato[2], R. Coppola[2], E. Ventola[1], P. Chiani[1], A. Knijn[1], F. Minelli[1], A. Gianviti[3], M. Argentieri[3], L. Pansani[3], G. Scavia[3], S. Morabito[1], A. Parisi[2], M.G. Basanisi[2], G. La Salandra[2]

[1]*Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria, Istituto Superiore di Sanità, Roma, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy*
[3]*Ospedale Pediatrico Bambino Gesù, Roma, Italy*

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) is a significant zoonotic foodborne pathogen worldwide, causing human infections with symptoms ranging from uncomplicated diarrhea to severe diseases as hemolytic uremic syndrome. STEC strains produce two main types of Shiga toxins (Stx), Stx1 and Stx2, encoded by genes classified into different subtypes (stx1a, c-e; stx2a–o) (1). In this study, we report the first isolation of two Stx2k-producing strains from raw milk and curd in Italy in the Apulia region.

Materials and Methods

Two STEC strains (ED1814, ED1815) were isolated from raw milk and curd, respectively, in the Apulia region in 2015. The isolates were subjected to Whole Genome Sequencing through short-reads (Illumina or IonTorrent) and long-reads (Nanopore) sequencing and analyzed using the available tools on the ARIES platform (https://aries.iss.it/platform/) for serotyping, virulotyping, de novo assembly, gene annotation and cgMLST analysis and the MAUVE program for the structural analysis of the genome of Stx-bacteriophage.

Results

The two strains showed the stx2k subtype. Characterization results are reported in Table 1. ED1814 and ED1815 showed a high correlation with a stx2k-producing *E. coli* isolated in China in 2013 (STEC316) (2) (Figure 1), also showing the same insertion site (dusA) and genetic organization of Stx-bacteriophages (Figure 2). In addition, as also reported for STEC316 by Yang et al. (2), these two Stx2k-STEC carried the heat-labile toxin (LT)- encoding gene elt, exhibiting a STEC/ETEC hybrid pathotype.

Isolate	From	MLST	Serotype	stx gene	Plasmidfinder	Virulencefinder
ED1814	Raw milk	ST1611	O100:H19	stx2k	IncFIB(K)(pCAV1099-114) IncFII(pHN7A8) IncHI1A IncHI1B(R27) IncI1-I(Alpha)	*capU, eltIIAB-a, fdeC, gad, hha, hlyE, ipfA, terC, traT,*
ED1815	Cagliata	ST1611	O100:H19	stx2k	IncFIB(pHCM2) IncFII(pHN7A8) IncFII(pECLA) IncI1-I(Alpha) repE(pEH60-7)	*capU, eltIIAB-a, fdeC, gad, hlyE, ipfA, terC,*

Table 1. Characteristics of two stx2k-positive *E. coli* isolated from food.

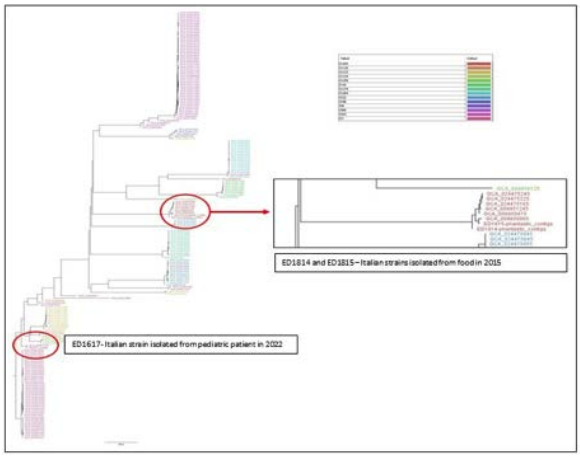


Figure 1. cgMLST analysis revealed a high correlation of strains ED1814 and ED1815 with Stx2k-producing *E. coli* isolated in China in 2013 (STEC316).

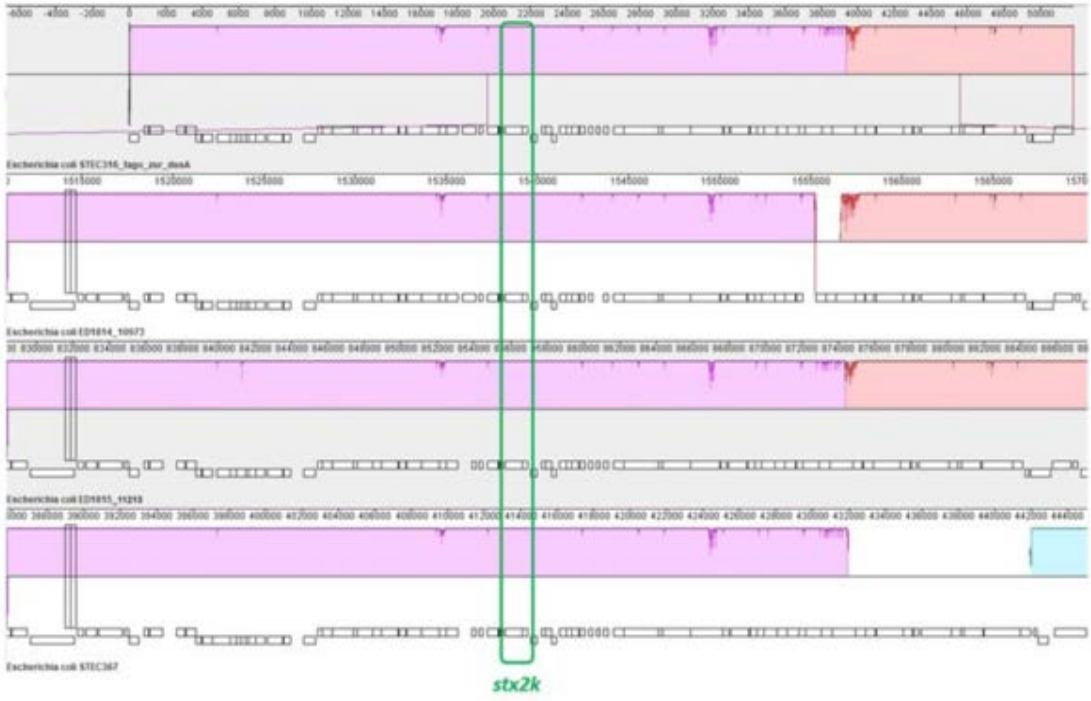


Figure 2. MAUVE alignment of the stx2k bacteriophage sequences identified in strains ED1814 and ED1815 with two stx2k bacteriophages described by Yang et al. (STEC316, STEC367).

Discussion and Conclusion

This study represents the first isolation of STEC harbouring the stx2k subtype from food samples in Italy and in Europe. In 2022, a stx2k-positive *E. coli* from a clinical pediatric patient (ED1617) was notified in Italy (2), highly divergent from the food strains here characterized, but also showing a high correlation with another strain isolated in China in 2013. These results reveal a high genomic stability of strains harbouring the stx2k subtype, which could be maintained in their ecological niche, still to be investigated.

References

1. McMahon T. et al, 2024. doi: 10.1016/j.ijfoodmicro.2024.110744
2. Yang X. et al., 2022. doi: 10.1128/spectrum.01571-22.
3. Michelacci V. et al., 2024. https://www.amcli.it/wp-content/uploads/2024/03/programma-definitivo.pdf

OP22

Genomics

BACTERIAL COMMUNITIES IN A FISH-FARM UNDER INTENSIVE PRODUCTION CONDITIONS

S. Scibetta[3], C. Piazza[3], T. Lupo[3], E. Oliveri[3], F. Vitale[3], G. Sarà[1], M. Del Mar Bosch Belmar[1], S. Sadok[2], S. Reale[3]

[1]*Department of Earth and Marine Sciences University of Palermo, Italy*
[2]*Institut National des Sciences et Technologies de la Mer (INSTM), Tunis, Tunisia*
[3]*Istituto Zooprofilattico Sperimentale della Sicilia, Italy*

Introduction

Aquaculture is experiencing rapid growth to meet increasing global demand for fish, necessitating sustainable development focused on optimizing fish nutrition, managing diseases, and reducing antibiotic use. Amplicon metagenomics, analyzing fish gut microbiota, plays a crucial role in understanding these dynamics [1]. This study was conducted at a nursery fish-farm in Sicily, examining bacterial communities in seabream and seabass under intensive production conditions, to investigate the complex interactions among hosts, microbiota, pathogens, and the environment.

Materials and Methods

Samples were taken to investigate different conditions: fish species, fish size (age), water tanks, unhealthy signs or deformations observed in fish (Table 1). Amplicon libraries were generated amplifying the 16S rRNA gene and sequenced on MiSeq Illumina platform. Data were processed through Qiime 2 pipeline [2].

Results

Comparisons across 3 different ages of seabream, highlighted distinct taxonomic compositions, with *Lactobacillus* predominant in younger fish and *Staphylococcus* and *Mucilaginibacter* prevalent in older (Figure 1). Examining healthy versus unhealthy seabream underscored substantial differences in microbial profiles. Healthy samples were characterized by beneficial taxa (75,4%) such as *Sphingomonas, Lactobacillus*, *Mucilaginibacter*, and *Rhizobiales*, whereas unhealthy fish exhibited pathogens (58%) like Photobacterium, Vibrio, *Polaribacter, Clostridiaceae* etc. (Figure 2).

seabream	**size A (<3 gr)**		**size B (3-6 gr)**		**size C (>6 gr)**		
	tank 1	*tank 2*	*tank 3*	*tank 4*	*tank 5*	*tank 6*	
samples n.	4	4	4	4	4	4	
seabass	**size A (<3 gr)**						
	tank 7						
samples n.	4						
water	**tank 1**	**tank 2**	**tank 3**	**tank 4**	**tank 5**	**tank 6**	**tank 7**
samples n.	1	1	1	1	1	1	1
unhealthy signs	**enteritis**	**lordosis**	**no bladder**	**wound**	**deformation operculum**		
samples n.	4	4	4	4	4		
healthy/unhealthy seabream	**total healthy**		**total unhealthy**				
	24		*20*				

Table 1. List of the total 55 samples taken to investigate different conditions in the fish-farm: fish species (seabream and seabass), 3 fish size (A, B, C), water from tanks (2 different tanks for each of the size investigated), unhealthy conditions or deformations observed in fish (lordosis, deformity of the operculum, swim-bladder loss, wounded tail, enteritis).

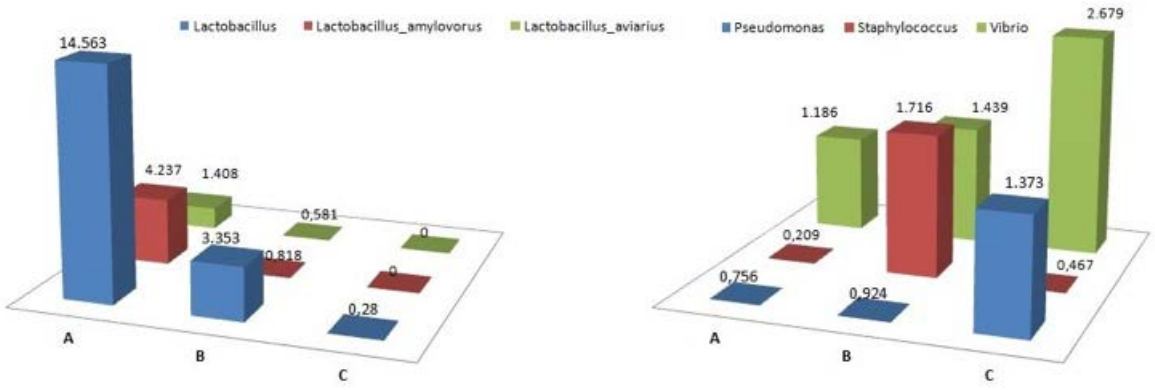


Figure 1. Comparison of seabream at different ages, size A) <3 gr., size B) 3-6 gr., size C) >6 gr.: *Lactobacillus* taxa (beneficial) are significant related to the youngest age A, whereas *Staphylococcus*, *Pseudomonas,* Vibrio (pathogens) characterize the older stages B, C.

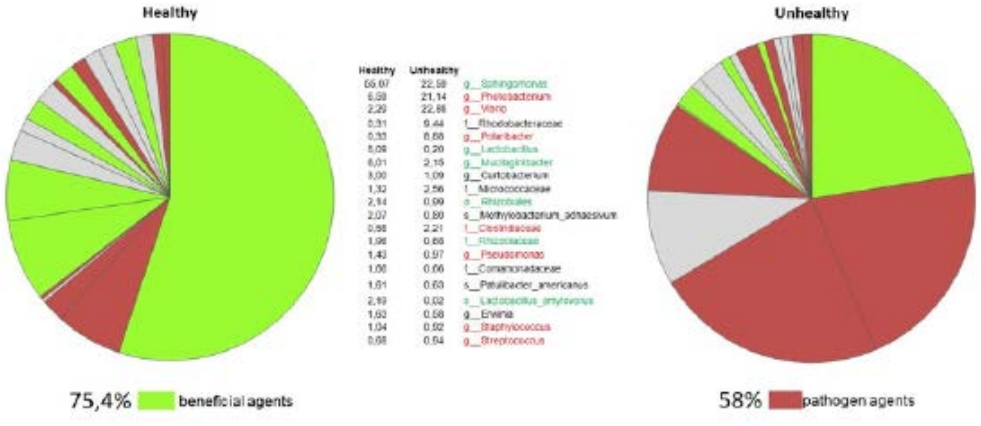


Figure 2. Comparison of seabream between the categories healthy (24) and unhealthy (20) samples, considering the 20 most abundant taxa: healthy samples are characterized by beneficial taxa (in green) for 75,4%, and unhealthy are characterized by pathogen taxa (in red) for 58%. In grey taxa considered neutral.

Discussion and Conclusion

Beneficial interactions were linked to maintaining host health, whereas pathogenic taxa highlighted risks requiring mitigation strategies to sustain farm productivity and environmental health. In conclusion, the study emphasized the relevant role of amplicon metagenomics in enhancing understanding microbial dynamics, helpful in strategies for disease control, efficient feeding practices, and sustainable production.

References

1. Ghanbari M, Kneifel W, Domig KJ. 2015. A new view of the fish gut microbiome: advances from next-generation sequencing. Aquaculture 448:464-475.
2. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, et al. 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 37(8):852-857.

OP23

Genomics

LEISHGENAPP AND LEISHGENR, TWO NOVEL DIAGNOSIS PLATFORMS TO DETECT CNVS AS DRUG-RESISTANCE BIOMARKERS IN *LEISHMANIA INFANTUM* FROM CULTURES AND CLINICAL SAMPLES

M. Carrasco-Martin[1], J. Martí-Carreras[1], X. Roura[3], L. Ferrer[2], O. Francino[1]

[1]*Nano[1]Health S.L*
[2]*Nano[1]Health S.L, Departament de Medicina i Cirurgia Animals, Universitat Autònoma de Barcelona, Bellaterra, Spain*
[3]*Nano[1]Health S.L, Hospital Clínic Veterinari, Universitat Autònoma de Barcelona, Bellaterra, Spain*

Introduction

Drug-resistant strains of *Leishmania infantum* are emerging in the Mediterranean. Copy-number variation (CNV) is associated with resistance to first-line drugs. We developed a novel diagnosis platform to detect CNVs as drug- resistance biomarkers in *Leishmania infantum* from cultures (LeishGenApp) or clinical samples (LeishGenR).

Materials and Methods

Forty-six *Leishmania infantum* isolates from the Mediterranean were whole-genome sequenced with nanopore long reads (Oxford Nanopore Technologies plc). Genomes were assembled with Flye 2.9, and completeness was assessed with BUSCO 5.4.4. Structural rearrangements and CNV were predicted through proprietary analysis pipelines (cloud.nano1health.com).

Results

We developed a novel diagnosis platform to detect CNVs as drug-resistance biomarkers in *Leishmania infantum* from cultures (LeishGenApp) and clinical samples (LeishGenR). LeishGenApp is a genome analysis platform that includes 15 biomarkers against antimonials, allopurinol, amphotericin B, miltefosine, and paromomycin and it is suitable for cultures (10e7 parasites/ml). At least one genetic resistance biomarker was detected in 78% of the Mediterranean isolates. Genetic resistance to antimonials and allopurinol (52%) in canine isolates and to antimonials (61%), followed by paramomycin (38%) in human isolates, were the most prevalent. Monoresistance (42%) predominates in dogs, and resistance to two drugs (46%) in human isolates (Figure 1). We also developed LeishGenR, a qPCR system for clinical samples (>600 parasites/mL) to quantify gene metk and locus H as biomarkers for resistance to allopurinol and antimonials. A strong correlation was obtained between CNV from LeishGenApp (sequencing-based test) and LeishGenR (qPCR test) (metk: r=0.856, p<0.001; H-locus: r=0.909, p<0.001), with 100% specificity and >80% sensitivity, resulting in accuracy of 92% for metk and 94% for H-locus.

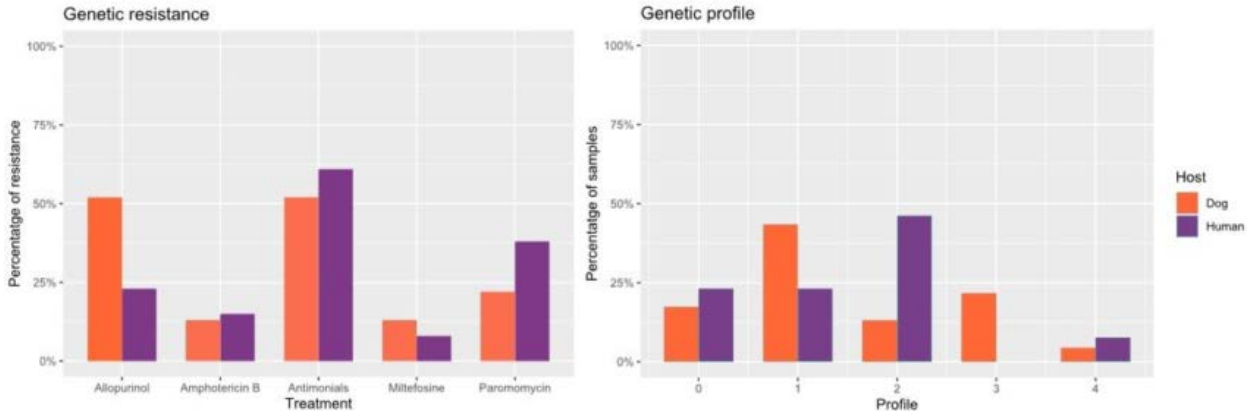


Figure 1.

Discussion and Conclusion

LeishGenApp is a diagnostic platform for resistance biomarkers targeting the five first-line drugs through nanopore whole-genome sequencing and a web app for bioinformatics. LeishGenR is a cost-effective qPCR system to detect resistance biomarkers for antimonials and allopurinol, suitable for clinical samples with parasite loads as low as 600 parasites/mL (Figure 2).

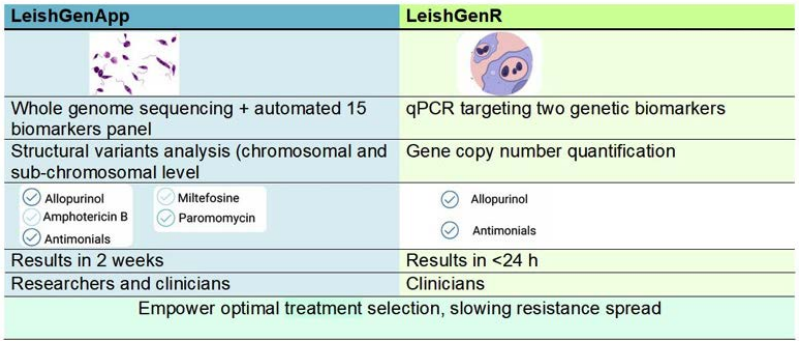


Figure 2.

OP24

Genomics

DECIPHERING THE EVOLUTION OF THE ARCTIC-LIKE LINEAGE OF CANINE DISTEMPER VIRUS

Z. Lanszki[2], F. Pellegrini[1], M.S. Lucente[1], G. Diakoudi[1], V.I. Vasinioti[1], G. Lanave[1], T. Görföl[2], K. Bányai[2], G. Kemenesi[2], V. Martella[1]

[1]*Department of Veterinary Medicine, University of Bari, Italy*
[2]*National Laboratory of Virology, Szentágothai Research Centre, University of Pécs, Hungary*

Introduction

Canine Distemper Virus (CDV) is a fatal viral infection that affects both domestic and wild animals globally. Based on the hemagglutinin gene, geographic patterns of genetic diversification have been observed. These CDV lineages can spread naturally or through human intervention into new geographical regions (1). One example is the so-called Arctic-like lineage that has spread throughout Europe over the last two decades. However, the epidemiological and molecular (sequence) data on Arctic-like CDV are still scattered, limiting our understanding of the evolution of this lineage. Complete genome analysis is much more effective for uncovering genomic-level evolutionary patterns, specific mutations, and recombination events than investigations based on a single gene target (i.e. the hemagglutinin). In this study, we generated genome sequence data of CDV strains of Europe and Arctic-like lineages collected in Italy over a nearly 15-year period.

Materials and Methods

Complete genome sequencing was carried out by a pan-genotype CDV-specific, amplicon-based Nanopore Sequencing Method (2) from 20 domestic dogs and 3 red foxes sampled between 2005 and 2019 from Southern Italian regions. Phylogenetic and recombination analyses were performed.

Results

Seven genome sequences were classified as Europe lineage, and 14 sequences as Arctic-like lineage. All the red fox sequences were grouped into the Europe lineage. A possible recombination event was identified between the Europe and Arctic-like lineages in the matrix (M) gene region.

Discussion and Conclusion

Complete genome sequencing has revealed a temporal pattern of diversification for Arctic-like CDV strains, with the oldest of these CDV strains (1988, 2004 and 2006) being rooted in a basal position. Interestingly, in the genome of Arctic-like CDVs a large portion of the M gene was derived from recombination with Europe lineage. RNA recombination occurred between the 5' part of the M gene and the large intergenic M-F region. Recombination has already been described among CDV strains in the P, and L genes and the F/H intergenic region. These findings support the importance of genome-scale monitoring of CDV evolution.

References

1. Martella, V. et al. North Am. - Small Anim. Pract. (2008)
2. Lanszki, Z. et al. Sci. Rep. (2022)
3. Yuan, C. et al. PLoS One (2017)

OP25

Forensic diagnostics

A CASE OF ACUTE INTOXICATION BY THE ORNAMENTAL PLANT *PIERI JAPONICA* IN A GOAT FARM: TOXICOLOGICAL SCREENING BY DIRECT INTRODUCTION HIGH RESOLUTION MASS SPECTROMETRY (DART-HRMS) IN A DIAGNOSTIC SETTING

A. Leone[2], A. Tata[2], D. Dellamaria[2], A. Collini[1], P. Covi[3], C. Zacometti[2], G. De Martino[2]

[1]*Azienda provinciale per i servizi sanitari di Trento, Italy*
[2]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy*
[3]*Libero professionista della provincia autonoma di Trento, Italy*

Introduction

In March 2024, a group of five goats of approximately one year of age escaped from their enclosure to reach a private garden in search of fresh vegetation because their pasture was covered in snow. The medical history reported predominantly neurological symptoms (convulsions, grinding of teeth, sialorrhea, motor incoordination), which arose suddenly within a few hours; furthermore, the veterinarian and the breeder reported the ingestion by the animals of two different ornamental plants present in the private garden. In addition to the tests routinely used in the diagnostic field such as bacteriological, parasitological and histopathological tests, high resolution mass spectrometry with direct introduction (DART-HRMS) was applied to identify the presence of potentially toxic substances in the ruminal contents and the liver of deceased subjects.

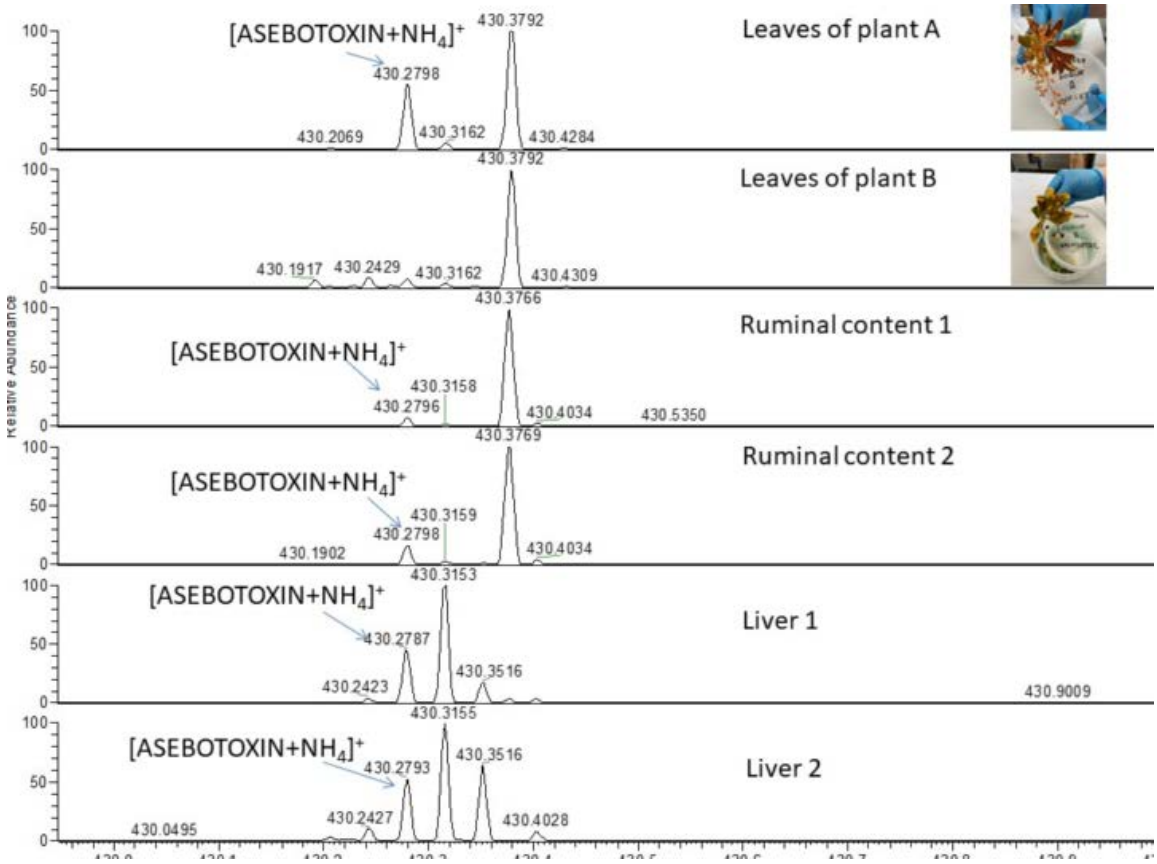


Figure 1. DART-HRMS spectrum of the chemical fingerprinting of: plant leaves A, plant leaves B, goat rumen contents 1, goat rumen contents 2, goat liver 1 and goat liver 2. The toxin Asebotoxin is observed (also called Grayanotoxin I or Andromedotoxin)

Materials and Methods

The corpses of the four goats were autopsied. The rumen and liver contents of the other two deceased subjects were sampled. In addition, plant materials suspected of being the cause of the accidental poisoning were provided. The methanolic extracts of the samples were analyzed by DART-HRMS.

Results
The outcomes of the post-mortem examinations revealed that in the rumen of all the subjects, several leaves belonged to two different plant species, the same species collected from the plants found damaged by the owner of the private garden (*Pieris Japonica* and *Euonymus japonicus*). The DART-HRMS spectra showed the presence of signals corresponding to Graianotoxin 1 in the ruminal contents and livers of the goats. The same molecule was also detected in samples of the *Pieris Japonica* leaves.

Discussion and Conclusion
The intake of grayanotoxins-containing materials from plants belonging to the Ericaceae family can cause intoxication with symptoms that are specifically characterized by dizziness, hypotension and atrioventricular block (1). These symptoms are caused by the inability to inactivate neural sodium ion channels resulting in a continued increase in vagal tone. In conclusion, the use of the DART-HRMS analysis allowed us to corroborate the previously formulated hypothesis and demonstrate that this is a useful technique for the detection of natural toxic compounds.

References
1. Jansen SA et al,CardiovascToxicol.2012;12(3):208.

OP26

Forensic diagnostics

A FIELD TRIAL CHARACTERIZATION OF WILD BOAR POST-MORTEM INTERVAL TO SUPPORT AFRICAN SWINE FEVER DETECTION

G. Assirelli[3], F. Obber[3], R. Celva[3], S. Carlin[3], C. Zacometti[3], E. Poletto[3], A. Franzoso[3], G. Mian[3], A. Casara[2], R. Verin[2], M. Gallo[4], G. Rocca[4], E. Michelotto[4], M. Rocca[4], G. Chiarello[3], A. Tata[3], G. Rosa[1], D. Deni[1], C.V. Citterio[3], N. Pozzato[3]

[1]*CEMEDFORVET - Istituto Zooprofilattico Sperimentale Lazio e Toscana, Italy*
[2]*Dept. Comparative Biomedicine and Food Science - University of Padua, Italy*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*
[4]*Parco Regionale dei Colli Euganei, Italy*

Introduction
The use of passive surveillance is essential for the timely implementation of African swine fever (ASF) management strategies. From this point of view, the definition of the post mortem interval (PMI) and the study of the decay process in the sylvatic environment are crucial to identify the range of possible spread of the virus after the detection of the index case and to improve surveillance.

Materials and Methods
Three wild boar cadavers per site were placed in two different spots of the Colli Euganei regional park (Padua, northeastern Italy) in 4 seasons: summer/autumn 2023 and winter/spring 2024. The cadavers were monitored over 2 months for temperature and humidity using internal/environmental data loggers and for scavengers' consumption by continuous photo trapping. The state of decomposition was periodically evaluated by filling a total body score (TBS) sheet (1) and by collecting and identifying necrophagous entomofauna (2). Samples from skin/subcutaneous/muscle were also collected for histologic and from muscle for metabolomic analysis by Direct Analysis in Real Time - High Resolution Mass Spectrometry.

Results
Wildlife interactions with the corpses were recorded for different species, including wild boar. Time to advanced decomposition ranged from day 3 (summer) to 24 (winter). Metabolomic analysis shown to be capable of detecting changes in volatile and non-volatile compounds during decomposition. An Accumulated Degree-Days calculator that integrates temperature, TBS, entomology, histology and metabolomics is under development.

Discussion and Conclusion
The present study has provided further information on the action of scavengers on wild boar carcasses and confirmed intraspecific interactions albeit without consumption, especially during colder periods. The estimation of PMI through the combined action of morphological changes, entomofauna sampling and metabolomics can reach a high level of accuracy, representing a useful tool for the integration of ASF management strategies.

References
1. Probst C et al. Estimating the Postmortem Interval of Wild Boar Carcasses. Vet Sci. 2020 Jan 5;7(1):6
2. https://www.izslt.it/medicinaforense/wp-content/uploads/sites/12/2019/06/Linee-guida-nazionali-per-le- autopsie-a-scopo-forense-in-medicina-veterinaria.pdf.

OP27

Immunology and vaccines

COXIELLA BURNETII-SPECIFIC IFNγ AND ANTIBODY RESPONSES TO NATURALLY-ACQUIRED INFECTION IN DAIRY COWS.

L. O'Shannessy[4], P. Sheehy[4], J. House[4], K. Plain[1], S. Rowe[4], B. Bauer[2], B. Logan[4], R. Zadoks[4], J. Morton[3], K. Bosward[4]

[1]*Elizabeth Macarthur Agricultural Institute, NSW Department of Primary Industries, Menangle NSW, Australia*
[2]*Institute of Immunology, Friedrich-Loeffler-Institut, Germany*
[3]*Jemora Pty Ltd, Geelong VIC, Australia*
[4]*Sydney School of Veterinary Science, The University of Sydney, Sydney NSW, Australia*

Introduction

Q fever is a zoonotic disease, caused by the bacterium *Coxiella burnetii* (Cb) (1). Cattle are a reservoir for human infection but factors influencing shedding persistence are unclear. Human studies suggest cell mediated immunity (CMI) has a vital role in pathogenesis, with the pro-inflammatory cytokine interferon gamma (IFNγ) linked to clinical syndromes (1). Despite this, most cattle studies focus on the humoral (antibody) response (2). This study optimised a whole blood cytokine recall assay to quantify Cb-specific IFNγ responses in cattle and used this to examine longitudinal responses and associations with shedding.

Materials and Methods

In an initial cross-sectional pilot study, paired blood samples were collected from 5 dairy cows in an endemically- infected herd and transported at room temperature (RT) and on-ice. Whole blood was stimulated ex vivo with Cb for 24 and 48 hours and IFNγ detected in the supernatant by ELISA. Next, a longitudinal sampling of 192 dairy cows, at four timepoints (3 weeks before calving, calving, 55-65 days in milk (DIM), 190 DIM) collected blood, placenta, vaginal mucus, faeces and milk to measure shedding by qPCR, serum antibody levels by ELISA and IFNγ responses by the optimised assay. Differences between IFNγ response over time were analysed by the Wilcoxon matched-pairs test and interrelationships between immune variables and shedding were visualised using cluster analysis.

Results

In the pilot study, the highest IFNγ response in all 5 cows was obtained using blood transported at RT when stimulated for 48hr. In the longitudinal study, IFNγ responses were higher at 55-65 DIM than at calving. At calving, 34% of cows had PCR-positive placentas and at 190 DIM, 10% of cows had PCR-positive milk, with the latter's immune profiles all within a cluster of high antibody level and low IFNγ response (Figure 1).

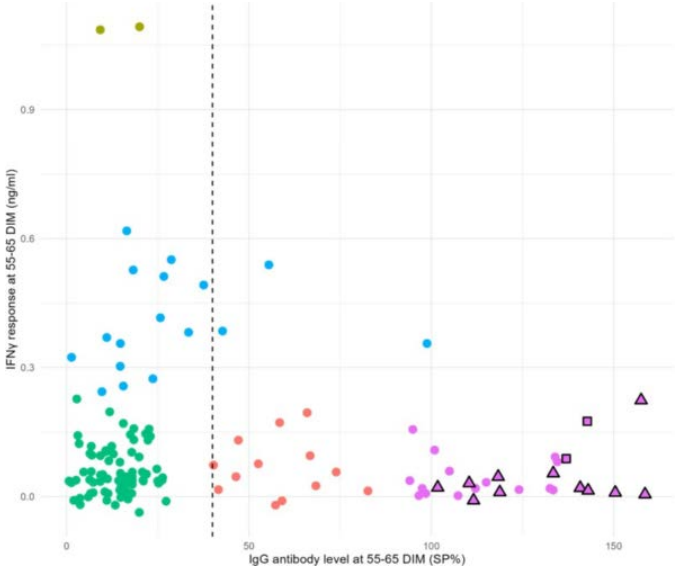


Figure 1. Cluster analysis of dairy cows' *C. burnetii* IgG antibody level (sample to positive ratio (SP%)) and whole blood IFN-gamma response to *C. burnetii* stimulation (ng/ml) measured at 55-65 days in milk (DIM). Shapes indicate milk PCR result at 190 DIM (n = 133 cows that were sampled at the final time point): circles = cows with PCR-negative milk; squares = cows with PCR-suspect positive milk; triangles = cows with PCR-positive milk. Serum IgG antibody levels were analysed by a commercial ELISA (dashed line = seropositivity threshold). IFN-gamma response was determined by stimulating whole blood with *C. burnetii* and measuring using an ELISA. Colours indicate clusters as determined by k-means cluster analysis.

Discussion and Conclusion

This study utilised an IFNγ recall assay to shed light on the pathogenesis of Cb infection in cattle. Cows that prioritised a humoral response in combination with a low IFNγ (CMI) response were more likely to shed in milk at mid-lactation. This suggests low levels of pro-inflammatory IFNγ may allow Cb persistence and chronic shedding.

References

1. Pennings et al. (2014) doi:10.1128/CVI.00078-15
2. Freick et al. (2017) doi:10.1111/rda.12878

OP28

Immunology and vaccines

ELECTROCHEMOTHERAPY FOR FELINE SQUAMOUS CELL CARCINOMA TREATMENT: NOVEL INSIGHTS INTO IMMUNE RESPONSE

F. Dell'Anno[4], F. Fruscione[4], A. Bufalari[3], G. Alterio[1], L. De Paolis[4], S. Mecocci[3], E. Monti[3], I. Di Matteo[2], E. Lepri[3], S. Bergagna[4], R. Giugliano[4], A. Dentini[2], L. D'Ippolito[4], K. Cappelli[3], C.G. De Ciucis[4], A. Ghelardi[5], E. Razzuoli[4]

[1]*Clinica Borghesiana, Rome, Italy*
[2]*Clinica Tyrus, Terni, Italy*
[3]*Department of Veterinary Medicine, Perugia, Italy*
[4]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy*
[5]*Nuovo Ospedale Apuano, Massa, Italy*

Introduction

Squamous cell carcinoma (SCC) represents approximately 70-80% of all oral tumors in cats (1). In humans, head and neck tumors (HNC) account for about 10-12% of all malignant tumors in men and 4-5% in women. Cat SCC and human HNC are frequently associated with the presence of papilloma virus (PV), and both the pathologies share similar mechanisms in their progression. Recent studies suggest electrochemotherapy (ECT) based on bleomycin (BLM) as a possible therapy both for humans and animals (2). Studies show tumor regression following ECT even of untreated lesions. This highlights a possible involvement of the immune system; hence the importance of characterizing the immune response related to the tumor and therapy as well as the need to identify immunomodulatory therapies which, in combination with ECT, can improve the outcome.

Table 1. List of primers for the detection of FcaPV isotypes

Target gene	Sequences (5'-3')
FCA PV1 F (L1)	AGGATGGTGACATGGTGGAT
FCA PV1 R (L1)	CACCATACGGCTCATTTGC
FCA PV2 F (L1)	TACACGCGGTACCAATTTCA
FCA PV2 R (L1)	TGACCACGCACACTTGGAATA
FCA PV3 F (L1)	AAGATTGGTATGGCGTTTGC
FCA PV3 R (L1)	CTGCTGTGGAAGTGTTGTAGG
FCA PV4 F (L2)	AGAGGGCACAATGGTGGATG
FCA PV4 R (L2)	ATCAGGCTCAACTGCACTCC
FCA PV5 F (L1)	ACTGCAGTACCCCCTAAGGA
FCA PV5 R (L1)	CAAAAACTTCCGCCCCAGTG
FCA PV6 F (L1)	TCGTCCAAGTCAACCACGAG
FCA PV6 R (L1)	TAGCCCCCACAAAAGCAACA

Table 2. List of primers (Forward (F) and Reverse (R)) for the detection of FcaPV' s oncogene E6 and E7

Target gene	Sequences (5'-3')
FcaPV1 E6 F	AATCCAGTGCTGCCTCGAAA
FcaPV1 E6 R	TGCCCAATCATCCTTCCACC
FcaPV2 E6 F	GAATGCGTCCTCATGGAGCA
FcaPV2 E6 R	CGCCACACACCTCTGACTAA
FcaPV3 E6 F	TTTGTGCTTTGCCAGTTCGG
FcaPV3 E6 R	CGCCACACACCTCTGACTAA
FcaPV4 E6 F	GAAAACTTGGTGGTGCGGTG
FcaPV4 E6 R	ACAATCGGCAACACCCTCTC
FcaPV5 E6 F	GGGGTGCTGTCTCCGTTTAT
FcaPV5 E6 R	ACGCACTAGATGCAAAGGCT
FcaPV6 E6 F	ATCCAGATGGCAAAGGCGAA
FcaPV6 E6 R	CCCCAGAAGTTGCTGTTCCT
Fc PV1-E7 F	ACATCTCGCATCTCCGAAGTC
Fc PV1-E7 R	CAGCTTCAACCAACACCCAAT
Fc PV2-E7 F	GACGTACCCTGTGGAGTGTG
Fc PV2-E7 R	CGCCGCAGACGAGACTTAAA
Fc PV3-E7 F	GAGTGCCACAAGCAGTCAGA
Fc PV3-E7 R	TTATCGCGATCCGCCATGTT
Fc PV4-E7 F	AGGAAGAGGAGGAGCCTGAG
Fc PV4-E7 R	TATCCCCAGACCTGCCAAGA
Fc Pv5-E7 F	TTGCCCGCCAATTTACTTGC
Fc Pv5-E7 R	CAGACGTGGCAAAAACCCAG
Fc Pv6-E7 F	CAGATTCGGACACAGCAGGA
Fc Pv6-E7 R	CAGATTCGGACACAGCAGGA

Materials and Methods

The present project proposes to evaluate the immune modulation mediated by ECt in a spontaneous model of felines SCC. The presence of *Felix catus* PVs and the expression of its relative oncogenes has been investigated with RT- PCR during the study.

Results

Results suggested positivity for more than 50% of cats enrolled. Subsequent analyses evaluating, pre-treatment with ECT, the gene expres-

sion of inflammatory cytokines such as IL 17, IL 6, TGFβ and TNFα revealed a significantly modulated expression in PVs positive vs negative cats. Following treatment with ECT the i) possible changes in cell populations of the immune system (e.g. lymphocytes, monocytes), ii) and any variation in the expression of circulating cytokines were monitored 7 and 14 days after treatment. Results have shown a significant reduction ($p<0.05$) for monocytes and basophils population; conversely, the number of neutrophils increased over time. Similarly, the expression of circulating cytokines was modulated compared to the pre-treatment control.

Discussion and Conclusion

Overall, the data presented here indicate an effective modulation of the immune system following ECT based on BLM. Further studies involving a larger number of subjects, will validate and strengthen our observations. This project was founded by Italian ministry of health (research code: 21C10).

References

1. Cannon CM. DOI: 10.3390/vetsci2030111
2. Lin et al. DOI: 10.21926/obm.genet.2102131

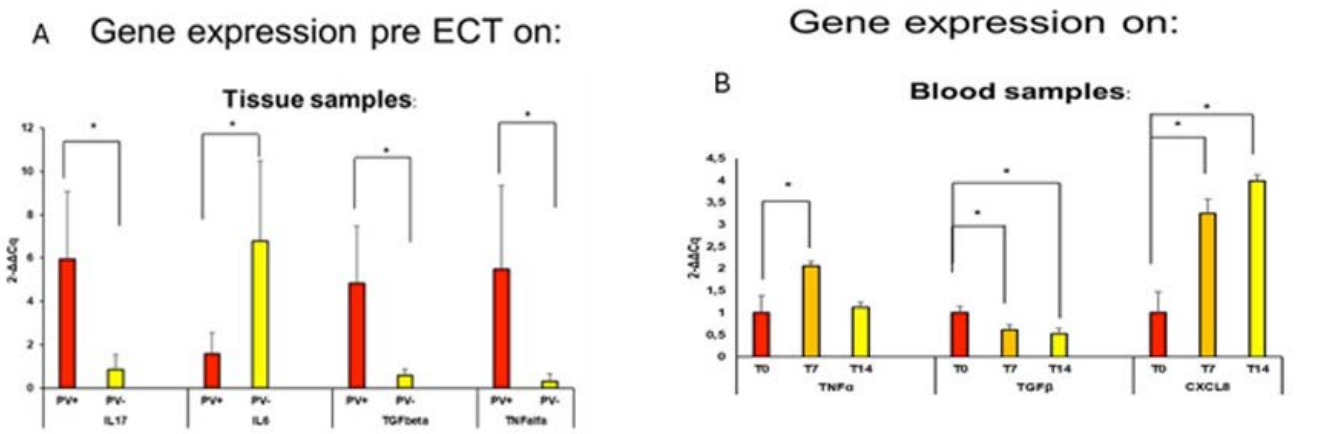


Figure 1. Gene expression on (A) tissue samples positive and negative to PV and (B) on blood samples after ECT

OP29

Immunology and vaccines

CIRCULATING MICRORNA: BIOMARKERS FOR EARLY DIAGNOSIS OF BOVINE PARATUBERCULOSIS

P. Mazzone[3], S. Mecocci[1], F. Carboni[1], R. Ratto[1], M. Torricelli[2], L. Petrucci[2], A. Fratto[2], L. Madeo[2], S. Capomaccio[1], K. Cappelli[1]

[1]*Dipartimento di Scienze Veterinarie – Università degli Studi di Perugia, Italy*
[2]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy*
[3]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy; Dipartimento di Scienze Veterinarie – Università degli Studi di Perugia, Italy*

Introduction

Circulating microRNAs (miRNAs), small non-coding RNAs involved in gene expression regulation and immune response modulation, are promising biomarkers for the early diagnosis and prognosis of bovine Paratuberculosis (PTB) (1), a chronic granulomatous enteritis, caused by *Mycobacterium avium* ssp. paratuberculosis (MAP). Here we present a miRNA assessment in a PTB affected herd of a native cattle breed.

Materials and Methods

Fifteen Marchigiana breed cattle were divided into three groups (Healthy, Affected, Infected) basing on the outcomes of PTB tests: ELISA, IFN-γ, qPCR for MAP detection from feces (Table 1). For the discovery study, RNA sequencing via Illumina technology was performed on RNA extracted from serum of cattle from the three phenotypic groups. After quality control procedures, uniquely aligned reads on miRbase 22 and Bos taurus genome were used to retrieve differentially expressed (DE) miRNAs (p-value < 0.05) in the three paired comparisons with DESeq R package.

Results

Table 2 shows the main DE miRNAs in the three groups, and their functions. In the Affected vs Healthy cows comparison, 2 miRNAs were up-regulated (mir-24-2, mir130b), and 2 down-regulated (mir-95, mir-215). In the Infected vs Healthy comparison, 1 miRNAs was up-regulated (mir-92b) and 1 was down-regulated (mir-126). In Affected vs Infected contrast, 4 DE miRNAs were found: 2 miRNA up-regulated (mir-130b, mir-302c) and 2 miRNA down-regulated (mir-494, mir-95).

Discussion and Conclusion

Promising PTB biomarkers might be: bta-miR-24-2, involved in immune response and in IFN-γ regulation already described in PTB affected cattle, and bta-mir-92b, described in MAP infected cattle, up-regulated in affected and infected animals, respectively; bta-miR-130b, significantly up-regulated in affected animals, known for reducing inflammatory responses and regulating macrophages differentiation; bta-miR-494, down-regulated in affected vs. infected comparison, implicated in various infectious diseases, (i.e. Enterovirus disorders and sepsis), highlighting its role in the immune response. Further validation by RT-qPCR on a larger cohort of cattle will be required to confirm the identified miRNAs as potential biomarkers for the early diagnosis of PTB.

Research funded by Italian Ministry of Health RCIZSUM 06/21

References

1. Gupta et al., 2018 J. Dairy Sci. 101:10259–10270

Phenotypic group	Paratuberculosis Tests	Test outcome	Number of animals / Health status
Healthy	ELISA	Negative	5 healthy subjects (not infected but exposed)
	qPCR	Negative	
	Gamma – Interferon test	Negative	
Infected	ELISA	Negative	5 healthy but MAP infected subjects (infected without symptoms)
	qPCR	Negative	
	Gamma-Interferon test	Reattive	
Affected	ELISA	Positive	5 PTB affected subjects (infected with symptoms)
	qPCR	Positive	
	Gamma – Interferon test	Reattive/Negative	

Table 1. Three phenotypic groups, based on Paratuberculosis (PTB) test outcomes, in a *Mycobacterium avium* ssp. paratuberculosis (MAP) infected herd of Marchigiana cattle breed.

Groups comparison	DE miRNA	Log_2 fold change	adjusted p-value (padj)	References	Functions
Affected vs Healthy	bta-mir-24-2	7.941862659	0.039522943	*Malvisi et al., 2016*	Immune response modulation, in particular anti-bacterial properties; inhibition of IFN-γ expression
	bta-mir-130b	7.958023847	0.000218551	*Yuan et al., 2020*	Inflammatory response decreasing inflammation; negative regulation of macrophage derived foam cell differentiation
	bta-mir-95	-6.977879073	0.038078184	*Zhang et al., 2016*	Involved in the differentiation of skeletal muscle-derived satellite cells (MDSCs) with pivotal role in controlling muscle growth
	bta-mir-215	-4.402486968	0.039522943	*Ioannidis and Donadeu 2018*	Tissue-enriched in the intestine of cattle
Infected vs Healthy	bta-mir-92b	3.551063005	0.046568394	*Shaughnessy et al., 2015*	Up-regulated in early and late MAP infected cattle sera
	bta-mir-126	-1.886561642	0.009560745	*Shaughnessy et al., 2015*	bta-miR-126-3p; bta-miR-126-5p up-regulated in PTB seronegative negative cattle vs MAP late infected
Affected vs Infected	bta-mir-302c	10.43139432	0.039427854	*Zhu et al., 2014*	Inhibits tumor growth of hepatocellular carcinoma by suppressing the endothelial-mesenchymal transition of endothelial cells; Involved in the process of endothelial-mesenchymal transition.
	bta-mir-130b	8.293114666	0.0000175	*Yuan et al., 2020*	Inflammatory response decreasing inflammation; negative regulation of macrophage derived foam cell differentiation
	bta-mir-95	-6.520481812	0.049368822	*Zhang et al., 2016*	Involved in the differentiation of skeletal muscle-derived satellite cells (MDSCs) with pivotal role in controlling muscle growth
	bta-mir-494	-8.956327982	0.015949888	*Zhao et al., 2018* *Ghafouri-Fard et al., 2021*	Initiation and development of Enterovirus 71-related disorders; Involved in immune responses during sepsis.

Table 2. The differentially expressed (DE) miRNAs (DEGs - log2FoldChange > |1| and an adjusted p-value < 0.05) derived from the three-paired comparisons among Healthy, Affected, Infected cattle.

OP30

Miscellaneous

VKORC1 MUTATIONS IN RODENTS FROM EMILIA-ROMAGNA, NORTHERN ITALY

A. Reggiani[4], G. Rugna[4], R. Veronesi[1], R. Bellini[1], C. Venturelli[2], F.M. Dini[3], G. Pampiglione[5], M. Dottori[4], E. Carra[4]

[1]*Agriculture Environment Centre "G. Nicoli", Crevalcore (BO), Italy*
[2]*Azienda Unità Sanitaria Locale della Romagna, Ravenna, Italy*
[3]*Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia (BO), Italy*
[4]*Experimental Zooprophylactic Institute of Lombardy and Emilia-Romagna, Brescia, Italy*
[5]*Pest management consultant, Forlì, Italy*

Introduction

Anticoagulant rodenticides (ARs) are a very effective tool to control pest rodent populations, mostly due to late onset of their lethal effect. Nevertheless, AR resistance has been documented in Europe, Asia and the Americas [1]. ARs block the cycle of Vitamin K (the most important co-factor involved in the blood coagulation pathway), thus leading the animal to death by internal bleeding: the presence of single nucleotide polymorphisms (SNPs) in *VKORC1* gene can cause resistance by inducing structural changes that prevent a proper binding of the anticoagulant to the enzyme. The presence of AR-resistant rodents could lead to an increased diffusion of zoonotic pathogens and to an unwanted exposure of non-target animals to ARs. This study aims to test the presence of *VKORC1* SNPs in synanthropic rodents from the Emilia-Romagna region (RER), Italy.

Materials and Methods

Rat and mouse carcasses were collected in partnership with rodent control companies in five RER provinces (Figure 1). The three exons of the *VKORC1* gene were amplified and sequenced from DNA extracted from tail muscle: the full sequence was then assembled and checked for the presence of SNPs. In addition, a Taqman Real-Time PCR (qPCR) assay on the most common resistance SNP (codon 139) was performed.

Results

A total of 67 animals were analysed: 24 *Rattus norvegicus*, 35 *R. rattus* and 8 *Mus musculus*. A SNP associated with AR resistance (in homo- or heterozygosis on codon 128 or 139) [1] was detected in 13/24 *R. norvegicus* and 6/8 mice, but not in *R. rattus*. Furthermore, several missense mutations never described in literature were detected in all the tested species, particularly in *R. rattus* and *M. musculus* (Tables 1 and 2). Finally, results of the qPCR assay on codon 139 consistently matched the sequence analysis.

				Rattus norvegicus (n=24)			Rattus rattus (n=35)		
Codon position	Codon & mutation	WT codon	Mutant codon	Frequency	Homozygous genotype	Heterozygous genotype	Frequency	Homozygous genotype	Heterozygous genotype
7	S7G	AGC	GGC	0,0%	0	0	2,9%	0	1
12	A12A	CGG	CGA	0,0%	0	0	100,0%	34	1
28	H28Q	CAC	CAG	4,2%	1	0	8,6%	1	2
35	R35R	CGC	CGT	0,0%	0	0	2,9%	0	1
36	N36H	AAT	CAT	0,0%	0	0	2,9%	1	0
41	A41A	GCG	GCA	0,0%	0	0	40,0%	8	6
42	L42P	CTC	CCC	0,0%	0	0	22,9%	5	3
59	W59R	TGG	AGG	0,0%	0	0	28,6%	6	4
61	R61W	CGG	TGG	16,7%	2	2	0,0%	0	0
82	I82I	ATA	ATT	41,7%	10	0	0,0%	0	0
90	I90L	ATA	TTA	0,0%	0	0	100,0%	35	0
94	L94L	TTA	CTA	0,0%	0	0	57,1%	18	2
96	C96G	TGC	GGC	4,2%	0	1	2,9%	0	1
107	I107I	ATC	ATA	0,0%	0	0	97,1%	34	0
123	I123F	ATC	TTC	0,0%	0	0	8,6%	0	3
123	I123S	ATC	AGC	4,2%	1	0	0,0%	0	0
137	T137T	ACC	ACT	0,0%	0	0	97,1%	34	0
139	Y139F	TAT	TTT	37,5%	9	0	0,0%	0	0
143	A143A	GCG	GCA	0,0%	0	0	85,7%	17	13

Table 1. SNPs identified in *Rattus* spp., compared to the *R. norvegicus* AY423047 reference sequence. Yellow: SNP associated with resistance; White: known SNP, but not associated with resistance [11]; Green: newly identified SNP; WT: wild-type. The number of subjects presenting a resistance-associated SNP is highlighted in red.

				Mus musculus (n=8)		
Codon position	Codon & mutation	WT codon	Mutant codon	Frequency	Homozygous genotype	Heterozygous genotype
78	Q78H	CAA	CAC	12,50%	0	1
85	C85R	TGC	CGC	12,50%	0	1
87	F87L	TTC	TTA	12,50%	0	1
128	L128S	TTA	TCA	50,00%	1	3
139	Y139C	TAT	TGT	62,50%	1	4

Table 2. SNPs identified in *Mus musculus.*, compared to NM178600 reference sequence. Yellow: SNP associated with resistance [11]; Green: newly identified SNP; WT: wild-type. The number of subjects presenting a resistance-associated SNP is highlighted in red.

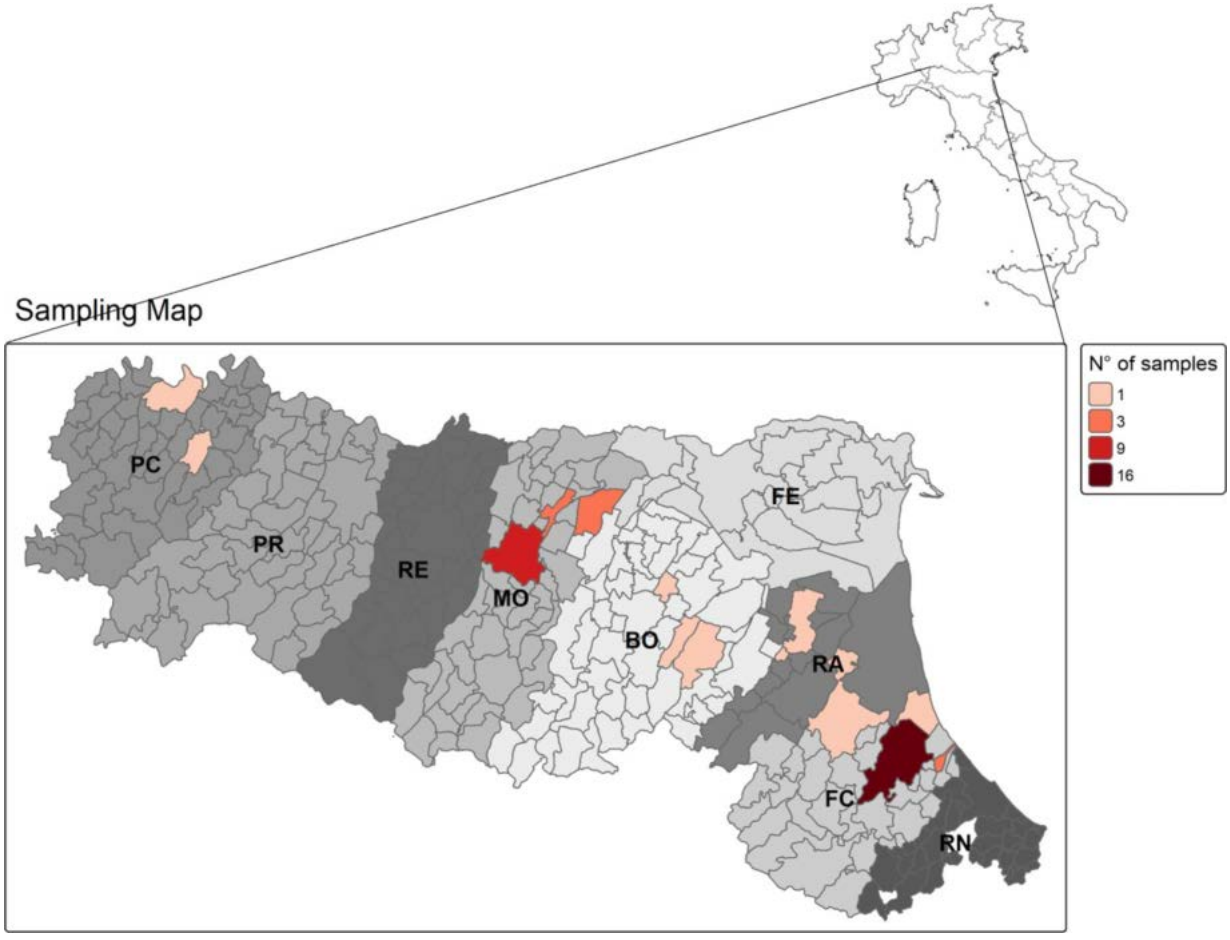


Figure 1. Map of municipalities where rodents were sampled. Each municipality is coloured in a shade of red, proportionally to the number of sampled animals. For 23 other rodents sampled in the provinces of Forlì-Cesena and Ravenna, the exact municipality could not be known due to privacy reasons, therefore they are not represented in this map.

Discussion and Conclusion

This study has shown the occurrence of AR resistance-related *VKORC1* SNPs in rodents from RER, whose presence has not been reported so far, and has not been well studied in the rest of Italy. To determine if uncharacterised SNPs may contribute to AR resistance, further research is needed. Finally, sequencing proved to be more informative than qPCR, and should be preferred in future investigations.

References

1. McGee et al., Anticoagulant rodenticides - A comprehensive review. J. Stored Prod. Res 2020, doi:10.1016/ j.jspr.2020.101688.

OP31

Animal health

AFRICAN SWINE FEVER: CURRENT EPIDEMIOLOGICAL SITUATION IN EUROPE AND IN ITALY

C. Iscaro[2], L. Ruocco[3], D. Di Sabatino[1], M. Sordilli[3], F. Pacelli[3], F. Plasmati[3], F. Feliziani[2]

[1]*Istituto Zooprofilattico Sperimentale Abruzzo e Molise "Giuseppe Caporale", National Reference Centre for Veterinary Epidemiology, Programming, Information and Risk Analysis, Teramo, Italy*
[2]*Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", National Reference Laboratory for Swine Fevers, Perugia, Italy*
[3]*Ministry of Health, ex Direzione Generale della Sanità Animale e del Farmaco Veterinario, Ufficio III, Sanità Animale e Gestione Operativa del Centro Nazionale di Lotta ed Emergenza Contro le Malattie Animali e Unità Centrale di Crisi, Rome, Italy*

Introduction
African Swine Fever (ASF) is gradually spreading in Europe since 2007. ASF virus (ASFV) was detected for the first time in mainland Italy in 2022 and some translocations events were notified in 2023. EU Commission provides for the development of legislation based on the regionalization approach, the publication of guidelines, the scientific advices by European Food Safety Authority (EFSA).

Materials and Methods
ASF section on EU website is one of official data sources relating Member States updates (1), as well as the latest annual epidemiological report by EFSA (2). Italian data are inputted in the VETINFO portal (3): SINVSA for data from surveillance activities; SIMAN for suspect and positive cases/outbreaks notifications.

Results
In 2023 ASF was notified for the first time in Croatia, Bosnia-Herzegovina, Kosovo (domestic pigs DP and wild boar WB), Sweden (WB only), Greece (re-occurrence in both populations). In 2023 than in 2022, number of outbreaks was 5 times higher mainly due to spread in Croatia and resurgence in Romania; in WB a 10% increase of cases was observed. In Italy ASF is continuing to spread since 2022 in Liguria and Piedmont causing multiple cases in WB, and in Rome municipality with new epidemic peaks. ASF affected new areas in 2023/2024: Reggio Calabria and Pavia provinces in DP and WB; Salerno, Parma and Piacenza provinces in WB; Dorgali municipality in DP.

Discussion and Conclusion
In Europe, despite new introductions of ASFV and the trend to become endemic, some local improvements are recorded (Germany, Slovakia, ecc). In Italy, the north-west cluster is expanding towards Tuscany and Emilia Romagna, with a likely involvement of the WB (Central Apennines) and DP (Po Valley); epidemic peaks have succeeded epidemiological silences in Rome and in the southern clusters; the eradication strategy has not been completed in Calabria yet; restriction measures make difficult farms management in Lombardy and Emilia Romagna. Passive surveillance, biosecurity attitude, preparedness to emergency and awareness are the effective tools to eradicate ASF.

References
1. https://food.ec.europa.eu/animals/animal-diseases/diseases-and-control-measures/african-swine-fever_en
2. https://doi.org/10.2903/j.efsa.2024.8809
3. https://www.vetinfo.it/

OP32

Animal health

BRUCELLA SUIS OUTBREAK IN AN AUSTRIAN PIG FARM - TRACING POTENTIAL SPILL-OVER FROM WILDLIFE USING WHOLE GENOME SEQUENCING

M. Gonano[1], R. Posch[1], D. Polzer[1], J. Lambertz[1], B. Ponweiser[1], M. Stätter[1], A. Allinger[1], S. Revilla-Fernández[1], A. Steinrigl[1], F. Schmoll[1]

[1]*Austrian Agency for Health and Food Safety, Institute for Veterinary Disease Control, Mödling, NRL for Brucellosis*

Introduction
Brucellosis is a worldwide occurring disease caused by *Brucella* spp. Austria is free from infection with *Brucella* abortus, *B. melitensis* and *B. suis* in cattle and small ruminants. Porcine brucellosis caused by *Brucella* suis Biovar 2 occurs sporadically. Infected wildlife is regarded as the primary infection source for domestic pigs.

Materials and Methods
In March 2024 abortion tissue from two sows was sent to the Austrian National Reference Laboratory (NRL) for Brucellosis at AGES

after the farm reported fertility problems. Samples were analysed using pathohistological, bacteriological and PCR methods. Blood samples were tested for antibodies using Rose-Bengal test.

Results

B. suis Biovar 2 was detected by PCR and bacterial cultivation and confirmed by Whole-Genome-Sequencing (WGS). Blood testing of the whole breeding unit (n65) revealed a 52% seropositivity rate. All sows, piglets and the boar were culled. Randomly collected blood samples from fattening pigs (n56) housed in a separate unit tested serologically negative. The farm followed a closed-in-out strategy, suggesting wildlife as the probable infection source. During decontamination and disinfection, twelve mice collected from the farm environment were sent to the NRL, along with two road-killed hares and one cat from the farm. One mouse tested positive for *Brucella* spp. by PCR and *Brucella* spp. was cultivated. By using WGS, *Brucella* microti was confirmed. Both hares and the cat tested negative for *Brucella* spp.

Discussion and Conclusion

Core genome multilocus sequence typing (cgMLST) showed that Austrian *B. suis* Biovar 2 strains from previous outbreaks (2017, 2004) were genetically clearly different to the 2024 outbreak strain. Some *B. suis* Biovar 2 strains from hares in the outbreak area showed genetic proximity to the 2024 strain, but allelic differences were too high to establish a direct epidemiological link. Wild boar strains mostly formed a separate cluster, besides fox strains that were found related to wild boar and hare strains. The Austrian *B. suis* Biovar 2 outbreaks highlight the importance of a ONE Health approach to control zoonotic bacteria spread from wildlife to domestic animals and potentially humans. Moreover, the power of WGS and cgMLST to characterize genetic relatedness of *B. suis* strains in high resolution is demonstrated.

OP33

Antimicrobial resistance / susceptibility testing

GASTROINTESTINAL NEMATODES IN CATTLE AND SHEEP FARMS OF THE CAMPANIA REGION: EVALUATION OF ANTHELMINTIC RESISTANCE

R. D'Ambrosio[2], S. Cavallo[2], R. Brunetti[2], A. Pesce[2], M. Buonanno[2], M. Santaniello[1], L. Rinaldi[1], A. Bosco[1], L. Baldi[2]

[1]*Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Naples, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Naples, Italy*

Introduction

Gastrointestinal nematode (GIN) infection endangers ruminant health and welfare. In recent years, the onset of the first cases of anthelmintic resistance (AR) in cattle and sheep farms in Campania region (Southern Italy) has been reported (1) compared to very high anthelmintic efficacy recorded up to a decade ago (2). This study provides a scenario of the current distribution of GINs in some cattle and sheep farms selected on the base of the number of anthelmintic treatments recorded in recent years.

Materials and Methods

The target population consists of cattle and sheep farms that have had veterinary prescriptions for anthelmintics (ivermectin - IVM and albendazole- ALB) in the period 2019-2021. In each farm, individual faecal samples were collected from 15 adult and 5 young animals. The samples were analysed using the Mini-FLOTAC technique for the diagnosis of GIN infections (Figure 1). The results of this investigation were represented on a map using QGIS software. In each farm the animals were divided into 2 groups of 20 animals randomly chosen, one group treated with IVM and one group with ALB, without using an untreated control group (3). Individual faecal samples were collected rectally on the day of treatment (D0) and after 14 days (D14) and analised. The Faecal Egg Count Reduction (FECR) was calculated from individual samples and the efficacy was classified as Reduced, Suspected and Normal (Table 1).

Results

The parasitological results of farms examined showed different values of eggs per gram (EPG) of faeces. Based on the FECR Test, high efficacy (from 96.7% to 100%) was observed for ALB and IVM in all cattle farms and in eighteen sheep farms. On two sheep farms, the efficacy for the IVM was classified as Normal, but Reduced efficacy was observed for ALB on Farm 1 (FECR = 75%) and Suspected efficacy on Farm 2 (FECR = 93.3%) (Figure 2) with the predominant GIN genus Trichostrongylus followed by Haemonchus at D14.

Discussion and Conclusion

In Southern Italy, where the negative impacts from AR have played a minor role, efficient monitoring of AR is important in order to evaluate potential risks.

This research was founded by the Italian Ministry of Health IZS ME 09/20 RC.

References

1. Bosco et al., ParasitVectors, 2020
2. Rinaldi et al., Vet Parasitol, 2014
3. Bosco et al., Parasit Vectors, 2020

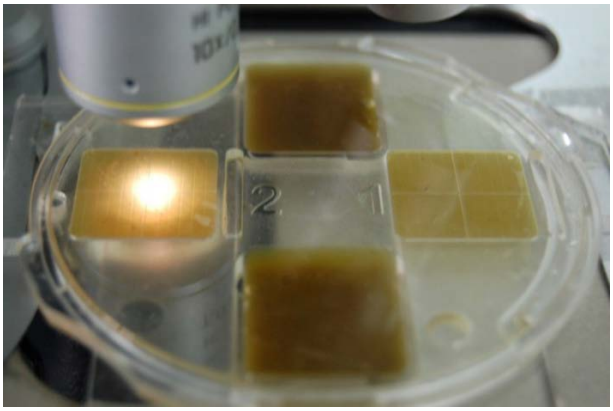


Figure 1. Mini-FLOTAC.

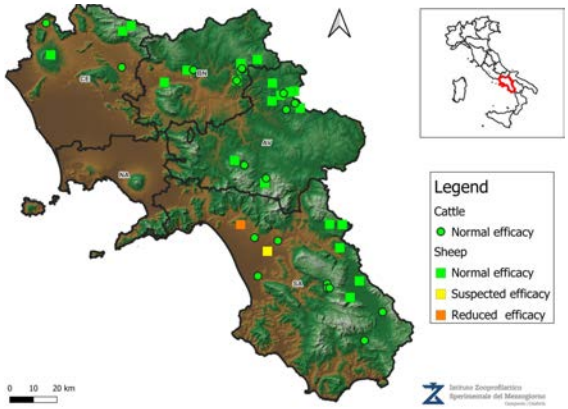


Figure 2. Distribution of anthelmintic efficacy in cattle and sheep farms of Campania region.

ID	Animal species	Province	Albendazole			Ivermectin		
			FECRT %	CI (LL) 95%	Efficacy	FECRT %	CI (LL) 95%	Reponse
1	Sheep	Salerno	86	84.2-88.3	Reduced	99.7	98.3-100	Normal
2	Sheep	Salerno	92.4	89.5-94.7	Suspected	99.8	98-7-100	Normai
3	Sheep	Salerno	97.7	96.7-98.4	Normai	98,,6	97.7-9<9.2	Normai
4	Sheep	Salerno	9'9,9	96.0-100	Normai	99.9	99.3,,100	Normai
5,	Sheep	Salerno	9'9,8	98.8-100	Normai	99.9	9'9,5,-100	Normai
6	Sheep	Avellino	99.9	99.5-100	Normai	99.9	99.7-100	Normai
7	Sheep	Benevento	98-2	95,,1-99.6	Normai	100	98.9-100	Normai
g	Sheep	Benevento	99.9.	98.1-100	Normai	99.9	99.2-100	Normai
9	Sheep	Caserta	9'9,9	96.0-100	Normai	99.8	9'9,,1-100	Normai
10	Sheep	Caserta	97.2	96.2-97.9	Normai	'96.9	95.8-9<7.7	Normai
11	Sheep	Caserta	100	99.0-100	Normai	100	99.0-100	Normai
12,	Sheep	Salerno	99.7	98.2-100	Normai	99.2	98.1-100	Normai
13,	Sheep	Salerno,	9'9,7	95.9-100	Normai	99.5	98.7-100	Normai
14	Sheep	Avellino	98.9	98.5-100	Normai	99',4	99.1-100	Normai
15	Sheep	Benevento	**98.8**	96,,1--9-9.4	Normai	100	98.3,,-100	Normai
16	Sheep	Benevento	99.6	98.3,,100	Normai	99-.8	99.1-100	Normai
17	Sheep	B,enevento	9'9,4	97.3,,-100	Normai	98.5	97.8--100	Normai
18	Sheep	Benevento	**100**	99.S-100	Normai	**100**	99.7-100	Normai
19	Sheep	Benevento	**98.8**	97,,1-99.6	Normai	100	99.4-100	Normai
20	Sheep	Benevento	99.4	98.1-100	Normai	99.6	99.2-100	Normai
21	Catrtle	Salerno,	100		Normai	**100**		Normai
22	Cattle	Avellino	99,7	98,2-100	Normai	99,8	98,3-100	Normai
23	Cattle	Salerno	99,9	98,7-100	Normai	99,6	98,5-100	Normai
24	Cattle	Salerno,	99,9	96.0-100	Normai	99,9	99,3,,-100	Normai
25	Catrtle	Salerno,	100		Normai	100		Normai
26	Cattle	Salerno	99,9	99,5-100	Normai	99,9	99,7-100	Normai
27	Cattle	Caserta	100		Normai	100		Normai
28	Cattle	Salerno	100		Normai	100		Normai
29	Catrtle	Avellino	100		Normai	100		Normai
30	Cattle	Avellino	99,9	98,1-100	Normai	99',9	99,2-100	Normai

Table 1. Results of the Fecal Egg Count Reduction Test (FECRT) in sheep and cattle

OP34

Antimicrobial resistance / susceptibility testing

MOLECULAR IDENTIFICATION OF *TRICHOPHYTON INDOTINEAE* STRAINS ISOLATED IN ITALY AND EVALUATION OF TERBINAFINE RESISTANCE OR SUSCEPTIBILITY

D. Cruciani[5], S. Spina[5], R. Calcaterra[3], C. Farina[6], M. Papini[1], L. Pisano[2], M. Sabbatucci[4], S. Crotti[5]

[1]*Clinica Dermatologica di Terni, Dipartimento di Medicina e Chirurgia, Università degli Studi di Perugia, Italy*
[2]*Dipartimento di Scienze della Salute, sezione di dermatologia, Università degli Studi di Firenze, Italy*
[3]*Istituto Nazionale per la promozione della salute delle popolazioni Migranti e il contrasto delle malat-tie della Povertà, INMP, Italy*
[4]*Istituto Superiore di Sanità, Dipartimento di Malattie Infettive, Roma, Italy*
[5]*Istituto Zooprofilattico Sperimentale Umbria e Marche, Italy "Togo Rosati", IZSUM* [6]*Laboratorio di Microbiologia Clinica e Virologia, ASST Papa Giovanni XXIII, Italy*

Introduction

Trichophyton indotineae is an emerging dermatophyte species that plays a relevant role in human healthcare. It has been associated with severe chronic skin infections and a high level of terbinafine resistance. *T. indotineae* is endemic to India, Iran, and Iraq but several cases have been reported in Italy, recently (1). To correctly identify dermatophytes and to investigate terbinafine resistance or susceptibility, a molecular approach is highly recommended

Materials and Methods

Between May 2023 and May 2024, four Italian hospital laboratories, located in Terni, Florence, Rome, and Bergamo isolated 12 dermatophyte strains in patients with *Tinea corporis*, onychomycosis and no responsive to terbinafine treatment. Considering the macro- and microscopical features (Figure 1) of the dermatophytes colonies, native countries of patients, and/or the illness severity, and/or the no response to terbinafine (Table 1), dermatologists hypothesized a *T. indotineae* infection. To confirm the diagnostic suspects, the strains were conferred at IZSUM where a PCR, using universal fungal primers (ITS1/ITS4) and DNA sequencing were carried out. Lastly, at IZSUM, a Multiplex real-time PCR assay was performed to establish strains' behaviour towards terbinafine (1).

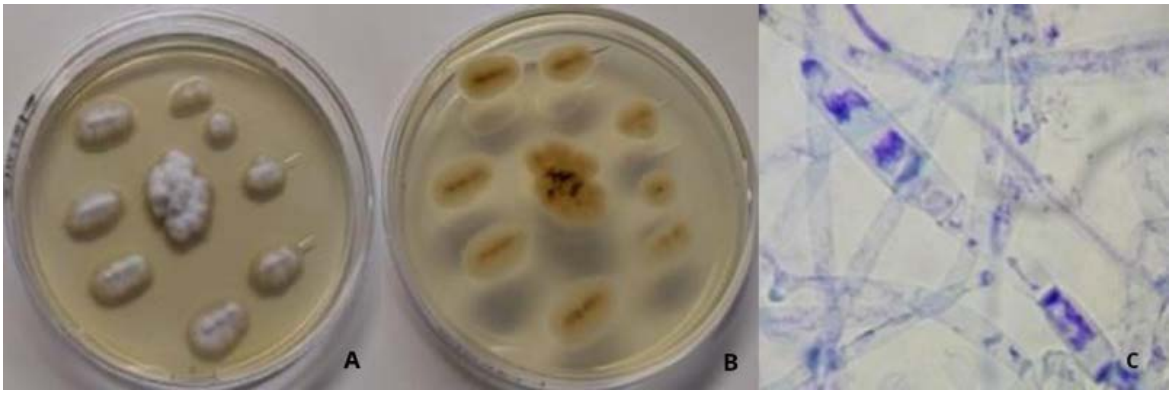


Figure 1. Macroscopical (A, B) and microscopical (C) features of *Trichophyton indotineae*.

Case	Native country	Illness gravity	Terbinafine R/S
1	Bangladesh	Yes	R
2	Bangladesh	Yes	R
3	Bangladesh	Yes	R
4	Bangladesh	Yes	R
5	Bangladesh	Yes	R
6	Bangladesh	Yes	S
7	Bangladesh	Yes	S
8	Perù	Yes	S
9	Perù	Yes	S
10	India	Yes	S
11	Sri Lanka	Yes	R
12	Italy	Yes	R

Table 1. Details of the *T. indotineae* cases.

Results
T. indotineae was confirmed for all 12 strains, demonstrating the nine characteristic SNPs mutations in ITS region (2). Out of the 12 strains, 7 (58.3%) were terbinafine resistant, showing several point of mutations in the gene encoding for squalene epoxidase. In these cases the Tm ranged between 54.0-64.0 °C, associated with mutant SQLE strains. The 5 remaining samples (41.7%) showed terbinafine susceptibility, with a range of T melting associated with wild type SQLE strains (64.5-68.0 °C).

Discussion and Conclusion
T. indotineae infections should be considered when lesions occur in patients coming from endemic areas or with travel history linked to these countries, or in case of terbinafine-resistance. In order to avoid misidentification, specialized laboratories are required. *T. indotineae* detection in Northern and Central Italy and its role in human health are two crucial items to encourage diagnostic improvement through a One Health approach

References
Crotti S, et al. 2023 https://doi.org/10.3390/jof9090865
Thi Minh Chau Ngo, et al. 2022 https://doi.org/10.1016/j.mmcr.2022.05.004

OP35

Emerging and re-emerging diseases

A NOVEL ARTERIVIRUS IN FATAL ENCEPHALITIS OF EUROPEAN HEDGEHOGS IN ENGLAND

N. Inglese[1], T. Partridge[4], S. Karuna[1], D.J. Everest[1], J. Frossard[1], M.P. Dagleish[3], M.F. Stidworthy[2], A. Dastjerdi[1]

[1]*Animal And Plant Health Agency (APHA), UK*
[2]*International Zoo Veterinary Group, UK*
[3]*Moredun Research Institute, UK*
[4]*Vale Wildlife Hospital and Rehabilitation Centre, UK*

Introduction
In October 2019, a fatal encephalitis affected more than 200 European hedgehogs (*Erinaceus europaeus*), admitted to a Wildlife Hospital and Rehabilitation Centre. The disease started with inappetence followed by neurological signs such as tremors, twitching, hyperaesthesia, ataxia/paresis, and paddling legs leading to their death. The outbreak lasted for four months. We describe here an investigation into this disease outbreak using metagenomics.

Materials and Methods
Freshly frozen brain tissues from three hedgehogs were processed at APHA- Weybridge to extract nucleic acid and NGS using Illumina MiSeq. The NGS data were analysed using SeqMan NGen Pro Software (DNA STAR) through de novo assembly.

Results
Analysis of the NGS data resulted in several contigs having the highest nucleotide identity to African giant-pouched rat arterivirus, but only at 52%. Accordingly, the virus, hedgehog arterivirus-1 (HhAV-1) clustered phylogenetically with the African giant-pouched rat (*Cricetomys gambianus*) arterivirus in the subfamily Heroarterivirinae, family Arteriviridae (Figure 1). A real time RT-qPCR, amplifying a 100-nucleotide segment of the ORF7 gene, detected high virus RNA load in brain, blood, liver, lung and spleen of the hedgehogs. No other microbial pathogen was detected.

Discussion and Conclusion
We report the first detection of a novel arterivirus from hedgehogs with encephalitis and conclude a nosocomial infection, introduced by one or several asymptomatic hedgehogs. This outbreak highlights the requirement for strict biosecurity measures during rehabilitation involving intensive hospitalization of animals of this species, which are a frequent wildlife casualty submission in the UK. Hedgehogs are protected by the Biodiversity Action Plan in the UK.

References
Novel Arterivirus Associated with Outbreak of Fatal Encephalitis in European Hedgehogs, England, 2019. A Dastjerdi, N Inglese, T Partridge, S Karuna, D Everest, JP Frossard, MP Dagleish, MF Stidworthy. Emerg Infect Dis. 2021 Feb;27(2):578-581.

OP36

Emerging and re-emerging diseases

A MULTICENTRIC EUROPEAN EPIDEMIOLOGICAL STUDY FOR FELINE CIRCOVIRUS 1 IN DOMESTIC CATS

A. Salvaggiulo[2], V.I. Vasinioti[2], F. Pellegrini[2], M. Camero[2], N. Decaro[2], J.R. Mesquita[3], I. Buzdugan[1], V. Martella[2], G. Lanave [2]

[1]*Department of Public Health, Iasi university of Life sciences, Iasi, Romania*
[2]*Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy*
[3]*ICBAS-School of Medicine and Biomedical Sciences, Porto University, Porto, Portugal*

Introduction

Members of the family Circoviridae (circoviruses) are small, non-enveloped viruses with a circular, covalently closed DNA genome of about 1.7 to 2.1 kb in length. The family Circoviridae includes the genera Circovirus (CV) and Cyclovirus (CyV), which have been identified in several animal species and in human specimens. CVs are associated with severe disease in pigs and birds and with respiratory and gastrointestinal disorders and systemic disease in dogs 1. Feline CV-1 (FeCV-1) was first reported in 2023 in fecal samples of cats from different geographical areas of Italy 2. FeCV-1 was distantly related genetically to a mongoose CV (<79% nt identity) and it was suggested that cats could be a primary host for this CV. In the present study, we extended the investigations for FeCV-1 to other European countries.

Materials and Methods

Between 2015 and 2023 a total of 186 samples from 174 cats were collected in Greece, Romania, and Portugal. Fecal, rectal swabs, oropharyngeal swabs, and blood samples were analyzed using quantitative PCR (qPCR) and endpoint PCR assays, specific for FeCV-12. For a selection of samples circular DNA was enriched by rolling cycle amplification (RCA), and used as template for PCR amplification of overlapping genome fragments (of about 600-800b) of FeCV-1.

Results

Overall, 20 (10.7%) out of 186 samples tested positive for FeCV-1. A prevalence as high as 20.4% (10/49) was observed in samples from Greece, 11.4% (5/44) from Romania, and 5.4% (5/93) from Portugal. FeCV-1 was repeatedly detected in fecal, respiratory, and blood samples. The whole genome sequence was generated for eight strains. The FeCV-1 strains shared 95.2% to 99.6% nt identity, forming a well-conserved clade, regardless of the geographic origin.

Discussion and Conclusion

The detection of FeCV-1 in different geographical locations indicates that this novel agent is a common component of the feline virome. These findings may pave the way for stratified and structured epidemiological investigations in cat populations, including case-control studies, to decipher the pathological role, if any, of FeCV-1.

References

1. Piñeyro and Ramamoorthy, 2022 doi:10.1002/9781119650836.ch48
2. Vasinioti et al., 2023 doi: 10.1016/j.rvsc.2023.06.011

POSTER

P001

Animal health

ENHANCING DEFENSE: THE ROLE OF ADJUVANTS IN FMD VACCINATION OF BUFFALO CALVES

Q. Akram[2], M. Ahsan Naeem[1], M. Younas[2], M. Rizwan Saeed[2]

[1]*Department of Basic Sciences (Pharmacology), University of Veterinary and Animal Sciences, Lahore (Narowal Campus) Narowal, Pakistan*
[2]*Department of Pathobiology (Microbiology), University of Veterinary and Animal Sciences, Lahore (Narowal Campus) Narowal, Pakistan*

Introduction
Foot and Mouth Disease (FMD) is the deadly disease of ruminants, causing huge economic losses in dairy and meat industry. This study is aimed to evaluate the effect of different adjuvant on the antibody response in buffalo calves vaccinated against FMD virus serotype "O".

Materials and Methods
Montanide ISA-70 (OB), montanide (50%) with clinoptilolite (1.0 µg/dose) (ISA 70 + CLNP), lanolin, and aluminum hydroxide gel (AHG) adjuvants were tested. Each vaccine formulation used the inactivated FMD virus with a mean tissue culture infective dose (TCID50) of 2x106.2 units/ml. The study involved 36 unvaccinated male buffalo calves, divided into groups, and vaccinated with respective adjuvant-containing vaccines. The antibody titers were monitored over 240 days using virus neutralization tests.

Results
Anti-FMDV-VN, or detectable levels of FMDV virus neutralizing antibodies, were expressed by all vaccines containing vaccines. The OB vaccine produced an antibody response that was greater and more sustained later on, with antibody titers remaining above protective limits for more than 240 days after priming as compared to the AHG vaccination. On subcutaneous injection of OB vaccine, sterile abscesses and granulomas developed that were avoided with deep intramuscular injections.

Discussion and Conclusion
Montanide ISA-70-based vaccines demonstrated superior immunogenicity, suggesting that bi-annual vaccinations with the OB-FMD vaccine could effectively enhance immunoprophylaxis in dairy animals. Montanide (50%) with clinoptilolite (1.0 µg/dose) provides the protection for 38 weeks making them strongest candidate for vaccine adjuvant. Improved vaccine formulations are essential to mitigate FMD outbreaks and their economic impact.

P002

Animal health

COMPARISON OF DIAGNOSTIC TOOLS FOR CALF DIARRHEA IN FLANDERS

L. Allais[1], E. Van Driessche[1], N. Botteldoorn[1], J. Schaep[1], T. Vanblaere[1]

[1]*DGZ Vlaanderen (Animal Health Care Flanders), Belgium*

Introduction
Neonatal calf diarrhea (NCD) is a significant health issue in the bovine industry with high morbidity and mortality rates among neonatal calves, leading to high economical losses. Primary infectious agents are rotavirus, coronavirus, *E. coli*, *Cryptosporidium* spp. and *Salmonella* spp. Sufficient diagnostic tools are needed for efficient and early detection on the farm. Currently, the mainly used test is ELISA on faeces samples. In literature, diagnostic sensitivity and specificity of ELISA appears to be suboptimal (1). Therefore, we compare ELISA with PCR as a more sensitive method.

Materials and Methods
Commercial ELISA was performed for rotavirus, coronavirus, *E. coli* F5 (K99) attachment factor and *Cryptosporidium parvum*. Commercial Real-Time PCR was performed for rotavirus, coronavirus and *Cryptosporidium parvum* using commercially available kits.

Results
In 2023, 873 feaces samples from cattle were analysed by ELISA for antigen detection of rotavirus, coronavirus, *E. coli* F5 (K99) and *Cryptosporidium* spp. For the cattle faeces samples, 4% were positive for coronavirus, 20,4% were positive for rotavirus, 8% were positive for *E. coli* F5 (K99) and 30,4% were positive for *Cryptosporidium* spp. On five samples collected in 2024, we compared ELISA and PCR for rotavirus, coronavirus and *Cryptosporidium* parvum. For ELISA and PCR, similar results were obtained. For one sample, rotavirus was detected by PCR but not by ELISA.

Discussion and Conclusion
We conclude that the PCR is slightly more sensitive than ELISA, with a false negative result in ELISA compared to PCR. Nevertheless, the ELISA still offers good results for use in the field and is a cheaper alternative for PCR. To further investigate the difference in sensitivity between both methods, additional samples will be compared.

References
1. van Mol, W., Clinquart, J., Pas, M., Bokma, J., & Pardon, B. (2022). Pathogen-oriented approaches for neonatal calf diarrhea. Vlaams Diergeneeskundig Tijdschrift, 91(4), 167-181.

P003

Animal health

PATHOSENSE AS A DIAGNOSTIC TOOL TO UNRAVEL PCV2 – PORCINE PARVOVIRUS COINFECTIONS

L. Allais[1], C. Bonckaert[1], E. Van Driessche[1], N. Botteldoorn[1], J. Schaep[1], H. Nauwynck[2], S. Theuns[3], T. Vanblaere[1]

[1]*DGZ Vlaanderen (Animal Health Care Flanders), Belgium*
[2]*Laboratory of Virology, Faculty of Veterinary Sciences, Ghent University, Belgium*
[3]*PathoSense BV, Belgium*

Introduction
PathoSense is a diagnostic tool to identify pathogens. Porcine circovirus type 2 (PCV2) causes porcine circovirus- associated disease. An increase in PCV2 viral loads is reported. The clinically most important porcine parvovirus (PPV) is PPV1. However, little is known about PPV2-7. Research data show that co-infection with PPV may underlie the rise in PCV2 load.

Materials and Methods
Pig serum samples collected July 22-June 23 were pooled to 5 and analyzed by qPCR for PCV2. PCV2+ samples were selected for viral & bacterial metagenomic sequencing (Oxford Nanopore Technologies) at PathoSense. Group 1: PCV2
<10^4 genome copies/ml ; group 2: 10^5-7; group 3: >10^8. In this way, the PCV2 loads were linked to the detection of PPV coinfections.

Results
Samples with a viral PCV2 load of $<10^3$ genome copies/ml (group 1) were tested negative for PPV. Sequencing by PathoSense confirmed the presence of PCV2 in the samples in group 2 (10^{5-7} PCV2 genome copies) and 3 (10^{8-10} PCV2 genome copies) and also at least 1 PPV type. In total, 5 different types of parvoviruses (not PPV1) were detected by PathoSense: PPV2, 3, 4, 5 and 7. Increase of total PPV in samples with a high PCV2 load was observed.

Discussion and Conclusion
We conclude that PathoSense is a reliable diagnostic tool for detection of PCV2 and PPV coinfections. Furthermore, we found evidence that different PPVs can be found in association with high PCV2 loads.

References
Ouyang, Co-Infection of Swine with Porcine Circovirus Type 2, Viruses 2019 Feb 21
Theuns S., Nanopore sequencing as a revolutionary diagnostic tool. Scientific Reports, 2018, 8:9830

P004

Animal health

THE MOLECULAR EPIDEMIOLOGY OF FELINE PARVOVIRUS INFECTIONS IN CATS

T. Fedai[4], C.U. Aslan[5], G. Acar[2], F. Dogan[3], S. Bilge Dagalp[1]

[1]*Ankara University, Faculty of Veterinary Medicine, Department of Virology, Ankara-Turkey*
[2]*Atatürk University, Faculty of Veterinary Medicine, Department of Virology, Erzurum-Turkey*
[3]*Mustafa Kemal University, Faculty of Veterinary Medicine, Department of Virology, Hatay -Turkey*
[4]*The Graduate School of Health Sciences, Department of Virology, Ankara University, Ankara-Turkey*
5The Graduate School of Health Sciences, Department of Virology, Ankara University, Ankara-Türkiye

Introduction

Feline panleukopenia, caused by feline parvovirus (FPV) or canine parvovirus 2 (CPV-2), is a highly contagious and fatal disease in cats. High genetic similarity between FPV and CPV-2 complicates diagnosis. (2) This study aims to determine the prevalence and genetic characterization of parvovirus infection in cats in Ankara, Turkey, including various clinical and vaccination statuses.

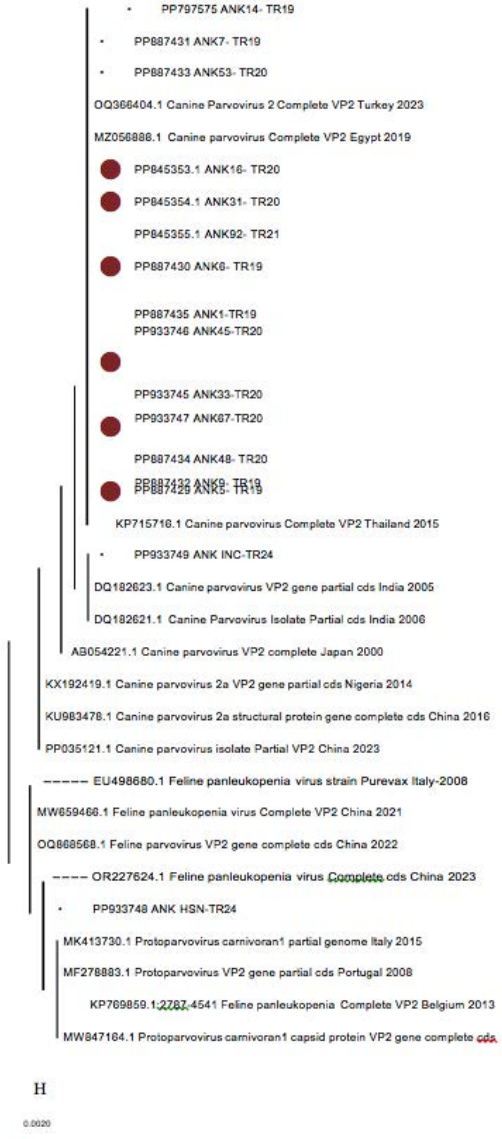


Figure 1. Phylogenetic Tree of Positive FPV Samples.

No	Cat no.	Clinical signs	Age	Gender	Positive sample	Vaccination/ Sampling date	FPV PCR	Other Agents PCR
1	ANK1 (SARI)	URD* Conjunctivitis	0-1	Male	Nasal Swab	-/ December 2019	+	FCV
2	ANK4 (CHRİSTİNA)	HA**	1-2	Female	Ocular and Nasal Swab	July 2018/ December 2019	+	FCV
3	ANK5 (SİS)	URD, Conjunctivitis, Stomatitis	-	Female	Oral Swab	July 2019/ December 2019	+	FCV
4	ANK6 (SARI-BEYAZ)	URD	0-1	Male	Nasal Swab	Single dose / December 2019	+	FCV
5	ANK7 (TEKİR)	HA	0-1	Male	Ocular Swab	-/ December 2019	+	FCV
6	ANK9 (LOKUM)	HA	0-1	Male	Ocular, Nasal and Oral Swab	-/ December 2019	+	FCV
7	ANK11 (BADEM)	URD	0-1	Female	Nasal Swab	November 2019/ December 2019	+	-
8	ANK12 (KARBEYAZ)	HA	0-1	Female	Nasal Swab	December 2019/ December 2019	+	-
9	ANK14 (JUNIER)	URD Conjunctivitis, Stomatitis	≥ 3	Female	Nasal Swab	-/ December 2019	+	FCV
10	ANK16 (PONÇİK)	HA	≥ 3	Male	Nasal Swab	July 2019/ January 2020	+	FCV
11	ANK31 (TARÇIN)	Stomatitis	≥ 3	Male	Ocular and Nasal Swab	-/ January 2020	+	FCV
12	ANK33 (KAMİ)	HA	0-1	Female	Ocular Swab	-/ January 2020	+	-
13	ANK45 (ŞERO)	URD	≥ 3	Male	Oral Swab	-/ January 2020	+	-
14	ANK47 (GÜNEŞ)	HA	0-1	Female	Ocular Swab	November 2019/ February 2020	+	-
15	ANK 48 (ZUZU)	HA	0-1	Female	Oral Swab	-/ February 2020	+	-
16	ANK50 (OSMAN)	HA	≥ 3	Male	Ocular and Nasal Swab	August 2019/ February 2020	+	FCV
17	ANK52 (RAMSES)	HA	2-3	Male	Ocular and Nasal Swab	April 2017/ February 2020	+	-
18	ANK53 (GECE)	HA	≥ 3	Male	Oral Swab	August 2019/ February 2020	+	-
19	ANK61 (İNCİ)	HA	2-3	Female	Oral Swab	June 2019/ March 2020	+	-
20	ANK62 (PERA)	HA	0-1	Male	Oral Swab	July 2020/ March 2020	+	-
21	ANK63 (KEDİŞ 2)	HA	2-3	Female	Oral, Nasal and Ocular Swab	June 2020/ March 2020	+	-
22	ANK 67 (BAMBAM)	HA	≥ 3	Male	Nasal Swab	February 2020/ September 2020	+	-
23	ANK71/99 (UMUT)	HA	≥ 3	Male	Nasal Swab	June 2017/ September 2020	+	FCV
24	ANK92 (KARAM)	Conjunctivitis	0-1	Female	Oral Swab	-/ April 2021	+	FCV
25	ANK111 (ZEYTİN)	Conjunctivitis, Stomatitis	0-1	Female	Nasal Swab	-/ Jamuary 2022	+	FCV

Table 1. The information of all FPV positive cats and coinfections

*URD Upper respiratory disease
**HA Healthy Appearence
***NI: No information
****The sampling was done before vaccination

Table 1. Information of all FPV positive cats and co-infections with Feline calicivirus

Materials and Methods

192 samples, comprising conjunctival, nasal, oral swabs, and EDTA-containing blood samples, were collected from 68 cats and were subjected to DNA extraction for FPLV nucleic acid detection. Previously designed primers for VP2 gene were used for PCR analysis.(1) One of the positive samples from each positive animal underwent sequence analysis. Sequences were compared with known strains in GenBank, using Aliview. Phylogenetic analysis using MEGA X was done to determine the genetic similarity between the obtained virus sequences and those from previous studies.

Results

Of 68 cats, 27 (39.71%) tested positive for FPV. Among the positive cases, 55.56% were vaccinated, and 44.44% were unvaccinated. Clinically, 36% of the cats showed signs of infection, while 64% were healthy-appearing. Coinfections were observed in 52% of the cats. Sequence analysis indicated that all positive swab samples were closely related to CPV-2b strains, showing high genetic similarity to each other and strains from Egypt and Turkey. Two PCR-positive blood samples were identified as CPV-2a and FPLV. The genetic analysis revealed a significant difference between our blood and swab samples.

Discussion and Conclusion
The study underscores the significance of molecular surveillance in understanding parvovirus epidemiology and cross-species transmission. The detection of CPV-2b and CPV-2a in cats highlights the potential for cross-species transmission and the role of asymptomatic carriers in virus dissemination. Comprehensive diagnostics and continuous surveillance are essential to manage parvovirus risks, especially in mixed-species environments. Despite vaccination efforts, infection rates remain high, emphasizing the need for stringent hygiene practices and improved vaccination strategies.

References
1. Buonavoglia, C. et al., (2001). The Journal of general virology, 82(Pt 12), 3021–3025.
2. Hoelzer, K. et al., (2008). The Journal of general virology, 89(Pt 9), 2280–2289.

P005

Animal health

SURVIVAL AT +4°C, +20°C AND +37°C OF ITALIAN GENOTYPE I AND II AFRICAN SWINE FEVER STRAINS

R. Biccheri[1], M. Pela[1], A. Felici[1], C. Maresca[1], F. Feliziani[1], M.S. Beato[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" (IZSUM), Italy*

Introduction
African swine fever (ASF) is a viral disease affecting domestic and wild pigs, causing a haemorrhagic fever-like disease with high lethality and belong to the *Asfarviridae* family, *Asfivirus* genus. ASF is a major threat to animal health with high economic impacts to the swine industry worldwide. It is transmitted through direct and indirect contacts with infected animals and fomites. The disease spread is a function of the virus resistance to temperature and pH. The absence of vaccines directed research towards the field of vaccinology and studies on virus resistance remained a marginal topic.The present study aimed at generating data on survival of italian ASFV of genotype I and II.

Materials and Methods
The laboratory-adapted genotype I as a reference strain, a genotype I virus isolated in Sardinia in 2008, and a genotype II strain isolated in wild boar in Italy in 2022, were exposed at +4°C, +20°C, and +37°C and tested at day 0, 7 and 15. For each exposure time, 3 independent aliquots were tested for residual infectivity by virus titration on cell cultures according to the Spaerman-Karber formula. A two-way ANOVA test and Post hoc analysis was run to study the effect of temperature and time-points on viral titres. Significance levels were adjusted by the Bonferroni correction.

Results
All ASFV were infectious at 15 days at +4°C and +20°C. A difference was observed at +37°C: at 15 days only the genotype I ASFV was completely inactivated. The decrease in virus titres at +37°C was statistically significantly dependent on the strain and the time; differences were detected at each time points for genotype I, between 7 and 15 days for the genotype II and between 0 and 7 days for the laboratory adapted ASFV. Comparing survival curves of each ASFV, a statistically significant difference was observed among temperatures and time points, indicating a higher survival rates at +4°C and +20°C than +37°C.

Discussion and Conclusion
In conclusion, this study partially confirms literature data on ASFV prolonged survival at temperature, suggesting a potential difference between genotype I and II that must be further investigated testing additional ASFV strains. Such studies are pivotal to understand the role of survival characteristics on ASFV transmission.

P006

Animal health

TYPING OF APEC ISOLATES REVEALED AN OUTBREAK OF BROILER COLIBACILLOSIS CAUSED BY ST23 O78:H4 IN FINLAND

M. Biström[1], T. Lienemann[1], T. Pohjanvirta[1], P. Vennerström[1]

[1]*Finnish Food Authority, Finland*

Introduction
Colibacillosis, a major bacterial disease in poultry, is caused by avian pathogenic *Escherichia coli* (APEC). In Finland, a colibacillosis outbreak occurred in 2021, impacting numerous flocks across the three major broiler companies in the country.

Materials and Methods
A comprehensive analysis involved 91 broiler flocks, with a median of six birds per flock subjected to necropsy. The average age of birds was 22 days. Inner organs and bone marrow were subjected to bacteriological cultivation. The *E. coli* isolates, primarily isolated from bone marrow underwent PCR typing for phylogeny group and eight virulence- associated genes.

Results
Necropsies unveiled classical colibacillosis indicators such as polyserositis, cellulitis, and femoral head necrosis. The *E. coli* typing results revealed a sudden surge in a specific APEC type belonging to phylogeny group A, with virulence genes cva, irp2, iss, IucD, and tsh. Out of 217 typed isolates, 165 (76%) belonged to this type. Subsequent whole genome sequencing (WGS) analysis of 25 single isolates of this type, representative of all three companies and both parent and broiler flocks, revealed that all isolates belonged to sequence type 23 (ST23) and serotype O78:H4. Core genome multilocus sequence typing (cgMLST) demonstrated high genetic similarity with allele differences ranging from 0 to 13 (mean 5).

Discussion and Conclusion
This study underscores that certain APEC strains with high virulence potential can trigger colibacillosis outbreaks affecting multiple farms. The epidemiological connection between affected farms was common grandparents. Vertical transmission of the strains in the broiler pyramid is evident, supported by the small allele differences revealed by cgMLST. PCR typing is a cost-effective and reliable method for detecting APEC strains responsible for colibacillosis outbreaks. Typing isolates serves as an initial step in confirming outbreaks and can be crucial in disease prevention by incorporating the strain into autogenous vaccines for broilers.

References
1. Clermont, O. et al: Rapid and Simple Determination of the *Escherichia coli* Phylogenetic Group. Appl. Environ. Microbiol. 66 (2000)
2. Ewers, C. et al: Rapid Detection of Virulence-Associated Genes in Avian Pathogenic *Escherichia coli* by Polymerase Chain Reaction. Avian Dis. 49 (2005)

P007

Animal health

APPLICATION OF NEXT GENERATION SEQUENCING (NGS) TECHNOLOGIES FOR THE IDENTIFICATION AND CHARACTERIZATION OF *APIS MELLIFERA* MICROBIOTA IN ITALY

F. Bordin[8], A. Peruzzo[8], G. Zamperin[8], M. Bertola[8], E. Palumbo[8], A. Milani[8], M. Orsini[8], A. Fusaro[8], P. Mogliotti[3], M.P. Cerioli[4], G. Formato[1], L. Ricchiuti[9], A. Cerrone[2], P. Troiano[5], A. Salvaggio[7], A. Pintore[6], F. Mutinelli[8], A. Granato[8]

[1]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, Roma (Capannelle), Italy*
[2]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy*
[3]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Asti (AT), Italy*
[4]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia (BS), Italy*
[5]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia (FG), Italy*
[6]*Istituto Zooprofilattico Sperimentale della Sardegna Sassari (SS), Italy*
[7]*Istituto Zooprofilattico Sperimentale della Sicilia, Catania (CT), Italy*
[8]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy*
[9]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Lanciano (CH), Italy*

Introduction
A metagenomic approach based on Next-Generation Sequencing (NGS), already used to characterize the microbiome of various organisms as well as to research pathogens involved in honey bee colony losses and mortality events, could provide new information on the communities of microorganisms, including pathogens, in honey bees within the Italian territory, integrating the data currently available.

Materials and Methods
Honey bees were collected from 19 apiaries located in 16 Italian regions.
DNA and RNA extracted from pools of 30 honey bees per apiary were used for:
- detection of known pathogens (viruses, fungi and trypanosomatids) through molecular assays;
- metagenomic analysis of the bacterial community through amplification and sequencing of the V3-V4 hypervariable regions of 16S rRNA gene using Illumina sequencing technology; bioinformatic analysis with DADA2 package and statistical analysis with phyloseq package, both in RStudio software;
- metagenomic analysis of the viral community through sequencing of RNA using Illumina sequencing technology; bioinformatic analysis with DIAMOND and MEGAN software.

Results
Vairimorpha apis as well as IAPV and KBV viruses were not detected in all apiaries. All samples tested positive for SBV and BQCV virus-

es, while the detection of other pathogens varied (Table 1). Honey bee bacterial communities showed no significant difference in composition and were mainly composed of a core of bacterial taxa, with the *Lactobacillus*, *Gilliamella*, and *Snodgrassella* genera being the most represented (Figure 1). Metagenomic analysis of the viral community revealed the presence of both known viruses and viral species never previously investigated in Italy. Their presence was further confirmed by newly developed RT-PCR protocols and Sanger sequencing (Table 1).

Discussion and Conclusion

In this study, conducted within the RC IZSVE 09/20 project and funded by the Italian Ministry of Health, the bacteriome of honey bees collected from different Italian regions appeared almost unchanged among apiaries, despite the heterogeneity of habitat, territory, climate, and diet. The detection of new viral species contributed to increasing the current available epidemiological data and developing diagnostic molecular protocols, enhancing the NGS data.

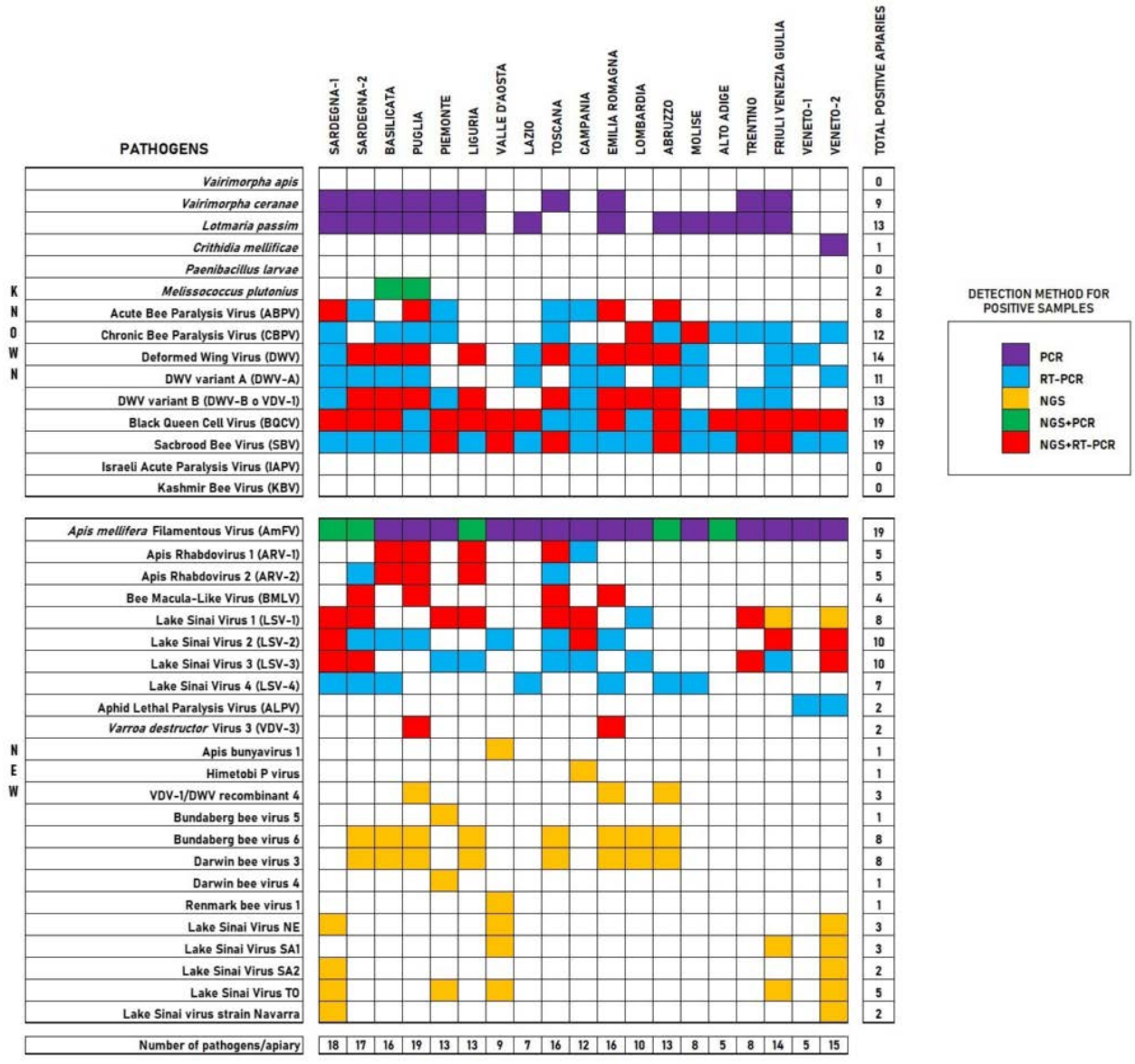


Table 1. Known and new pathogens of A. mellifera detected in 19 apiaries across 17 different Italian regions using PCR (violet), RT-PCR (blue) and NGS (yellow). Some of them were first identified with NGS and then confirmed by PCR (green) or RT-PCR (red) and Sanger sequencing of amplification product. The number of positive apiaries for each pathogen and the number of pathogens detected per apiary are shown.

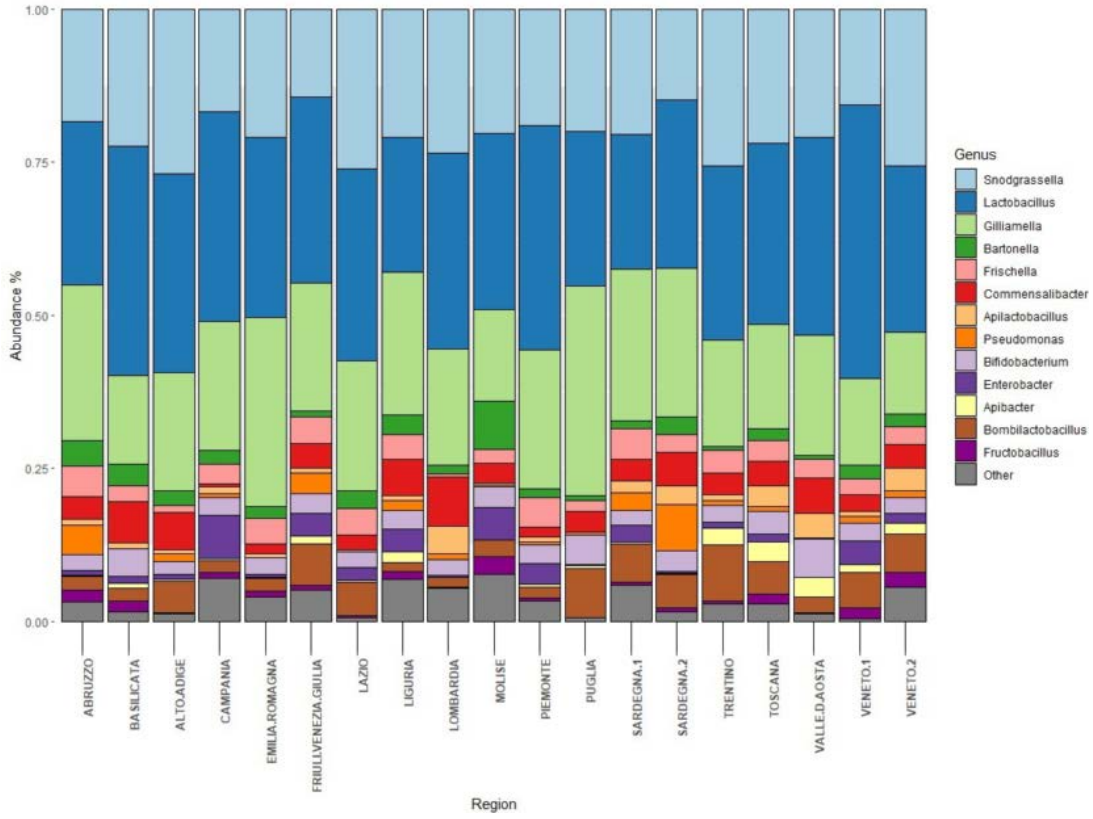


Figure 1. Taxonomic composition of the *A. mellifera* microbial community of 19 apiaries from 17 different Italian regions. The barplots represent the relative abundance (expressed as a percentage) of the bacterial genera detected in at least the 5% of apiaries and whose number of total counts was >20.

P008

Animal health

WHOLE GENOME SEQUENCING AND CHARACTERIZATION OF ANTIBIOTIC RESISTANCE GENES IN *BRUCELLA* STRAINS ISOLATED FROM WATER BUFFALO IN SOUTHERN ITALY DURING 2019 - 2021: A PRELIMINARY STUDY

M.G. Riccardi[1], R. Paradiso[1], A. Rinaldi[1], B. Cecere[1], A. Cerrone[1], F. Bove[1], G. Fusco[1], G. Borriello[1]

[1]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy*

Introduction

Brucellosis is a zoonosis caused by *Brucella* spp. infecting several animal species including humans. The pathogen can be transmitted through direct contact with infected animals or consumption of contaminated raw milk or dairy products. Antibiotic treatment of *Brucella* is hindered by poor intracellular spread and development of resistance. To date, no standard antimicrobial testing protocol is available and few data on resistance to antibiotics are reported in the literature. The aim of this study was to characterize by Whole Genome Sequencing (WGS) *Brucella* strains from water buffaloes in order to perform epidemiological trace-back studies.

Materials and Methods

40 pure *B. abortus* bv.1 colonies were isolated from lymph nodes of water buffaloes from 39 different farms in the Campania Region, as part of the diagnostic and prophylactic activities during the three-years period 2019-2021. All strains were processed for WGS analysis for in silico MLVA and MLST typing, determination of virulence and antibiotic resistance genes.

Results

Tested strains exhibited a unique sequence type (ST1) and 4 MLVA genetic profiles. All strains showed the presence of the main genes involved in the mechanisms underlying the pathogenicity of this bacterium. The study of antibiotic resistance genes by WGS allowed detection of mprF, bepG, bepF, bepC, bepE and bepD genes in all isolates.

Discussion and Conclusion

All the strains under study exhibited two AMR genes, multiple peptide resistance factors (mprF) and the outer efflux membrane proteins Bep (G, F, C, E, D), consistently with previously reported data (4). The mprF gene is involved in antibiotic cation resistance such as gentamicin, moenomicin and vancomycin while Bep proteins increase resistance to certain antibiotics such as tetracycline and doxycycline. Our data indicate a very high homogeneity of the analyzed *Brucella* strains and suggest the persistence of a few bacterial strains in close geographical areas, characterized by intense inter-human and commercial exchanges.

References

1. De Massis F et al. PLoS One. 2019 Mar 22;14(3):e0213689.
2. Carvalho Neta AV et al. Vet J. 2010 May;184(2):146-55.
3. Borriello G et al. Appl Environ Microbiol. 2013 Feb;79(3):1039-3.
4. Dadar M et al. Pathogens. 2023 Jan 3;12(1):82.

Sequence Type: *1*

Locus	Identity	Coverage	Alignment Length	Allele Length	Gaps	Allele
aroA	100	100	565	565	0	aroA_1
cobQ	100	100	422	422	0	cobQ_1
dnaK	100	100	470	470	0	dnaK_2
gap	100	100	589	589	0	gap_2
glk	100	100	475	475	0	glk_1
gyrB	100	100	469	469	0	gyrB_1
int_hyp	100	100	430	430	0	int_hyp_1
omp25	100	100	490	490	0	omp25_1
trpE	100	100	486	486	0	trpE_3

Table 1. All strains analyzed show a unique sequence type by MLST analysis.

Virulence and Pathogenicity Factors	Related Genes
LPS (lipopolysaccharide) pathogenicity factors, entry, intracellular survival and immunomodulatory	acpXL, fabZ, gmd, htrB, kdsA, kdsB, lpsA, lpsB/lpcC, lpxA, lpxB, lpxC, lpxD, lpxE, manAoAg, manCoAg, per, pgm, pmm, wbdA, wbkA, wbkB, wbkC, wboA, wbpL, wbpZ, wzm, wzt
Type IV secretion system effector secretion	virB1, virB2, virB3, virB4, virB5, virB6, virB7, virB8, virB9, virB10, virB11, virB12
TIR domain-containing protein immune evasion	btpA, btpB
Rab2 interacting conserved protein A intracellular survival	RicA
CβG (cyclic β-1,2 glucan) intracellular survival	Cgs

Table 2. All strains show the presence of the main genes involved in the mechanisms underlying the pathogenicity of *Brucella*.

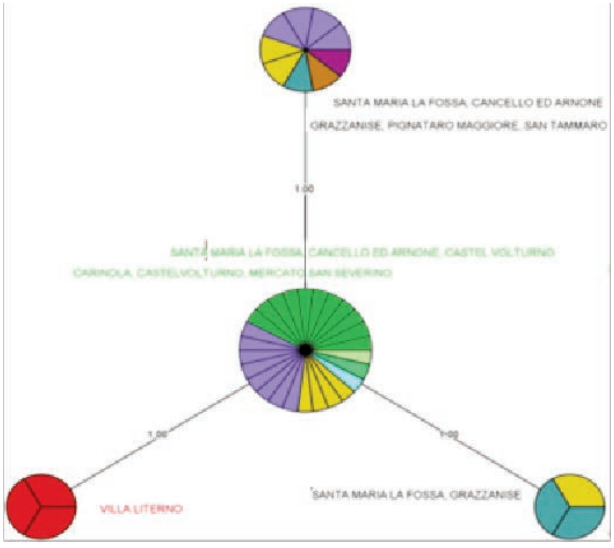


Figure 1. MLVA analysis shows 4 distinct genetic profiles distibuted in the Campania region

P009

Animal health

PREVALENCE OF *STREPTOCOCCUS EQUI* SUBSP. ZOOEPIDEMICUS IN MARTINA FRANCA AND ANDALUSIAN DONKEY POPULATIONS

M. Burgio[1], G.G. Aiudi[1], M.S.M. Yusuf[1], J.V. Delgado Bermejo[2], C. Rivas López[2], A. Carbonari[1], M.S. Lucente[1], V. Landi[1], M. Corrente[1], M. Tempesta[1], G. Greco[1]

[1]*Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano, Bari, Italy*
[2]*University of Córdoba, Faculty of Veterinary Sciences, Department of Genetics, Córdoba, Spain*

Introduction

Streptococcus equi subsp. zooepidemicus (SEZ) is a common saprophytic component of genital mucosa of stallions often associated with different diseases including septicemia, mastitis and endometritis in mares [1]. To date, there are no reports describing the presence and impact of SEZ on the reproductive tract of donkeys. This study aims to investigate the occurrence, antimicrobial susceptibility and risk factors associated with the presence of SEZ in the genital tract of donkeys.

Materials and Methods

From March 2023 to June 2024, a cross-sectional study, investigating 73 (60.8%) Martina Franca and 47 (39.2%) Andalusian healthy donkeys from Italy and Spain, was performed. All the animals were reared through a semi-extensive system. In details, 106 vaginal (88.3%) and 14 preputial (11.7%) swabs were collected and screened for the occurrence of SEZ by using culture (Edward's modified medium) and molecular (cPCR targeting 16S rRNA region) [2] methods. All isolates were tested for their susceptibility to 13 antimicrobials, belonging to 6 different classes by using disk diffusion method (Table 1).

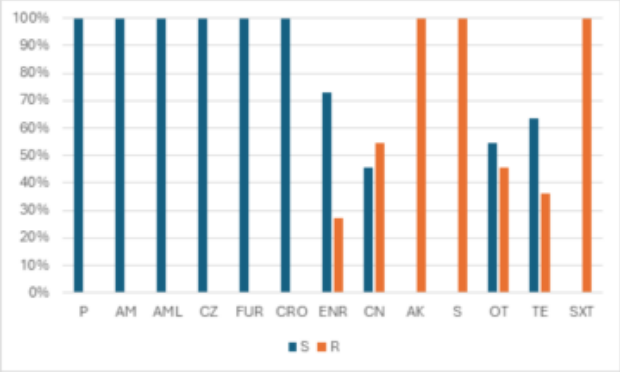


Table 1. Antimicrobial susceptibility profile.

Results

An overall prevalence of 9.17% (CI 95%: 4-14) was recorded with farms without biosecurity measures adoption being at higher risk of infection (OR 5.35, CI 90%: 0.92-30.99), while no difference for animal origin, age and gender, were recorded. The total antimicrobial resistance rate was observed for amikacin, streptomycin and sulfamethoxazole- trimethoprim (100%). Furthermore, decreased antimicrobial susceptibility was observed for gentamicin (55%), tetracyclines (up to 40%) and enrofloxacin (27%), while full susceptibility was recorded for all β-lactams and cephalosporines.

Discussion and Conclusion

This study represents the first report on SEZ infection in healthy donkeys, mainly from farms without a biosecurity plans, from different European Countries. These data highlight a possible role of donkey as reservoir of SEZ, stressing the need of control measures also considering its zoonotic potential [3]. Further studies are required to elucidate connections with reproductive pathophysiology of donkey.

References

1. Kim M. et al. (2022). https://doi.org/10.1111/zph.12895
2. Alber J. et al (2004). https://doi.org/10.1111/j.1439-0450.2004.00799.x
3. Rasmussen C. D. et al. (2013) https://doi.org/10.1186/1297-9716-44-26

P010

Animal health

DEVELOPMENT OF A DIAGNOSTIC PIPELINE FOR EQUINE PAPILLOMAVIRUSES

P. Capozza[1], A.A. Omar[1], F. Pellegrini[1], V.I. Vasinioti[1], A. Salvaggiulo[1], L. Occhiogrosso[1], G. Patruno[1], P. Pinto[3], G. Diakoudi[1], M. Camero[1], N. Decaro[1], A. Alberti[2], G. Lanave[1], V. Martella[1]

[1]*Department of Veterinary Medicine, University of Bari, Italy*
[2]*Department of Veterinary Medicine, University of Sassari, Italy*
[3]*Servizio di Igiene degli Alimenti di Origine Animale, ASL, Bari, Italy*

Introduction
Papillomaviruses (PVs) are DNA viruses with oncogenic potential and vaccines have been licensed for the prevention of PV infection in humans. Equine aural plaques (EAPs) are associated with *Equus caballus* papillomavirus (EcPV) infection. At least ten EcPV types have been described thus far. However, only EcPV-1, -3, -4, -5, and -6 have been detected from EAPs (1,2). In the present study, we developed a diagnostic pipeline for the identification of EcPVs in horses affected by EAPs.

Materials and Methods
Samples were collected from the internal pinnae of 22 horses, displaying both unilateral (n=19) and bilateral (n=3) lesions. DNA extracts from 25 EAP samples were screened using three pan-papillomavirus PCR protocols (targeting the L1 and E1 genes) and the amplicons were sequenced. Samples of interest were enriched with rolling circle amplification (RCA) using a sequence-independent protocol and sequenced with Oxford Nanopore Technologies (ONTTM) platform. All samples were re-screened using qPCR assays specific for the various EcPVs types.

Results
Overall, 72% (18/25) of the samples were positive for EcPVs by pan-papillomavirus PCRs. Sequencing of the amplicons identified EcPV-6 (8/18; 44.4%), EcPV-4 (5/18; 27.8%), and EcPV-3 (5/18; 27.8%) as the most common types. Whole genome sequences of 16 EcPV strains were generated. Nine strains showed high nucleotide (nt) identity to known EcPVs (EcPV-3, EcPV-4, EcPV-6), and seven strains were classified into five novels putative EcPV types. By qPCR, the infection rate reached 92% (23/25), with 91.3% of EcPV-positive samples being co-infections of different types.

Discussion and Conclusion
To date, 15 species of PVs have been reported to infect horses, including three bovine PVs, two donkey PVs, and ten EcPVs (1). Based on our diagnostic pipeline, at least 5 additional types of PVs are present in horses. Epidemiological investigations are required to assess the epidemiological and clinical relevance of these novel EcPVs.

References
1. Bromberger CR et al., Detection of Equus caballus Papillomavirus in Equine Aural Plaque Samples. J Equine Vet Sci. 2023 Sep.
2. Turco S et al. Genetic Characterization of a novel Equus caballus papillomavirus isolated from a thoroughbred Mare. Viruses. 2023 Feb 28.

P011

Animal health

ISOLATION AND CHARACTERIZATION OF SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* (STEC) IN A PIGLET FROM BASILICATA (ITALY)

M. Caruso[2], S. Castellana[2], L.M. Difato[2], G. Caldarola[2], A. Donatiello[2], A. Bianco[2], L. Capozzi[2], L. Del Sambro[2], V. Ianuzziello[1], A. Parisi[2], R. Fraccalvieri[2]

[1]*ASM Matera, Matera, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy*

Introduction
Porcine edema disease (ED) is an enterotoxaemia caused by Shiga toxin-producing *Escherichia coli* (STEC) that frequently occurs in 4-12 week-old piglets resulting in high mortality and economic losses to pig farming (1).

Materials and Methods
In a wild breeding farm of Basilicata, five of a group of 10 eight week-old piglets suddenly died showing swelling of eyelids and neurological signs (ataxia, trembling, lateral recumbency). Organs (gut, spleen, liver and kidney) from 1 piglet were collected and seeded on

Blood Agar in aerobiosis and anaerobiosis at 37°C for 48-72h. Moreover, detection of STEC was performed according to ISO 13136:2013, adapted to organs (2). *E. coli* strains were genotyped using Whole Genome Sequencing (WGS) (3) and antimicrobial susceptibility was evaluated by the Minimum Inhibitory Concentration (MIC) method according to the CLSI (4).

Results

Two *E. coli* strains were isolated from kidney (EC1) and gut (EC2). Results of MIC and WGS analysis of the 2 strains are shown in Tables 1 and 2 respectively.

Discussion and Conclusion

The isolation of two *E. coli*, one of which identified as a STEC and characterized by the presence of the two mayor typical virulence factors F18ab fimbriae and Stx2e confirms the role of this pathogen in the occurrence of porcine ED.

References

1. Casanova N., Redondo L.M., Dailoff G., Arenas D., Fernández Miyakawa M. "Overview of the role of Shiga toxins in porcine edema disease pathogenesis" Toxicon. 2018; 148:149-154.
2. UNI CEN ISO/TS 13136:2013 "Microbiology of food and animal feed.- Real-time polymerase chain reaction (PCR)- based method for the detection of food-borne pathogens.-Horizontal method for the detection of Shiga toxin- producing *Escherichia coli* (STEC) and the determination of O157, O111, O26, O103 and O145 serogroups"
3. Bianco A, Capozzi L, Monno MR, Del Sambro L, Manzulli V, Pesole G, et al. Characterization of *Bacillus* cereus Group Isolates From Human Bacteremia by Whole-Genome Sequencing. Front Microbiol. 2020;11: 599524
4. CLSI VET01S Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Edition 7, January 2024.

Antimicrobial class (Subclass)	Agent	Range µg/ml	E. coli	
			EC1	EC2
Penicillins	AMP - Ampicillin	0,25-32	S	S
β-lactam combination agents	AUG2 - Amoxicillin / Clavulanic acid	0,25/0,12-32/16	S	S
Aminoglycosides	AMS - Aminosidine	1-32	S	S
	GEN - Gentamicin	0,25-32	S	S
	KAN - Kanamycin	2-32	S	S
Phenicols	FFN - Florfenicol	1-64	S	I
Quinolones	FLUQ - Flumequine	1-16	S	S
Fluoroquinolones	ENRO- Enrofloxacin	0,015-32	S	S
Cephems (Cephalosporins I)	FAZ - Cefazolin	0,5-8	I	S
Tetracyclines	TET - Tetracycline	0,5-16	S	S
Sulfonamides	FIS - Sulfisoxazole	128-512	S	S
	SXT - Trimethoprim / Sulfamethoxazole	0,06/1,19-16/304	S	S
Lipopeptides (Polymyxins)	COL - Colistin	0,03-8	S	S
Cefalosporine III generazione	FOT - Cefotaxime	0,5-4	S	S

Table 1. Results of Minimum Inhibitory Concentration (MIC) test performed on EC1 and EC2.

	EC1	EC2	
Pathovar	E. coli - STEC	E. coli - EPEC	
Serotype	O139:H1	O51:H49	
Sequence Type	1	20	
Virulence factors			**Protein function**
stx2	✓ stx2e		shiga toxin 2 variant e B-subunit
eae		✓	intimin
eilA	✓		Salmonella HilA homolog
espF		✓	EPEC secreted protein F; Type III secretion system
fdeC	✓	✓	intimin-like adhesin FdeC
fedAab	✓		F18ab (F107) Major serotype specific fimbrial subunit A
fimH	✓	✓	Type 1 fimbriae
hlyA	✓		Hemolysin A
lpfA	✓		Long polar fimbriae
iss		✓	Increased serum survival
nleA		✓	Non-LEE encoded effector A
nleB		✓	Non-LEE encoded effector B
nleC		✓	Non-LEE encoded effector C
Antimicrobial resistance genes			
(Bla)AmpC1		✓	β-lactam resistance
(Bla)AmpC2_Ecoli	✓	✓	β-lactam resistance
(Bla)Penicillin Binding Prote	✓	✓	Penicillin Binding Protein in E. coli
mdf(A)_1	✓	✓	Multidrug efflux pump in E. coli
acrD	✓	✓	Aminoglycoside efflux pump expressed in E. coli
blaEC	✓		BlaEC family class C beta-lactamase

Table 2. Pathovar, serotype, Sequence Type, virulence factors and antimicrobial resistance genes of EC1 and EC2 detected by WGS analysis.

P012

Animal health

ATYPICAL PORCINE PESTIVIRUS IN WILD BOAR IN CENTRAL ITALY: PRELIMINARY RESULTS.

C. Casciari[2], C. Iscaro[2], S. Pirani[2], E. Tinelli[2], E. Scoccia[1], C. Colabella[3], C. Maresca[1], A. De Giuseppe[3], M. Giammarioli[2]

[1]Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", Epidemiological Observatory, Perugia, Italy
[2]Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", National Reference Laboratory for Pestivirus and Asfivirus, Perugia, Italy
3Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", Pharmaceutical Unit, Perugia, Italy

Introduction
Atypical Porcine *Pestivirus* (APPV), firstly discovered in USA in 2015, was reported in Europe, America and Asia, both in pigs and wild boars (1). A virological screening and a molecular characterization are performed in central Italy to investigate the presence of APPV genome in wild boar population. Laboratory results and population data are compared, to estimate the infection prevalence and the dynamics of disease maintenance/persistence/diffusion in this population.

Materials and Methods
Spleens from found dead wild boar are sampled. RNA extraction is performed by manual commercial kit and RT- PCR end-point is used for viral detection (2). Positive samples undergo Sanger sequencing for NS3, products are run on a genetic analyzer, sequences data are analyzed, aligned, edited and submitted in GeneBank. A descriptive analysis from enrolled wild boar populations considers parameters related to sampling (distribution of samples in time and space) and to individual animal sampled (age, sex, carcass status).

Results
231 wild boars carcasses were sampled from January to August 2023 in Umbria and Marche regions. Relating to carcasses data, 87% are fresh ones; 71% are road-killed wild boars; most of the animals are aged between 6-18 months (31%) and 18-30 months (28%); 47% were female wild boars. Submitted to screening, only 1 APPV positive sample was detected. It derives from a male fresh carcass, aged 6-18 months, road killed and found in January 2023 in Offagna municipality (AN). Following the partial sequencing and phylogenetic analysis of the NS3 portion, it clusters with viral strains already isolated from wild boar in Northern Italy (3).

Discussion and Conclusion
Based on these preliminary results, a low rate of virological positive samples is expected, even if an evaluation of infection prevalence has never been calculated in Italian wild boar populations. APPV positive wild boar was likely a persistently infected animal, according to the pathogenic features of the other *Pestivirus*es. Further positive samples are required to assess the epidemiological role of wild boar for this emerging disease.

References
1. https://doi.org/10.1136/vr.104824
2. https://doi.org/10.1111/tbed.12854
3. https://doi.org/10.3390/v11121142

P013

Animal health

ISOLATION OF A POTENTIALLY NOVEL MYCOPLASMA SPECIES RELATED TO *M. BOVIRHINIS* FROM DAIRY CATTLE IN NORTHERN ITALY.

M. Gastaldelli[3], A. Tondo[3], B. Colò[3], M. Picchi[3], E. Rinaldi[3], V. Righetti[3], A. Barberio[2], K. Qualtieri[2], K. Sulyok[1], M. Gyuranecz[1], M. Merenda[3], S. Catania[3]

[1]HUN-REN Veterinary Medical Research Institute, Hungária körút 21, Budapest, Hungary
[2]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy
[3]Mycoplasma Unit, WOAH reference laboratory for avian mycoplasmosis (M. gallisepticum, M. synoviae), SCT-1 Verona, Istituto Zooprofilattico Sperimentale delle Venezie, Buttapietra, Italy

Introduction
Mycoplasmas are small microorganisms frequently occurring as opportunistic pathogens in animals and due to their limited metabolic capacity, they can be fastidious to be cultured. Because of frequent genomic recombinations and the lack of proof-reading genes, mycoplasmas display high mutation rates potentially leading to emergence of new species (Nicholas at al, 2017). In this study we report the isolation of a *M. bovirhinis*-related strain in association with respiratory symptomps in a dairy farms in Northern Italy.

Materials and Methods
The animals presented a febrile state with pulmonary involvement, ruminal blockage and a decrease in milk production. Even though clinical signs improved after antimicrobial treatment, total recovery was not achieved. The analysis of nasal swabs for the common viral and bacterial forms involved in BRD yielded negative results. Therefore, further bacteriological investigations were conducted, which led to the isolation of a specific *Mycoplasma* strain.

Results
This was initially identified as *M. bovirhinis* by 16s-rDNA-PCR DGGE (denaturing gradient gel electrophoresis). However, sequence analysis of the genes 16s rRNA (1189 nt) and rpoB (1193 nt) revealed that the percent identity respect to *M. bovirhinis* was below 99% (16s rRNA) and 84% (rpoB). Such divergence was further observed both at the DNA and protein level by phylogenetic analysis of 16s rRNA and rpoB genes and by biotyping of the isolates with MALDI TOF MS. Finally, in order to assess distribution and prevalence of this strain, a specific primer pair for PCR was designed and utilized in a retrospective analysis on isolates collected over the last years.

Discussion and Conclusion
To conclude, these data suggest that this strain could belong to a different, novel species in potential association with respiratory clinical signs in cattle and underline the importance of culture and isolation to reveal the emergence of potentially new mycoplasma species.

References
Nicholas, R.A.J, Rosales, R. and Loria, G.R. (2017). Mycoplasmology: the big issues. Animal Husbandry, Anim Husb Dairy Vet Sci. 1(1). 1-4. 10.15761/AHDVS.1000102.

P014

Animal health

TRANSPLACENTAL TRANSMISSION OF *THEILERIA EQUI*

I. Ricci[2], A. Cersini[2], G.A. Marcario[2], R. Nardini[2], G. Manna[2], G. Brocherel[2], I. Congiu[2], S. Greco[2], C.A. Minniti[1], R. Conti[2], M.T. Scicluna[2]

[1]*Dipartimento per l'Organizzazione Sanitaria e Veterinaria - Comando Generale dell'Arma dei Carabinieri, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Italy*

Introduction
Tick-borne piroplasmosis, caused by *Babesia caballi* and *Theileria equi* (EP), affects equids and may result in reproductive disorders [1], including abortions that may be underestimated when investigating these conditions, as not they regularly included in differential diagnoses. Aim of the study is to improve knowledge on the vertical transmission of EP and subsequent cause of abortion and conduct laboratory analysis of mare and foetal samples to verify EP transplacental transmission.

Materials and Methods
Mare's blood sample and uterine swab, placenta, foetal brain, lung, spleen and liver were tested using direct and indirect methods for the diagnosis of principal viral and bacterial etiological agents causing abortions, including EP. In particular, for EP, a commercial competitive serological ELISA (VMRD®) and a real time PCR protocol, amplifying a fragment of the 18S rRNA V4 hypervariable region gene [2] were used. EP PCR products were sequenced using Genetic Analyzer Sequencing v5.4 (Applied Biosystems, Foster City). Sequencing alignment was performed using nucleotide Basic Local Alignment Search Tool (BLASTn) [2].

Results
T. equi was detected by PCR in the mare's blood, placenta and foetal organs examined. No significant positivity was obtained for other infectious agents investigated.

Discussion and Conclusion
Detection of *T. equi* in mare and foetal samples, excluding other infectious agents, further enforces its potential role as a cause of abortion in equids and should be included in a diagnostic panel for infectious agents responsible of abortion to better understand its role in reproductive disorders.

References
1. Leon A, Pillon C, Tebourski I, Bruyas JF, Lupo C. Overview of the causes of abortion in horses, their follow-up and management. Reprod Domest Anim. 2023 Sep;58 Suppl 2:93-101. doi: 10.1111/rda.14406. Epub 2023 Jul 12. PMID: 37312640.
2. Bartolome Del Pino LE, Meana A, Zini M, Cersini A. Evidence of transplacental transmission of equine piroplasms Theileria equi and Babesia caballi in an Italian breed mare. Folia Parasitol (Praha). 2023 Feb 10;70:2023.005. doi: 10.14411/fp.2023.005. PMID: 36960775.

P015

Animal health

ANTIMICROBIAL EFFICACY OF CINNAMON ESSENTIAL OIL AGAINST ENTERO-PATHOGENIC *ESCHERICHIA COLI* (EPEC) FROM RABBIT

E. Circella[1], G. Casalino[1], G. Bozzo[1], M. Schiavitto[2], A. Bove[1], D. Romito[1], V. Perna[1], F. D'Amico[1], A. Camarda[1]

[1]*Department of Veterinary Medicine, University of Bari, Italy*
[2]*Italian Rabbit Breeders Association, ANCI, Volturara Appula, FG, Italy*

Introduction
Entero-Pathogenic *Escherichia coli* (EPEC) strains are responsible for colibacillosis in rabbit. Until recently, antimicrobials were used to both treat and prevent the disease in farms. The interest in the potential use of natural substances in the prevention has increased. The aim of this study was to evaluate the potential antimicrobial efficacy of cinnamon essential oil (CEO) against EPEC strains.

Materials and Methods
Thirty EPEC strains from 30 rabbits died for colibacillosis in 4 industrial rabbit farms were tested. The efficacy of CEO concentrations ranging from 0.7 to 0.2 µL/mL and 0.6 to 0.1 µL/mL was evaluated against the bacteria tested at cell densities of 108 CFU/mL and 104 CFU/mL, respectively. MIC (minimal inhibitory concentration)50 and MIC90 values of CEO were obtained.

Results
The MIC50 and MIC90 values of CEO for strains analysed at the cell density of 108 CFU/mL were 0.5 and 0.6 µL/mL, respectively. Testing the strains at the cell density of 104 CFU/mL, MIC50 was 0.3 µL/mL while MIC90 was 0.6 µL/mL. Anyway, 0.2 µL/mL of CEO was still able to inhibit one strain.

Discussion and Conclusion
This study highlights the potential antimicrobial efficacy of cinnamon in the prevention of colibacillosis because it was efficient against *E. coli* strains tested at bacterial density of 104 CFU/mL that is the amount of *E. coli* found in the intestinal content of healthy rabbits. Also, CEO was able to inhibit the strains in cell density of 108 CFU/mL, corresponding to the amount detected in rabbits affected by colibacillosis. Therefore, although further investigation will be useful, this finding suggests the possibility to use CEO in the treatment of affected rabbit flocks, reducing the use of antibiotics aiming to protect animal health and the consumer of animal products.

References
Bassolé I.H., Juliani H.R. Essential oils in combination and their antimicrobial properties. Molecules 2012, 17, 3989– 4006
Casalino G., Dinardo F.R., D'Amico F., Camarda A., Lombardi R., Bozzo G., Dimuccio M.M., Bove A., Circella E. Antimicrobial Efficacy of Cinnamon Essential Oil against Avian Pathogenic *Escherichia coli* from Poultry. Animals 2023, 13, 2639.
Vasconcelos N.G., Croda J., Simionatto S. Antibacterial mechanisms of cinnamon and its constituents: A review. Microb. Pathog. 2018, 120, 198–203

P016

Animal health

USE OF RECOMBINANT E2 APPV PROTEIN IN ENZYME IMMUNOASSAY FOR SEROPREVALENCE STUDIES: PRELIMINARY DATA

C. Colabella[1], L. Anzalone[1], S. Pirani[1], E. Tinelli[1], M. Giammarioli[1], C. Iscaro[1], F. Feliziani[1], M. Cagiola[1], A. De Giuseppe[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, PG, Italy*

Introduction
APPV is a novel atypical porcine pestivirus first discovered in 2015 and is associated with congenital tremor in piglets [1,2]. APPV is a ssRNA/+ virus and the genome encodes for a single polyprotein, which is cleaved into 12 mature proteins, including the structural envelope proteins Erns, E1 and E2 [3]. To date, there are no commercial vaccines and validated diagnostic methods for APPV, and its epidemiology in domestic and wild populations needs to be further explored. The aim of this work was to develop an indirect enzyme immunoassay method based on the use of E2 recombinant protein (rE2) to improve the study of seroprevalence of APPVs.

Materials and Methods
E2 encoding gene was amplified and sequenced from porcine serum sample analysed in a previous study [4]. The production of the rE2 was performed on HEK-293 cells (prov. by GenScript) and verified by SDS-PAGE/Coomassie and Western blot. The antigenic properties of the rE2 were evaluated using four APPV antibody-positive porcine sera prov. by Dr. K.Wernike (Friedrich-Loeffler-Institut, Germany) and field sera. The indirect ELISA was finally performed using a Goat Anti-Pig IgG Ab-HRP.

Results
SDS-PAGE/Coomassie and Western blot revealed a 40 kDa band related to rE2 in fusion with Strep-TagII. APPV antibody- positive porcine sera showed high OD450nm values up to 1:25000 while field sera in the dilution range 1:200-1:1600.

Discussion and Conclusion
These preliminary results showed that the rE2 can be efficiently recognised by APPV antibody-positive sera. Further investigations are needed to confirm the performance of the assay through the use of a monoclonal antibody and the development of a competitive ELISA test that can be applied in APPV seroprevalence surveys.

References
1. Ma, H et al. 2022. Front. Microbiol., 13.
2. Arruda, B.L et al. 2022. J. Clin. Microbiol., 60(11), e00697-22.
3. Simmonds, P et al. 2017. J. Gen. Virol., 98(1), 2.
4. Iscaro, C et al. 2019. Large Anim. Rev., 25.

P017

Animal health

DEVELOPMENT OF AN INDIRECT ELISA TEST FOR THE DETECTION OF CIRCULATION OF EQUINE HEPACIVIRUS AND EQUINE PARVOVIRUS-HEPATITS IN PIEDMONT, ITALY

L. Brienza[2], R. Cardone[1], G. Memoli[2], I. Colasanto[2], G. Lanave[1], D. Avazato[2], S. Rosati[2], M. Bullone[2], G. Elia[1], B. Colitti[2]

[1]*Department of Veterinary Medicine, University of Bari, Italy*
[2]*Department of Veterinary Sciences, University of Turin, Italy*

Introduction
Equine Hepacivirus (EqHV) and Equine Parvovirus-hepatitis (EqPV-H) are recently discovered viruses associated with mild to severe liver pathology [1]. Although their circulation has been demonstrated worldwide, epidemiological information on their distribution in Italy is still limited [2]. The diagnosis mainly relies on the molecular detection of pathogens while a lack of serological assays is the main challenge for the serological investigations. EqPV-H antigenic protein VP1 is commonly used as a target for virus detection by PCR, but also for antibody detection methods [3] while EqHV NS3 protein is used as a target molecule in molecular and serology tests. In this context, the study aimed to develop an ELISA test able to detect antibodies against the two viruses and to explore their serological prevalence in Piedmont (Italy).

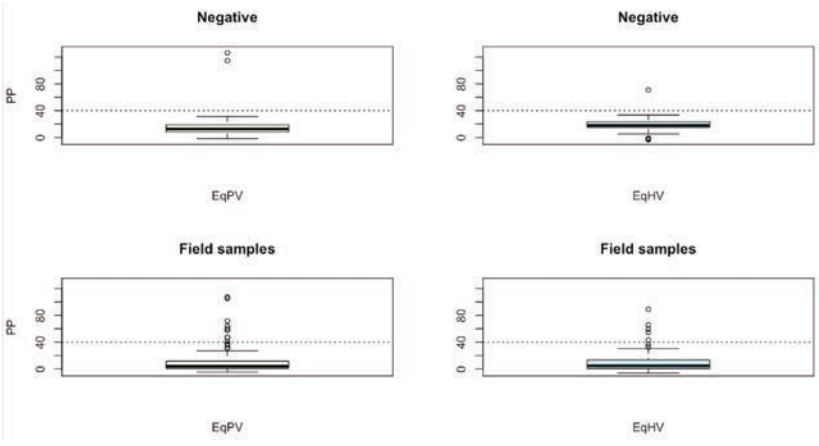


Figure 1.

Materials and Methods
The recombinant NS3 and VP1 antigens were produced via mammalian expression strategy, in a transient expression system based on high density, suspension-adapted, HEK cells. Performances of the prototype ELISA were evaluated with reference sera provided by the Dept. of Veterinary Medicine of Bari. Field serum samples from different Piedmontese stables were then tested.

Results
Test validation allowed us to set the method cut-off at 40% of the positive control. Out of 150 field samples tested, EqPV-H specific antibodies were detected in up to 10.91% of horses (95% CI, 5.08-16.74%), while a prevalence of 5.45% (95% CI, 1.21-9.70%) was detected for EqHV. No animals with co-infection were reported.

Discussion and Conclusion
The results of the serological investigation are consistent with those reported in previous studies in Italy and other European countries [2-3]. In conclusion, we detected the presence of antibodies against EqHV and EqPV-H and confirmed their circulation in Piedmont. Further analyses are needed to confirm our data and to deeply explore the epidemiology and the antibody response against these two viruses.

References
1. Pacchiarotti et al., Equine Hepacivirus: A Systematic Review and a Meta-Analysis of Serological and Biomolecular Prevalence and a Phylogenetic Update, Animals (2022).
2. Elia et al., Identification and Genetic Characterization of Equine Hepaciviruses in Italy, Vet. Microbiol. (2017).
3. Ramsauer et al., Equine Parvovirus Hepatitis, Equine Vet. J. (2021).

P018

Animal health

ENVIRONMENTAL EXPOSURE AND PET ANIMALS: RISK ASSESSMENT THROUGH THE STUDY OF EFFECT BIOMARKERS

F. Corrado[4], A. Iannuzzi[3], R. Pistucci[3], L. Vassallo[4], M.C. Cuomo[4], D. Sansone[5], A. Siciliano[2], G. Rofrano[2], J.L. D'Auria[2], D. Cardinale[1], A. Gallo[2]

[1]*Azienda Sanitaria Locale di Caserta - Dis. Matesino C Igiene allevamenti, Italy*
[2]*Centro di Referenza Nazionale per l'analisi e studio di correlazione tra ambiente, animale e uomo, Italy*
[3]*CNR - Istituto per il Sistema Produzione Animale in Ambiente Mediterraneo (ISPAAM), Italy*
[4]*Istituto Zooprofilattico Sperimentale del Mezzogiorno - "Studi clinici e Medicina Traslazionale" Coordinamento Ambiente, Salute e Relazione uomo-animale", Italy*
[5]*Istituto Zooprofilattico Sperimentale del Mezzogiorno - Dipartimento di Chimica, Italy*

Introduction
The aim of the study was to deepen the correlation between the environment and the presence of effect markers (1) (telomeric length) in pet animals, considering both healthy subjects and subjects suffering from oncological diseases, and assess any correlations between them. Considering the principle of One Health, which sees human health related to animal welfare and the health of the ecosystems in which we live, domestic animals can act as sentinels to identify environmental factors that are hazardous to humans and to prevent the development of certain cancers, since they often live in similar environments and are therefore subject to the same conditions. The study considers a sample of both healthy and healthy dog population affected by oncological diseases, living in different areas of the Campania Region. For the identification of the areas of enrollment of subjects, the environmental pressure index determined on the basis of a series of environmental and territorial variables considered significant for the preliminary analysis of the study was used. Research funded by the Italian Ministry of Health IZSME 13-20 RC

Materials and Methods
Subjects enrolled in these areas were subjected to telomere length analysis:
Extraction of DNA
Telomere length (LTL) evaluation using qPCR Statistical analysis

Results
Lymphoma patients showed a variability of T/S Ratio (0.68-0.7), while those with Breast Cancer showed a gradual decrease in CTR (0.77-0.62). The data suggest a possible correlation between age and tumour development, with the age group 5 appearing to be significant. Telomeres are significantly shortened in all cancer patients aged 4-7 years, indicating significant DNA damage in these subjects compared to controls.

Discussion and Conclusion
There are no significant differences in cancer or healthy patients aged 10 years or older. Further analysis will be needed to better understand the correlations between age, T/S Ratio and CTR and their impact on cancer progression, including control patients, and the results are consistent with what has recently been described in the literature and confirm the usefulness of telomere evaluation (elongation or shortening) in molecular epidemiology and biomonitoring studies.

References
1. Evaluation of bovine sperm telomere length and association with semen quality. Iannuzzi A. et all Theriogenology 2020

P019

Animal health

A POST MORTEM LABORATORY APPROACH FOR THE EVALUATION OF FREE-LIVING CATS HEALTH STATUS IN PADOVA PROVINCE (PRELIMINARY DATA)

E. Spagnolo[2], M.L. Moronato[1], R. Friso[1], P. Bassi[1], S. Varotto[1], A. Bortolini[1], A. Costa[1], M. Corrò[2]

[1]*AULSS 6 Euganea, Italy*
[2]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

Introduction

In the territory of competence of AULSS 6 there are a lot of free roaming cats and cat colonies officially registered managed by volunteers. From the sanitary point of view this population is monitored by The Urban Veterinary Hygiene Service with the application of Trap-Neuter-Return program. In this work we want to investigate the healthy status and the death cause in free-living cats to improve health management in cat colonies.

Materials and Methods

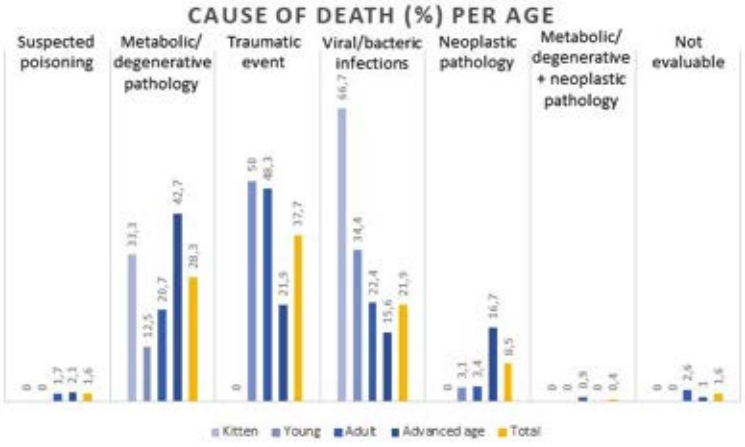
247 cats were collected dead or in precarious health conditions in the territory of AULSS 6 Euganea and delivered to the Public Veterinary Service, between January 2022 and March 2024. Necropsies were carried out on deceased or euthanized cats, laboratory exams were performed on organs and tissue: microbiological, molecular and histopathologic. Cats were grouped (Table1) according to their sex and presumed age: Young: ≤5 month's old: immature, absence of permanent dentition; Adults: 6 months-8 years old: definitive dentition and sexual maturity; Advanced age: >8 years old, based on dental wear and physical conditions; Kitten: newborn.

Sex / Age	Neutered male	Male	Female	Unknown
KITTEN	0	0	2	1
YOUNG	15	6	11	0
ADULT	49	13	54	0
ADVANCED AGE	36	8	50	2
Total				247

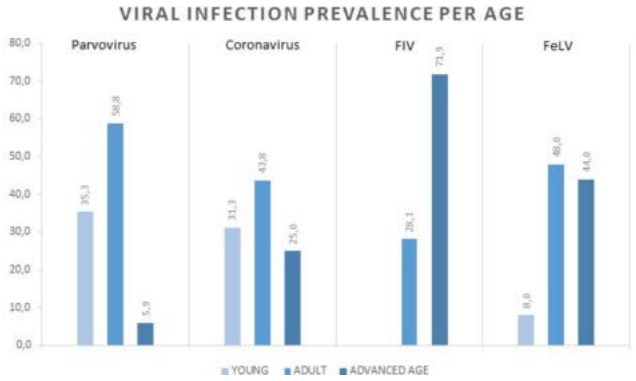
Table 1. Cat population examined. No distinction was made between sterilized female or not.

Results

The major cause of death is traumatic event 36.6% (58% male, 41.9% female). Other pathological finding such as metabolic/degenerative diseases 28.3% (47.2% male, 50.0% female, 2.9% unknown) and viral infections 21.9% (50% male, 48.1% female, 1.9% unknown) were observed (Graph 1). The prevalence of viral pathogens are summarized in Graph2.



Graph 1. Cause of death (%) per age.



Graph 2. Viral infections prevalence per age. Kitten are not shown (one positivity to herpesvirus)

Discussion and Conclusion

Most of male cats died for traumatic event. Metabolic/degenerative pathology were detected predominantly in elderly cats. Viral infection were detected even without no specific lesion

In conclusion, the post mortem evaluation of free living cats offers a preliminary view of the cause of death and orients the practitioner in deciding which diagnostic tests to perform. Moreover, as it is difficult to carry out a comprehensive serological screening of free-living cats over time, the gross pathology evaluation combined with the application of further in depth laboratory tests helps in understanding the circulation of the most relevant feline viral agents within ages and sex and in defining the health status of the population.

References

Grieco V et al. Causes of Death in Stray Cat Colonies of Milan: A Five-Year Report. Animals (Basel). 2021;11(11):3308 doi:10.3390/ani11113308

P020

Animal health

A RETROSPECTIVE STUDY ON THE PREVALENCE OF TOXIN-PRODUCING *ESCHERICHIA COLI* INFECTION IN CATS AND DOGS IN THE CAMPANIA REGION

G. De Luca[1], O. Valvini[1], N. Riccone[1], F. Del Piano[1], C. Auriemma[1], A. Gallo[1], F. Alfano[1], P. Izzo[1], G. Fusco[1], E. De Carlo[1], M.G. Lucibelli[1]

[1]*Istituto Zooprofilattico del Mezzogiorno, Italy*

Introduction

This retrospective study investigates the prevalence of toxin-producing *Escherichia coli* in cats and dogs in the Campania region, providing valuable insights into its epidemiology and potential public health implications. Toxin- producing *Escherichia coli* is an important cause of gastrointestinal in humans and domestic animals could be carriers of these pathogens by harboring the germ in the intestinal tract.

Materials and Methods

From 2017 to 2023, clinical samples from dogs and cats were collected and analyzed at the IZSM in Portici (Na). A total of 868 owned and stray animals were analyzed, including 446 cats and 422 dogs. Organ swabs from these animals were subjected to bacteriological examination to search for pathogens. The samples positive for *E.coli* in one or more organs were subjected to molecular investigation for the characterization of toxins.

Results

Out of a total of 868 animals analyzed, 46% tested positive for *Escherichia coli* in one or more organs through cultural examination. Molecular typing of these samples revealed 25% positivity for one or more virulence factors, specifically 14% tested positive for one or more genes encoding pathogenicity factors (CNF, CDT, LT, ST),11.1% tested positive for one or more virulence genes (VTX1, VTX2, EAE).

Discussion and Conclusion

These results underscore the importance of routine screening for toxin-producing *E. coli* in cats and dogs, especially in regions with high prevalence rates. This retrospective study reveals a notable prevalence of *Escherichia coli* infection among cats and dogs in the Campania region, with 46% of the analyzed animals testing positive. Among these, 25% harbored one or more virulence factors, indicating the importance of continuous monitoring to reduce the risk of transmission from pets to humans.

References

1. Detection and characterization of Shiga toxigenic *Escherichia coli* by using multiplex PCR assays for stx1, stx2, eaeA, enterohemorrhagic *E. coli* hlyA, rfbO111, and rfbO157- Paton et al. JCM 1998 Feb;36(2):598-602.
2. *Escherichia coli*: on-farm contamination of animals J.M. Fairbrother & É. Nadeau - Rev. sci. tech. Off. int. Epiz., 2006, 25 (2), 555-569
3. Infection by Verocytotoxin-Producing *Escherichia coli*t Mohamed A. Karmali-Clinical Microbiology Reviews, Jan. 1989, p. 15-38

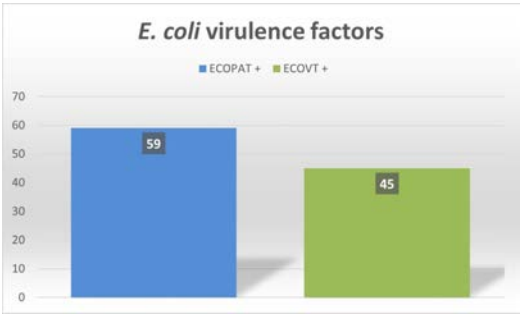


Figure 1. *E. coli* positivity.

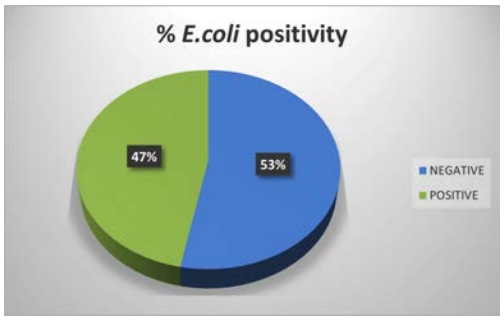


Figure 2. *E. coli* virulence factors.

***E. coli* virulence factors**

Virulence factors	Gene product	Activity
LT	Heat labile toxin	Alteration of the electrolyte content, adhesion factor
ST	Heat-stable toxin	Action on Na+ absorption, water imbalance
STX1	Shiga-toxin	Inhibition of protein synthesis, cell death
STX2	Shiga-toxin	Inhibition of protein synthesis, cell death
EAE	Intimina	Adhesion factor
CNF	Necrotizing cytotoxic factor	Exotoxins that induce cell death
CDT	Cytolethal toxin	Toxins that induce cell death

Table 1. *E. coli* virulence factors.

P021

Animal health

OCCURRENCE AND CHARACTERIZATION OF RHDVA STRAINS IN ITALY OVER 10 YEARS

V. Di Giovanni[1], C. Bertasio[1], A. Lavazza[1], L. Capucci[1], P. Cavadini[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy*

Introduction

Rabbit haemorrhagic disease virus (RHDV) is an RNA virus belonging to the family *Caliciviridae*, genus *Lagovirus,* causing severe hepatitis. In 1997, a variant (RHDVa) was identified, with genetic and antigenic differences compared to the classical strain circulating in Italy (RHDV BS89) (1). Although the new RHDV2 has replaced RHDV worldwide, RHDVa still occurs sporadically, especially in rabbit farms. This study aims to characterise some Italian RHDVa strains at both molecular and antigenic levels.

Materials and Methods

Fifteen RHDVa strains collected between 2013-2023 were amplified by RT-PCR and sequenced with Illumina Miniseq. Phylogenetic analysis of VP60 was conducted using MEGAX, and relations between sequences (implemented from GenBank) were estimated using the maximum likelihood method. The antigenic profiles of the strains were obtained with a sandwich ELISA using a panel of 16 specific anti-RHDVa monoclonal antibodies (MAbs) produced against the first RHDVa strain identified in Italy (GenBank EU250330.1). The absorbance value was transformed into reactivity percentage for each MAb after normalisation. Recombination events were investigated using RDP4 software.

Results
The phylogenetic tree showed that all 15 RHDVa VP60 sequences clustered within the branch with the RHDVa sequences deposited in Genbank. The branches differentiating this variant from RHDV and RHDV2 were supported by high bootstrap values (100). Otherwise, only one isolate showed a different profile using the MAbs panel at the antigenic level. In addition, no evidence of recombination with other known lagoviruses was detected.

Discussion and Conclusion
Based on this preliminary comparative analysis of nucleotide and amino acid sequences and the antigenic profile, it seems that RHDVa is relatively stable. The only exception is one strain identified in Foggia province, which does not react with the Mab 2B2. Finally, it remains to be clarified why RHDVa has not completely disappeared and how it sporadically reappears.

References
Capucci, L., Fallacara, F., Grazioli, S., Lavazza, A., Pacciarini, M. L., & Brocchi, E. (1998). A further step in the evolution of rabbit hemorrhagic disease virus: the appearance of the first consistent antigenic variant. Virus Research, 58 (1-2), 115-126.

P022

Animal health

A CASE OF *ERYSIPELOTHRIX RHUSIOPATHIAE*–ASSOCIATED SYSTEMIC INFECTION IN A WEIMARANER DOG

S. Di Nola[3], A. Mazzei[2], C. Calzolari[1], I. Radu[3]

[1]*Clinical Pathology unit, Pathology department, San Marco Veterinary Clinic and Laboratory, Veggiano, Italy*
[2]*Internal Medicine department, San Marco Veterinary Clinic and Laboratory, Veggiano, Italy*
[3]*Microbiology unit, Infectious diseases department, San Marco Veterinary Clinic and Laboratory, Veggiano, Italy*

Introduction
Erysipelothrix is a genus of ubiquitous Gram-positive rods historically associated with economically important farm animals' infections and occupational disease in humans. Its role as an emerging agent of endocarditis and systemic disease in dogs has been sporadically investigated.

Materials and Methods
An 8-year-old male Weimaraner was presented with a one-week history of lethargy, anorexia, polyuria/polydipsia, vomiting and diarrhea. The patient was fed a raw food-based diet, with meat and fish purchased locally and online. On physical examination, a heart murmur was noted. The patient underwent complete blood analysis, abdominal ultrasonography, echocardiography and spleen cytology. Aerobic and anaerobic cultures of a splenic specimen were performed, along with blood culture from two different samples using the BacT/ALERT®3D detection system and PF PLUS bottles. MALDI-TOF mass spectrometry was used to identify the isolates.

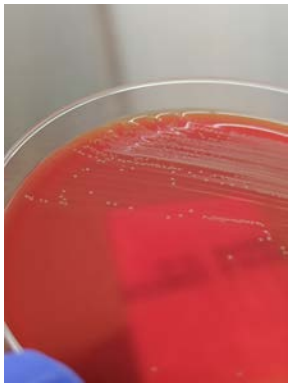


Figure 1. Smooth punctiform *Erysipelothrix* colonies on Columbia CNA agar.

Results
CBC revealed neutrophilia with toxic changes, monocytosis, thrombocytopenia, elevated CRP. Ultrasonography showed diffuse splenopathy and focal peritonitis. Aortic and mitralic vegetations and thickened valve leaflets supported a diagnosis of endocarditis. Cytological evaluation of the spleen detected neutrophilic inflammation with phagocytosis of rod-shaped bacteria. Splenic and blood cultures yielded growth

of punctiform, translucent, smooth colonies on Columbia CNA agar after respectively 24 and 48h of incubation at 37°C, identified as *Erysipelothrix rhusiopathiae* by MALDI-TOF MS. Molecular differentiation between *E. rhusiopathiae* and *E. tonsillarum* was not available. Antimicrobial-susceptibility testing showed resistance to aminoglycosides and trimethoprim/sulfamethoxazole, but susceptibility to beta-lactams, clindamycin and quinolones. Long-term antibiotic and antiaggregant therapies were initiated, achieving general improving.

Discussion and Conclusion

This case underlines the potential role of a known zoonotic pathogen in canine diseases, while presenting a hypothetical association with raw meat-based diet. The implications and relevance in public health should be further investigated.

References

Cabrera-García AI et al. Infective endocarditis due to Erysipelothrix rhusiopathiae in a dog - a case report. BMC Vet Res. 2020;16(1):328

P023

Animal health

TWO CASES OF PARAGANGLIOMA AS NECROSCOPIC FINDINGS IN DOGS

M. Dimatteo[3], M. D'Amore[3], M. Guida[3], G. Rosato[1], D. Izzillo[1], S.M. Casciaro[2], G. Fusco[3], E. De Carlo[3], B. Degli Uberti[3]

[1]ASL Napoli [1] Centro-CRIUV Regione Campania, Naples, Italy
[2]CRIUV Regione Campania, Naples, Italy
[3]Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy

Introduction

Paraganglioma is an uncommon neuroendocrine tumor in dogs. In fact,they represent only 0.2% of canine tumors described (1-2). There is no breed and sex predisposition. They arise from sympathetic and parasympathetic extra- adrenal paraganglia.In literature common sites reported are at the base of the heart, orbit and retroperitoneal areas as in the two cases described.

Materials and Methods

(First case) A stray male 8-year-old mixed dog was referred to clinical and radiological evaluation for an evident orbital mass. TC revealed severe osteolysis in both nasal cavity,lacrimal bones and rostral encephalic involvement,total obstruction of left frontal sinus and partially to the right on. (Second case)A stray male 15-year-old mixed dog was referred for clinical severe posterior leg oedema.Ultrasonography revealed a 15 cm diameter mass in retroperitoneum. At autopsy examination several masses were also identified in heart and intestinal wall.Samples with evident macroscopic neoplastic lesion were sent to Histopathological Unit (IZSM), fixed in 10% neutral buffered formalin. Slides were stained with hematoxylin and eosin. Immunohistochemistry(IHC) was performed for both cases.

Results

In the first case, histological examination of the orbit mass revealed a densely infiltrative,not encapsuled proliferation of pleomorphic neoplastic cells (Figure 1); retrobulbar,periocular bones and encephalic infiltration was also observed.Multifocal metastases were found in adrenal medulla and peritoneal serosa of intestine and mesenteric lymph nodes. Retroperitoneal mass of the second case showed neuroendocrine pattern confirmed by IHC. Metastasis to heart atrium (Figure 2), intestinal muscular wall and spleen were detected. IHC was positive for Chromogranin A, Synaptophysin and S-100 (Figure 3).

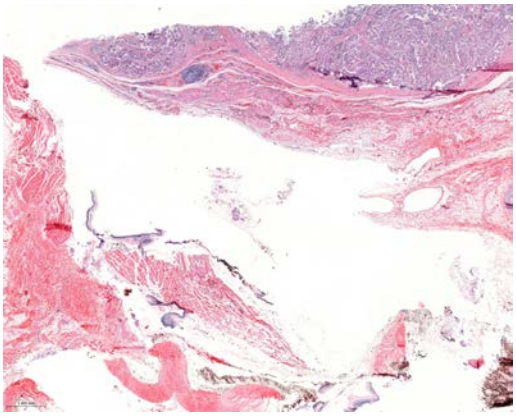


Figure 1. Retrobulbar paraganglioma, hematoxylin and eosin, panoramic view. Neoplastic epithelial polygonal cells divided in packets and nests by fibrovascular stroma.

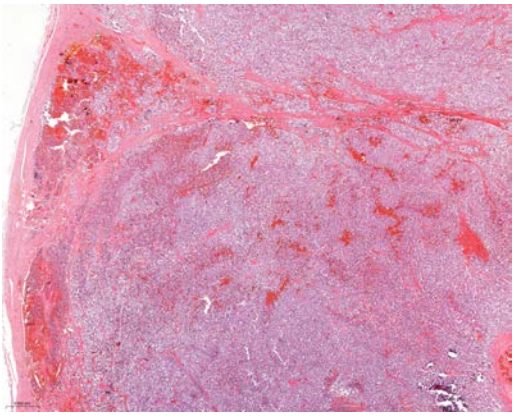


Figure 2. Left and right atrium paraganglioma hematoxylin and eosin (10x). Polygonal neoplastic cells grouped in nests separated by thin fibrovascular septae, "Zellballen aspect", infiltrating myocardiocytes fibers.

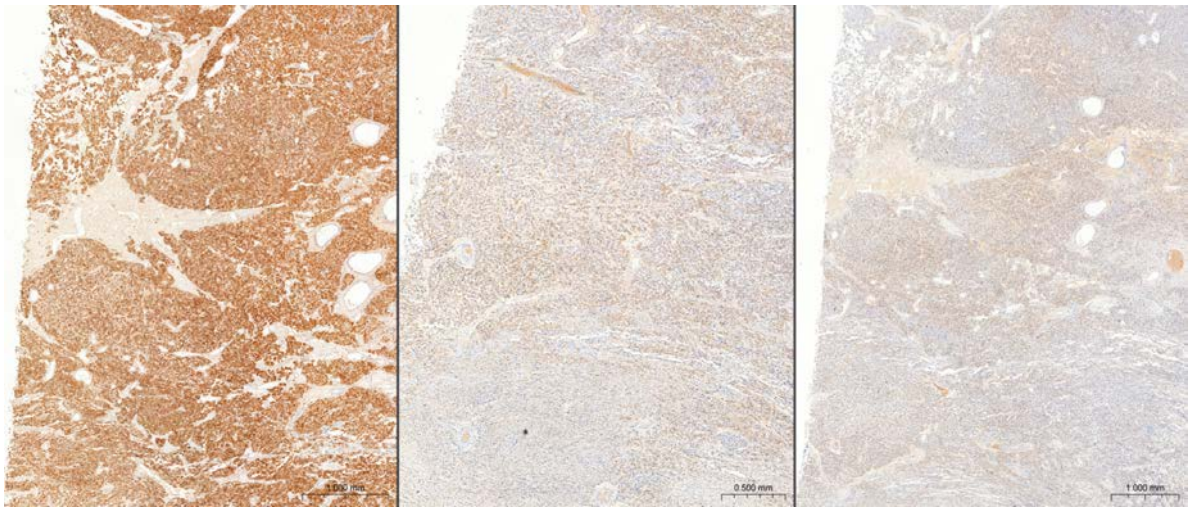


Figure 3. Left and right atrium paraganglioma (10x) IHC, Strong positivity to Synaptophysin (A), and mild positivity to S-100 (B)and Chromogranin-A (C).

Discussion and Conclusion
The synergic activity of clinical,radiological,postmortem and histologic examinations revealed casuistic of paraganglioma in dogs in short period of time(2021-2023). Paraganglioma is a rare tumor,and its incidence could be underestimated if autopsy is not performed.IHC in these two cases allowed differential diagnosis from other neuroendocrine tumors.

References
B. Porte et al. Incidentally detected retroperitoneal paraganglioma in a 10-year-old French bulldog. Can Vet J 2023;64
S. Hu et al. Paraganglioma of the urinary bladder in a dog. J Comp. Path.2022;195

P024

Animal health

THE OCCURRENCE OF POSITIVE PCR AND NEGATIVE ELISA SAMPLES (PPNES) WHEN TESTING FOR BOVINE VIRAL DIARRHEA VIRUS (BVDV) IN A COMPULSORY ERADICATION PROGRAMME

A. Donaghy[1], E. Holmes[1], J. Leigh[1], C. Brown[1], S. Leinster[1], A. Jeffers[1], J. Clarke[1], J. Mcconville[1]

[1]*AFBI*

Introduction
BVD causes infertility, poor health and production loses in cattle1. Immunosuppressive effects and secondary infections drive antimicrobial use. BVD infections after birth are Transient. Within 3 weeks the animal recovers and is virus negative. Pregnant animals infected before 4 months gestation can produce Persistently Infected calves (PIs). Veterinary advice is to cull PIs to prevent further viral shedding. A BVD eradication programme in Northern Ireland was established in 2016. Ear notch (EN) samples are tested for the presence of BVDV by antigen ELISA or RT-PCR 2. It was observed that positive RT-PCR EN pools were returning negative individual ELISA results. Investigations were performed as identifying a positive pool meant a positive individual sample should be present.

Materials and Methods
20 ENs were pooled for RT-PCR testing. Positive pool ENs were individually tested by ELISA. If all ENs tested negative by ELISA they were tested by RT-PCR in groups of 5. Any pool returning a positive result was tested individually by RT- PCR. This would identify the ENs that were PPNEs.

Results
From 2017 112 PPNE BVD ENs were identified. Individual EN CTs range from 24.2 to 37.5. The ENs were from 96 herds, 12 herds had multiple PPNE ENs. Confirmation testing (blood sampled >3weeks after positive EN) found PIs amongst PPNE samples.

Discussion and Conclusion
Findings show the need for diligence when testing large volumes of samples. We have highlighted the potential for PPNE ENs to be reported as negative if a check is not in place to ensure all BVD EN positive pools have ≥ 1 individual positive result. This finding would have

consequences in laboratories that only test ENs by ELISA. We recommend a two-test strategy for BVD EN testing. It is hypothesised that storage, postal delays, or contamination before laboratory arrival could play a part in ENs being PPNEs. Further work is required to identify the cause.

References

1. Baker John C., The Clinical Manifestations of Bovine Viral Diarrhea Infection, Veterinary Clinics of North America: Food Animal Practice, Volume 11, Issue 3, 1995, Pages 425-445
2. Strain Sam , Verner Sharon , Campbell Emma , Hodnik Jaka Jakob , Santman-Berends I. M. G. A., The Northern Ireland Control Programmes for Infectious Cattle Diseases Not Regulated by the EU, Frontiers in Veterinary Science Volume 8, 2021.

Table of summary of samples

Year	Number Of Herds	Number of Samples
2017	14	17
2018	7	9
2019	10	10
2020	15	17
2021	20	21
2022	13	16
2023	13	16
2024 (June)	6	6
	Total	112

Table 1.Table of summary of samples

P025

Animal health

BOVINE TUBERCULOSIS OUTBREAK CAUSED BY *MYCOBACTERIUM CAPRAE*: A "FROM FARM TO WEB" DIAGNOSTIC CASE REPORT

V. D'Ascenzo[2], D. Pintus[2], T. Piseddu[2], M.B. Boniotti[3], C. Bertasio[3], F. Balia[1], B. Mossa[1], S. Dore[2]

[1]*Azienda Socio-Sanitaria Locale di Sassari, Sassari, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*
[3]*Laboratorio di Riferimento Nazionale per la tubercolosi da M.bovis, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy*

Introduction

Mycobacterium caprae, a member of *Mycobacterium Tuberculosis* Complex (MTBC), causes tuberculosis (TB) in animals and humans, with the highest prevalence recorded in Central Europe and Mediterranean Region; recently, the attention on *M. caprae* is increasing worldwide due to the lack of reliable data on its burden, the geographical diffusion, and zoonotic transmission often associated with extra-pulmonary-TB.

Materials and Methods

In April 2023, during the post-slaughter inspection, calcified granulomas were found in an Intradermal-Tuberculin- Test-negative cow's liver and lymph-nodes from a farm in North-West Sardinia (Italy). Samples underwent anatomical, histological examination, decontamination (4%-NAOH), and cultured on Stonebrink and Lowestein-Jehnsen selective medium and VersaTREK cultural broth at 37 °C. The isolate was analysed by Real-Time PCR for the MTBC (i.e., gene IS1081) and by High-Resolution Melting for discriminating between *M. bovis*/*M. caprae* specie (i.e., gene gyrB), and submitted to MIRU-VNTR analysis and spoligotyping. Whole genome sequencing was performed on Illumina Miniseq instrument (2×300bp paired-end mode). Raw reads were trimmed for quality and analysed with MTBseq pipeline.

Results

The diagnosis of *M. caprae* spoligotype "SB0415" (MIRU-VNTR: 535456424375) was confirmed, leading to the hypothesis of a potential epidemiological relationship with the last Sardinian *M. caprae* isolated in 2009. The phylogenetic analysis conducted on the complete genome sequence (NCBI Id.: BioProject PRJNA1071513) of the isolate with the *M. caprae* SB0415 samples available on-line (all from Spain) resulted uninformative, showing a difference >200 SNPs (range: 225-267) in the pairwise comparison between strains.

Discussion and Conclusion

More targeted research and genomic studies are needed to better characterize all the *M. caprae* isolates for epidemiological purposes, as well as for investigating the chains and dynamics of transmission and the space-time evolution. Moreover, the integration of a systematic and standardized collection of data from veterinary and human medicine could be useful to better assess the real burden of each species of MTBC with a One-Health approach.

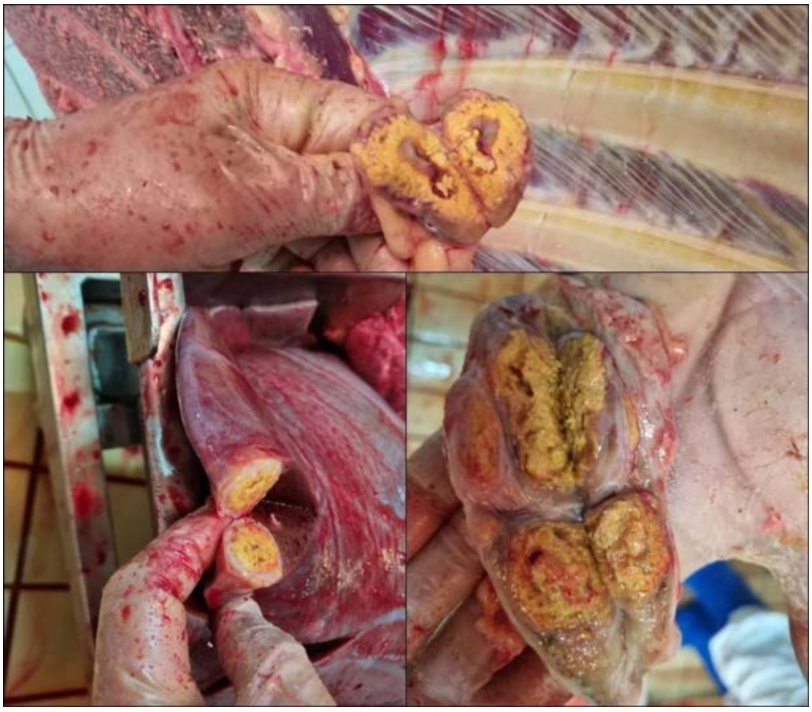


Figure 1. Calcified granulomas in the liver and lymph-nodes.

Figure 2. Phylogenetic relationship of *Mycobacterium caprae* SB0415 strains.

P026

Animal health

DIGITAL PCR (DPCR) TO QUANTIFY THE LOAD OF *MYCOBACTERIUM AVIUM* SUPSP. PARATUBERCULOSIS (MAP) PRESENT IN FECES AS A "TOOL" TO DEFINE PRIORITIES OF INTERVENTIONS IN AN INFECTED CATTLE HERD.

A. Filippi[3], C. Garbarino[3], G. Ventura[2], M. Gradassi[2], R. Frontoni[1], E. Tuccia[4], A. Giuliani[4], S. Russo[3], A. Luppi[3], M. Ricchi[3]

[1]*Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Territorial unit of Cremona, Italy*
[3]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Territorial unit of Piacenza, Italian National reference centre for paratuberculosis, Italy*
[4]*Veterinary Practitioner Cremona, Italy*

Introduction
Paratuberculosis is a chronical enteritis affecting ruminants caused by *Mycobacterium avium* subsp. paratuberculosis (MAP) with a serious impact on welfare, animal health and economic losses. MAP has been suspected to be a zoonotic agent with no final consensus. Both subclinically and clinically infected animals can spread MAP into the environment through feces. The ingestion of food and water contaminated by infected feces is the major route of transmission. We investigated the use of digital PCR (dPCR) for detection of animals shedding high load of MAP, that are the first to be segregated to reduce risk of infection for animals.

Materials and Methods
We report here our experience in a Northern Italy dairy farm with paratuberculosis infection. In May 2023, sera and feces from all 1019 animals ≥24 months old were collected and analysed. In November 2023, sera and feces from all 974 animals ≥24 months old were sampled. Sera were analised by ELISA while feces, environmental fecal samples and milk by IS900-qPCR (1). Samples showing a qPCR results ≤ 30 Cq underwent to F57 dPCR (2), to quantifying the load of MAPs.

Results
The apparent sero-prevalence decreased from 7.3% (1st round) to 4.6 % (2nd round), while fecal prevalence decreased from 6.5% to 2.8%. In the 1st round, 5 animals resulted Super shedders ($\geq 10^7$ MAP genome copies/g of feces), 6 animals were High shedders (from 10^5 to 10^7 copies/g of feces); in the 2nd round were detected 2 Super shedders and 5 High shedders animals.

Discussion and Conclusion
Since MAP has a very high environmental resistance, despite is unable to replicate outside hosts, the level of environmental biocontamination relies on both the number of shedding animals in the farm and the MAP load excreted by every single animal. The use of the dPCR allowed the direct quantification of the MAP cells load present in the feces in a very quicker way than cultural assay. Animals shedding high load of MAP were the first segregated. This method appeared to be effective in providing useful results for paratuberculosis management plan.

References
1. Russo S. et al. Prev Vet Med. 2022 Nov;208:105732. doi: 10.1016/j.prevetmed.2022.105732. Epub 2022 Aug 6. PMID: 35988391.
2. Russo S. et al. J Microbiol Methods. 2023 Oct;213:106825. doi: 10.1016/j.mimet.2023.106825. Epub 2023 Sep 20. PMID: 37739126.

P027

Animal health

DESIGN OF A NOVEL MULTIPLEX-RT-PCR ASSAY FOR IMPROVED PRRSV DIAGNOSTICS

S. Hänsel[2], F. Priller[2], C. Stieler[2], H. Ziebarth[2], K. Valentine[1]

[1]*BioChek B.V.*
[2]*Hygiena Diagnostics GmbH*

Introduction
PRRSV remains one of the biggest challenges to the global swine industry, as it proves both difficult to control and to diagnose. This is in large part owed to the rapid evolution of the virus, creating new genetic variants at fast pace, which can lead to high rates of false-negative RT-PCR results [1]. Diagnostics is also being complicated by the existence of two major viral types, 1 and 2, each with multiple distinct sub-linages. Of these the Asian Type 2 "highly pathogenic" (HP) lineage presents a major threat, warranting independent identification by multiplex RT-PCR [2].

Materials and Methods
The vetproof® Porcine Reproductive and Respiratory Syndrome Virus qPCR Kit uses an optimized design approach, covering multiple ORFs in the PRRSV genome, to reliably detect all known PRRSV variants with almost no predicted diagnostic gaps. This new 4-plex PCR kit can detect and differentiate Type 1, Type 2 and Type 2 HP strains and includes an MS2 phage extraction control to verify test results. Extraction of sample material was performed using the vetproof® MagBead Extraction Kit I.

Results
The new assay achieved an LLOD of 10-25 copies per reaction in typical matrices such as serum, blood, tissue, semen oral fluids and swabs for all three targeted subtypes. Excellent inclusivity was confirmed by testing of a diverse set of viral sequences and the absence of diagnostic gaps in proficiency testing panels that contained difficult to detect strains.

Discussion and Conclusion
The novel PCR kit allowed for very sensitive detection of all PRRSV lineages in all relevant sample types. The in-silico predicted analytical inclusivity of almost 100% was confirmed in initial field trials.

References
1. Stadejek T, Stankevicius A, Murtaugh MP, Oleksiewicz MB.: Molecular evolution of PRRSV in Europe: current state of play. Vet Microbiol. 2013;165(1-2):21-28
2. Wernike K, Hoffmann B, Dauber M, Lange E, Schirrmeier H, Beer M.: Detection and typing of highly pathogenic porcine reproductive and respiratory syndrome virus by multiplex real-time rt-PCR. PLoS One. 2012;7(6):e38251

P028

Animal health

NEW CHEMOKINES AS BIOMARKERS FOR DETECTION OF *MYCOBACTERIUM BOVIS* INFECTION IN WATER BUFFALO (*BUBALUS BUBALIS*): PRELIMINARY RESULTS ON MIP-1α AND MIP-1β

G. Franzoni[2], A. Donniacuo[4], P. Mazzone[3], E. De Carlo[4], S. Dei Giudici[2], S. Zinellu[2], L. Schiavo[4], F. Signorelli[1], F. Napolitano[1], G. De Matteis[1], G. Galiero[4], J. Bezos[5], A. Martucciello[4]

[1]*CREA-Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, Centro di ricerca Zootecnia e Acquacoltura (Research Centre for Animal Production and Aquaculture), Monterotondo (RM), Italy*
[2]*Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*
[3]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy*
[4]*National Reference Centre for Hygiene and Technologies of Water Buffalo Farming and Productions, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Salerno, Italy*
[5]*VISAVET Health Surveillance Centre, Complutense University of Madrid, Madrid, Spain; Facultad de Veterinaria, Departamento de Sanidad Animal, Universidad Complutense de Madrid, Madrid, Spain*

Introduction
Animal tuberculosis (TB) is a worldwide zoonosis affecting many domestic and wild animal species, including water buffalo. *M. bovis* is the main cause of infection. The IFN-γ release assay in response to its antigens has been used as an *in vitro* test to identify infected animals. New immunological biomarkers for *M. bovis* infection have been recently investigated in cattle and wildlife (1). This study investigated the utility of two poorly studied chemokines (MIP-1α, MIP-1β) as biomarkers of *M. bovis* infection in water buffalo. MIP-1α and MIP-1β are pro-inflammatory chemokines mainly produced by monocytes/ macrophages (2).

Materials and Methods
Thirty-six buffaloes were enrolled and divided into three groups: TB-infected (IFN-γ positive, TB lesions; N=11), TB- suspected (IFN-γ positive only; N=14), and uninfected (Officially Tuberculosis-Free herds; N=11). Blood samples were stimulated *in vitro* with PBS (Antigen Nil Acontrol), bovine bovine purified protein derivative (PPD-B) and Pokeweed Mitogen (PWM). At 16-24h post-stimulation, plasma was collected and levels of IFN-γ, MIP-1α and MIP-1β were evaluated by MILLIPLEX® kit. Data were analyzed with GraphPad Prism 10.01, differences were considered significant at $P<0.05$.

Results
Our results revealed that TB-infected and TB-suspected buffaloes, but not healthy controls, release high levels of IFN-γ, MIP-1α, MIP-1β in response to PPDB stimulation compared to PBS (Table 1). Comparison between groups showed that both TB-infected and TB-suspected animals released higher levels of MIP-1β in response to PPDB compared to healthy subjects. TB-infected animals, but not TB-suspected buffaloes, released higher levels of MIP-1α in response to PPDB compared to healthy subjects (Figure 1).

Discussion and Conclusion
Our preliminary results suggest the potential use of these chemokines as biomarkers of TB infection in water buffaloes. Future studies will

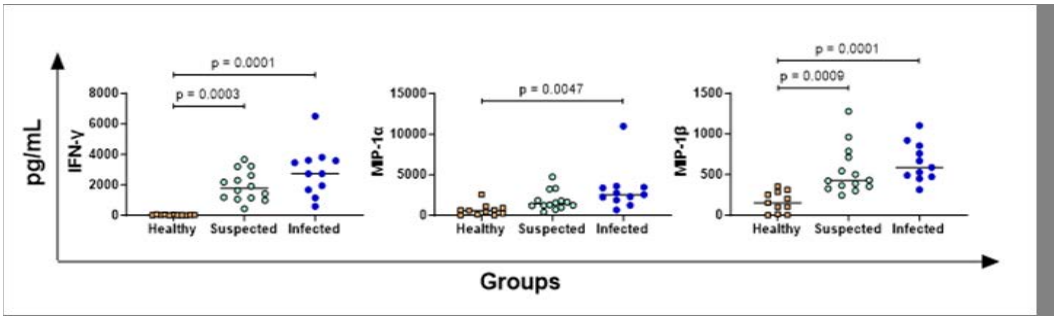
be conducted on a larger set of samples to assess the potential use of these chemokines (alone or combined) for detecting the status of TB infection in water buffaloes. Research funded by the Italian Ministry for Health (RC IZSME 14/22), ICRAD (European Union's Horizon 2020 - Grant Agreement n°862605) and the EU-RL for Bovine Tuberculosis.

References

1. Smith et al. 2021. Front Immunol 12:639605.
2. Menten et al. 2022. Cytokine Growth Factor Rev 13:455-481.

	HEALTHY				
	PBS	PPDB	PWM	PBS-PPDB	PBS-PWM
	LSM ± SEE	LSM ± SEE	LSM ± SEE	p-value	p-value
Cytokines					
IFN-γ	2 ± 82	25 ± 82	832 ± 82	0.9705	**0.0001**
MIP-1α	2169 ± 369	2831 ± 369	3263 ± 369	0.3531	0.0804
MIP-1β	545 ± 229	707 ± 229	937 ± 229	0.8369	0.3852
	TB-SUSPECTED				
IFN-γ	7 ± 171	1935 ± 171	1190 ± 171	**0.0001**	**0.0001**
MIP-1α	800 ± 365	2603 ± 365	2487 ± 365	**0.0024**	**0.0044**
MIP-1β	172 ± 71	716 ± 71	687 ± 71	**0.0001**	**0.0001**
	TB-INFECTED				
IFN-γ	2 ± 289	2895 ± 289	789 ± 294	**0.0001**	0.1132
MIP-1α	2404 ± 892	5594 ± 892	4044 ± 892	**0.0315**	0.3366
MIP-1β	319 ± 71	969 ± 71	718 ± 71	**0.0001**	**0.0008**

Table 1. Production of IFN-, MIP-1alpha, MIP-1beta in whole blood from healthy, TB suspected and TB infected water buffaloes. Whole blood was stimulated with PBS, PPDB, or PWM. Levels of cytokines were determined through by multiplex ELISA. LSM (Least Squares Mean) and SEE (Standard Estimated Error) values and statistical differences between conditions (p-value) are presented.



1. Russo S. et al. Prev Vet Med. 2022 Nov;208:105732. doi: 10.1016/j.prevetmed.2022.105732. Epub 2022 Aug 6. PMID: 35988391.
2. Russo S. et al. J Microbiol Methods. 2023 Oct;213:106825. doi: 10.1016/j.mimet.2023.106825. Epub 2023 Sep 20. PMID: 37739126.

Figure 1. IFN-, MIP-1alpha and MIP-1beta production in whole blood from water buffalo. Heparin blood from TB infected (n=11), TB suspected (n=14) and healthy (n=11) animals were collected. Whole blood was stimulated with PPDB, alongside antigen nil control (PBS). After 16-24 h, plasma were collected, and levels of cytokines were determined by multiplex ELISA. Specific cytokine responses to TB were determined by subtracting baseline cytokine levels (PBS) from those measured in the TB-antigen condition (PPDB stimulation). Differences between groups are displayed and p value < 0.05 were considered statistically significant.

P029

Animal health

LABORATORY VALIDATION OF REAL-TIME PCR ASSAYS FOR DETECTION OF AFRICAN SWINE FEVER IN FAECAL SAMPLES

A. Fulmini[1], S. Mrabet[1], E. Tinelli[1], C. Casciari[1], M.S. Beato[1], F. Feliziani[1]

[1]National Reference Laboratory for African and Classical Swine Fever, Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche (IZSUM), Perugia, Italy

Introduction
African swine fever virus (ASFV) is an enveloped dsDNA virus of the Asfariviridae family, Asfivirus genus, causing a haemorrhagic fever disease in wild and domestic pigs with high mortality rates. It spreads via direct and indirect transmission routes through infected animals and contaminated fomites, and in some areas soft ticks are also involved. ASFV has significant impacts on pig industry, amplified by the lack of vaccines, making a prompt and reliable diagnosis of paramount importance. Diagnosis targets virus-rich tissues: lymph nodes, bone marrow and spleen. Recent studies proposed non-invasive sampling of oral-nasal, rectal swabs and faeces for a faster diagnosis, all lacking data on test performances. In this study we validated two WOAH-recommended ASFV qPCR assays (King vs UPL), based on WOAH guidelines, targeting the *p72* gene, providing analytical performances for swine faeces.

Materials and Methods
Analytical specificity was assessed in silico and *in vitro* with main swine enteric pathogens. Analytical sensitivity (ASe) was assessed by determining the Limit of Detection (LoD) using a quantified 10-fold diluted genotype II ASFV, viable and heat-inactivated, extracted in triplicate using 2 extraction kits (manual and automated) and amplified in duplicate. Interference on ASe was assessed by spiking the 10-fold diluted ASFV in ~1 gr of faeces. The same dilutions were tested for absolute quantification by Droplet Digital PCR using King protocol.

Results
The ASe was similar for both protocols using live ASFV (2.5HAD/50µl, 0.13 copies/µl) while the UPL protocol was less sensitive using inactivated ASFV (2.5 10HAD/50µl, 14.5 copies/µl). The ASe on faeces decreased of two Logs using King but not with the UPL protocol. The higher Ct values observed with heat-inactivated ASFV suggest such treatment might interfere with downstream laboratory analysis.

Discussion and Conclusion
Generated data for faeces suggest the UPL method might be more appropriate for novel testing samples. However, further analytical and diagnostic validation is necessary to provide robust data on the inclusion of faeces and other alternative matrices in ASFV diagnostic algorithm.

References
1. WOAH 3.9.1
2. Niederwerder (2021)
3. Davies (2017)
4. de Carvalho Ferreira (2014)
5. Flannery (2020)
6. King (2003)
7. Fernandez-Pinero (2013)
8. WOAH 1.1.6

P030

Animal health

A SURVEY OF THE REPRODUCTIVE LESIONS IN CAPTIVE FEMALE NON-HUMAN PRIMATES IN ITALY

V. Galietta[3], C. Cocumelli[3], R. Parmigiani[3], E. Bovi[3], T. Palmerini[3], S. Antognetti[3], P. Di Cerbo[2], M. Aloisi[1], K.G. Friedrich[2], C. Eleni[3]

[1]Animanatura Wild Sanctuary, Semproniano (GR), Italy
[2]Fondazione Bioparco, Rome, Italy
[3]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy

Introduction
Non-human primates (NHPs) in zoos are often part of breeding programs to preserve their biological diversity. For this reason, the study of pathologies through the systematic post-mortem collection of reproductive lesions represents an excellent tool for obtaining useful

information. Single pathological cases are frequently reported, while reviews in captive NHPs are scarce [1]. We describe post-mortem reproductive lesions found between 2007 and 2024 in captive female NHPs housed in central Italy.

Materials and Methods

The cases were sourced from the Histopathology Laboratory database of the Istituto Zooprofilattico Sperimentale del Lazio e della Toscana, from dead animals received for post-mortem examination between 2007 and 2024. Necroscopies were conducted on 94 captive female NHPs held in three facilities; histopathology were performed on macroscopically evident lesions.

Category of NHP	Common name	Species	Age	Diagnosis
Old World NHP	Cynomolgus macaque	*Macaca fascicularis*	17	Leyomioma, Pyometra
	Cynomolgus macaque	*Macaca fascicularis*	adult	Ovaric Adenocarcinoma with peritoneal carcinomatosis
	Assam macaque	*Macaca assamensis*	33	Cystic endometrial hyperplasia, Adenomiosis
	Japanese macaque	*Macaca fuscata*	24	Leyomioma
	Rhesus macaque	*Macaca mulatta*	30	Leyomioma
	Pig-tailed macaque	*Macaca nemestrina*	20	Endometriosis, Adenomiosis
	Orangutan	*Pongo pygmaeus*	45	Metastatic histyocitic sarcoma in ovary, Leyomioma
	Orangutan	*Pongo pygmaeus*	37	Ovarian follicular cysts
	Lar gibbon	*Hylobates lar*	31	Cystic endometrial hyperplasia, Adenomiosis
	Chimpanzee	*Pan troglodytes*	27	Leyomioma
New World NHP	Tufted capuchin	*Sapajus apella*	19	Cystic endometrial hyperplasia
	Tufted capuchin	*Sapajus apella*	27	Endometrial carcinoma with metastasis
Prosimian	Ring-tailed lemur	*Lemur catta*	18	Metastatic mammary carcinoma in uterus

1. WOAH 3.9.1
2. Niederwerder (2021)
3. Davies (2017)
4. de Carvalho Ferreira (2014)
5. Flannery (2020)
6. King (2003)
7. Fernandez-Pinero (2013)
8. WOAH 1.1.6

Table 1. Reproductive pathologies in captive non-human primates observed during the period 2007-2024.

Results

Reproductive pathologies were observed in 13 adult females (13/94, 14%); some animals had multiple simultaneous lesions (Table 1). The most frequently lesions were neoplastic pathologies (9/13, 62%). Leiomyoma was the most frequent uterine tumor; malignant primary tumors were rare. In two cases, metastatic lesions were found. Non- neoplastic pathologies were diagnosed in 5 animals.

Discussion and Conclusion

The findings align with previous studies in these species [1]. All subjects were old aged, and information on their reproductive treatments are missing. Differently from what reported in the literature, non-neoplastic pathologies were not frequent in our cases, but tissues without macroscopic lesions were not systematically sampled. Early stages of endometriosis and adenomyosis often require histological examination to be assessed as they could not be macroscopically evident. Due to the retrospective nature of the study, and the limited number of cases, species-specific patterns and prevalence could not be determined. Future research should actively investigate these pathologies in all subjects during the necropsy to increase the cases detection and to obtain useful information for the reproductive management of NHPs.

References

1. Moresco A et al. Reproductive one health in primates. Am J Primatol. 2022;84:e23325.

P031

Animal health

BIOMONITORING STUDY CONDUCTED ON THE POPULATION OF PET ANIMALS RESIDENT IN CAMPANIA

A. Gallo[2], D. Cardinale[1], L. Vassallo[3], M. Cuomo[3], D. Sansone[3], A. Siciliano[2], G. Rofrano[2], J.L. D'Auria[2], D. Signorelli[3], F. Corrado[3]

[1]*Azienda Sanitaria Locale di Caserta area C Igiene allevamenti, Italy*
[2]*Centro di Referenza Nazionale per l'analisi e studio di correlazione tra ambiente, animale e uomo, Italy*
[3]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy*

Introduction

In a population sample of domestic dogs residing in different areas of the Campania region, we aimed to quantify environmental exposure through biomarkers of exposure, such as trace elements.

Materials and Methods
Seventy-nine master dogs were enrolled from which blood samples were taken.
0.500 ml of serum, brought to a final volume of 5ml with milliq water, was injected into ICP-MS. The metals determined are: Lithium, Beryllium, Aluminum, Gallium, Arsenic, Rubidium, Strontium, Silver, Cadmium, Indium, Cesium, Barium, Thallium, Lead, Bismuth, Uranium, Manganese, Zinc, Selenium, Vanadium, Chromium, Iron, Cobalt , Nickel, Copper.

Results
The results of the analysis of exposure biomarkers showed different contexts: cadmium was absent in all ser, while arsenic was found uniformly in all samples analyzed. In addition, a geographic pattern was found in the "Alto Casertano" area showing the significant presence of lithium and lead in the samples analyzed. The presence of these elements could result from several sources, including diet, or from environmental exposure; in fact, studies conducted previously have shown the presence in the area of bioavailable sources of lead and lithium in the topsoil analyzed. In serum samples from the area of the municipalities of the Gulf of Salerno, the presence of Aluminum in statistically significant concentrations indicates a different type of environmental exposure than in the other areas studied, but finds no evidence of geogenic origin. An unusual presence of Iron was found in the canine serum of dogs residing in the southern portion of the Diano Valley, as it is found in the topsoil of that territory [1].

Discussion and Conclusion
This study confirms the importance of using companion animals as sentinels to monitor environmental quality and identify potential human health risks. In conclusion, the project supports the One Health approach, demonstrating that the health of companion animals is closely related to human health and the environment.
This research was funded by the Italian Ministry of Health, grant number IZS ME 13-20 RC

References
1. MONITORAGGIO GEOCHIMICO-AMBIENTALE DEI SUOLI DELLA REGIONE CAMPANIA. PROGETTO CAMPANIA TRASPARENTE. VOLUME 2 / S. Albanese; D. Cicchella; B. De Vivo; M. Esposito, ; A. Fortelli; A. Guarino ; D. Hope; A. Lima; A. Pizzolante; Q. Qu; D. Zuzolo. - 19:(2021).

P032

Animal health

MOLECULAR DIFFERENTIATION OF *STREPTOCOCCUS SUIS* SEROTYPE 2, 1/2, 1 AND 14 IN STRAINS ISOLATED FROM PIGS FROM 2019 TO 2023 IN NORTHERN ITALY

S. Faccini[1], L. Gintoli[1], M. D'Incau[2], E. Cursio[3], C. Rosignoli[1]

[1]*Istituto Zooprofilattico Sperimentale Lombardia e dell'Emilia Romagna Sede di Mantova, Mantova, Italy*
[2]*Istituto Zooprofilattico Sperimentale Lombardia e dell'Emilia Romagna, Batteriologia specializzata, Italy*
[3]*University of Parma, Parma, Italy*

Introduction
Streptococcus suis serotype(ST) 2 is an important swine pathogen and zoonotic agent. Its capsular structure is closely related to those of the zoonotic ST14 and non-zoonotic ST1/2 and 1. The distinction of these 4 STs is crucial for epidemiology and vaccine production. Aim of this study was the assessment of molecular methods for the detection of the SNPs differentiating these STs and the estimation of their proportion in strains isolated from pigs in Northern Italy.

Materials and Methods
An archive of 260 *S. suis* strains, retrieved, from 2019 to 2023, from brains of pigs with neurological symptoms or sudden dead, and classified as ST2-1/2 (208) or ST1-14 (52) by PCR, was analyzed by 3 molecular tests [1; 3; 4]. The samples came from 73 pig farms, from Mantua province and other 7 provinces of Northern Italy.

Results
All the strains previously classified as type ST1-14 were concordantly assigned to ST1. Among those of ST 2-1/2, 79 were concordantly identified as ST2, 128 as ST1/2, and 1 was classified as ST1/2 by two methods [1; 4]and as ST2 by the HRM [3]. The sequencing of the cpsK gene of this strain showed the presence of a rare C, in the third position of the codon affected by the SNP. The encoded amino acid was a C as in ST1/2 and not a W as in ST2. Overall, 62,1% of the ST2-1/2 were ST1/2. In order to reduce the possible interference of repeated sampling, the frequencies were recalculated after removing all the isolates of the same ST, from the same farm, within one year. Among the remaining 79 ST2-1/2 strains 43% were ST2 and 57% ST1/2.

Discussion and Conclusion
MAMA PCR [1] was evaluated as the most suitable for diagnostic routine. Data suggest that ST14 is minimally involved in meningitis in swine in the sampled area. Similarly, a recent Spanish study found that ST14 constituted 3.9% of the ST1-14 isolates, but, was isolated solely from joints [2]. Our study showed ST1/2 was prevalent compared to ST2. Data highlight the importance of the identification of ST1, ST14, ST2 and ST1/2 for a proper epidemiology and support to autogenous vaccine production.

The study was funded by IZSLER - project STREP COMM

References
1. Okuhama-Yoshida, E., et al, 2023.
2. Petrocchi Rilo, M., et al, 2024.
3. Scherrer, S., et al, 2020.
4. Thu, I.S.L., et al, 2021.

P033

Animal health

DEVELOPMENT AND STANDARDIZATION OF A SEROLOGICAL ASSAY FOR BOVINE TUBERCULOSIS IN WATER BUFFALOES USING POTENTIAL DIAGNOSTIC ANTIGENS

S. Giovannozzi[4], A. Martucciello[5], M. Domínguez Rodríguez[7], I. Moreno Iruela[7], M. Boifava[4], G. Parisio[4], N. Vitale[2], E. De Carlo[5], J. Bezos Garrido[1], E. Gormley[6], P. Mazzone[3], B. Romero[1], M.B. Boniotti[4]

[1]*Animal Health Department of Veterinary Faculty and VISAVET Health Surveillance Centre, Complutense University of Madrid, Madrid, Spain*
[2]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy*
[3]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche, Perugia, Italy*
[4]*National Reference Centre for Bovine Tuberculosis, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy*
[5]*National Reference Centre for Hygiene and Technologies of Water Buffalo Farming and Productions, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Salerno, Italy*
[6]*School of Veterinary Medicine, University College Dublin, Dublin, Ireland*
[7]*Unidad de Inmunología Microbiana, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain*

Introduction
Tuberculosis (TB) infection due to *Mycobacterium bovis* is a zoonosis with a significant impact on public and animal health. Intradermal tests (IDT) and the Interferon-Gamma Release Assay (IGRA), both based on a cell-mediated response, are the principal methods used for TB diagnosis in buffalo. However, some animals may be anergic and not responsive to IDT and IGRA but, at the same time, potentially highly infectious. Therefore, the detection of specific antibodies against *Mycobacterium bovis* maximizes the identification of infected animals when IDT and IGRA fail. In order to elucidate this, MPB70, MPB83, ESAT6, CFP10, PPDB, and P22 antigens were used to evaluate their performance for humoral TB diagnosis.

Materials and Methods
We analysed 790 sera from buffaloes: 393 from TB-free herds and 397 from TB-infected herds. Among the latter, 338 animals were positive to IDT and IGRA, 57 animals had IDT and IGRA discordant results, and 2 were negative to IDT and IGRA. All buffaloes from TB-infected herds showed TB lesions or were culture positive. Each sample was collected 15-21 days after IDT. All sera were tested with an indirect ELISA for MPB70, MPB83, ESAT6, CFP10, PPDB, and P22. The ELISA optical density (OD) results were analysed with the criterion: (average OD sample – OD blank/average OD positive control – OD blank) × 100. The ROC curve was used to establish cut-off, sensitivity (Se) and specificity (Sp) for each antigen.

Results
Fixing the Sp at 98,2%, the Se of MPB70, MPB83, ESAT6, CFP10, PPDB, and P22 were 58,0%, 40,5%, 32,2%, 13,9%, 63,0% and 61,2%, respectively. In 57 buffaloes with discordant IGRA and IDT outcomes, serology for MPB70, P22 and PPDB revealed positive results in 39, 33 and 35 animals respectively.

Discussion and Conclusion
Our results showed comparable Se and Sp to other serological assays in different animal species. MPB70, P22, and PPDB showed the higher Se values. This study explored the use of single antigens and protein complexes for serodiagnosis of TB in water buffaloes and suggests that the detection of antibodies against *M. bovis* could be used as a complementary diagnostic tool to maximize Se in this species.
This research was funded by the Italian Ministry of Health for IZSME 02/2012 RC and ICRAD project (Horizon 2020).

P034

Animal health

ALTERATIONS IN THE BIOCHEMICAL PROFILE OF CALVES WITH DIARRHEA SYMPTOMS WITHIN THE FIRST 10 DAYS OF LIFE

G. Valli[3], V. Lorenzi[3], F. Fusi[3], M. Tempini[3], D. Vecchio[1], S. Bergagna[2], A. Barberio[4], L. Bertocchi[3], I. Archetti[3]

[1]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Salerno, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Torino, Italy*
[3]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini", Brescia, Italy*
[4]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy*

Introduction

Diarrhea in calves is a multifactorial disease associated with high morbidity and mortality. Investigating biological changes linked to calf diarrhea is crucial for predicting disease progression and providing timely treatment. Therefore, this study aimed to examine changes in blood biochemical profile of calves with diarrhea.

Materials and Methods

The study was part of an official control plan for calf health monitoring in dairy farms in Lombardy, Italy. It involved 35 dairy farms and 199 calves (5-6 calves/farm), mean age 8.5 days. Blood samples were collected after 2-3 hours from the morning milk feeding and serum was obtained for biochemical analysis (Table 1). Data on age, sex, diarrhea symptoms, and ongoing treatments were recorded. Calves with diarrhea were assigned to the diarrhea (D) group, the others to the No Diarrhea (ND) group. Biochemical profile results of the two groups were compare using Student's t test or Mann- Whitney U test, depending on data distribution.

ANALYTE	UNTTOF MEASUREMENT	METHOD
Hapto_globin	mg/mL	Phase Haptoglobin Kit (Tridelta Development Ltd., Ireland)"
Tota! protein	g/L	Biurcl.b
Albumin	g/L	Endpoint colorimetric IFCCb
Globulin	g/L	Computalion
Albumin/Globulin	-	Computation
Urea	11111101/L	Enzvmalic UV (urease/GLDH)h
Non-esterified fatty acids (NEFA)	mmol/L	Colorimetric - Kit Randox (UK)b
Beta-hydroxybutyrate	11111101/L	Colorimetric - Kit Randox (UK)b
Glucose	mmol/L	GOD,PODb
Tota! cholesterol	11111101/L	CHOD,PAPb
Cholesterol HDL	mmol/L	Imrnunoenzvmaticb
Cholesterol LDL	11111101/L	Computation
Triglvcerides	mmol/L	GPO, PAPb
Asoartate arninotransferase (AST)	IU/L	Kinetic IFCCb
Total bilirubin	µ11101/L	Jendrassik-Grof"
Creatine kinase	IU/L	NAC inactivated (IFCC)b
Calcium	mmol/L	o-Cresolphthalei nb
Phosphorns	mmol/L	Phosphomolybdate[6]
Magnesium	mmol/L	Enzymatic LJVb

"Analytical instrument: Absorbance reader Sunrise (Tecan Trading AG, Switzerland), **with** a 630 nm filter.
b Analytical instrumenl.: lLab 650 (Instrnmentation Labora tory, USA)

Table 1. Analytes included in the biochemical profile with the indication of the method used.

Results

D Group consisted of 65 calves (mean age=8 days), while ND Group included 134 calves (mean age=9 days). Results of the serum analyses are reported in Table 2. Significant differences between the 2 groups were found for urea ($P<0.01$), albumin ($P<0.05$), albumin/globulin ratio ($P<0.05$), glucose ($P<0.05$), triglycerides ($P<0.05$), calcium ($P<0.05$) and haptoglobin ($P<0.10$).

Discussion and Conclusion

D group had lower levels of glucose and triglycerides than ND group, consistent with Saleh et al.[1]. The decrease in blood glucose can be due to dehydration or sepsis, while lower triglycerides may result from lipid malabsorption. No significant changes in other lipid parameters were found. Earlier studies observed an increase in serum albumin concentration in severely diarrheic and dehydrated calves up to 10 days old[2], which aligns with our findings. The D group also exhibited lower calcium levels, as reported by other authors[3]. Additionally, urea levels were significantly higher in D group, likely due to dehydration and tissue damage[1]. The acute phase protein haptoglobin was elevated in D group compared to ND group.

This study was funded by the Italian MOH grant PRC IZSLER 2021/003.

ANALYTE	ND Group (n=134)	D Group (n=65)
Haptoglobin (mg/mL)	0.1 ± 0.3	0.2 ± 0.4*
Total protein (g/L)	56.2 ± 7.7	55.6 ± 9.2
Albumin (g/L)	28.3 ± 2.6	29.1 ± 3.2**
Globulin (g/L)	28.0 ± 7.7	26.5 ± 8.3
Albumin/Globulin (A/G)	1.1 ± 0.3	1.2 ± 0.4**
Urea (mmol/L)	4.7 ± 4.0	6.4 ± 5.6***
Non-esterified fatty acids (NEFA) (mmol/L)	0.3 ± 0.1	0.3 ± 0.1
Beta-hydroxybutyrate (mmol/L)	0.1 ± 0.1	0.1 ± 0.0
Glucose (mmol/L)	5.3 ± 1.5	4.8 ± 1.5**
Total cholesterol (mmol/L)	1.7 ± 0.7	1.5 ± 0.5
Cholesterol HDL (mmol/L)	1.1 ± 0.4	1.0 ± 0.4
Cholesterol LDL (mmol/L)	0.5 ± 0.4	0.5 ± 0.2
Triglycerides (mmol/L)	0.3 ± 0.2	0.2 ± 0.2**
Aspartate aminotransferase (AST) (IU/L)	44 ± 20	43 ± 14
Total bilirubin (µmol/L)	6.3 ± 3.7	6.6 ± 5.1
Creatine kinase (IU/L)	240 ± 279	302 ± 325
Calcium (mmol/L)	2.8 ± 0.3	2.7 ± 0.3**
Phosphorus (mmol/L)	2.6 ± 0.3	2.7 ± 0.6
Magnesium (mmol/L)	0.9 ± 0.1	0.9 ± 0.2

Table 2. Biochemical profile of ND group calves and D group calves. Values are reported as mean ± SD. Significant difference between the 2 groups were indicated by * at P<0.10; ** at P<0.05 and *** at P<0.01.

References
1. Saleh et al. 2022, JCVR, 4(1):58-68
2. Thornton et al., 1972, Can J Comp Med, 36(1):17-25
3. Grove-White et al., 2001, Vet Rec,149(7):203-7

P035

Animal health

DETECTION OF *SALMONELLA ENTERICA* SEROTYPES IN DAIRY CATTLE FARMS IN NORTHERN ITALY

F. Parolini[3], G. Ventura[2], C. Rosignoli[4], S. Rota Nodari[1], M. D'Incau[1], L. Marocchi[4], G. Santucci[4], M. Boldini[2], M. Gradassi[2]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Cremona, Italy*
[3]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Cremona, Italy and Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Teramo, Italy*
4Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Mantova, Italy

Introduction
The aim of this study is to conduct a retrospective analysis on the detection of *Salmonella* spp. in dairy cattle farms of the Cremona and Mantua provinces of Lombardy Region in the period 2021-2022.

Materials and Methods
Dairy farms with at least one *Salmonella* isolation were included in the analysis. A total of 2710 samples from different sources, calf carcasses/organs (n=128), rectal swabs (n=1937), feces (n=390), bulk milk (n=93) and environmental overshoes/swabs (n=100), were analyzed. After the first detection of *Salmonella*, additional samples (n = 2405) were collected in 60 farms (follow-up analyses). Lesions observed during necropsy on 91 calves carcasses were recorded. The isolation of *Salmonella* was carried out according to the ISO 6579-1:2017/Amd 1:2020 and serotype identification was performed with ISO/TR 6579-3:2014.

Results
A total of 120 cases of *Salmonella* infection from 118 dairy cattle farms were reported. Overall, 17 different serotypes were detected, with *S. Dublin, S. Typhimurium* and its monophasic variant accounting for 75.83% of the total. In 13 cases, simultaneous infection with two or more *Salmonella* serotypes was found. In the majority of cases (74/120), the first detection of *Salmonella* occurred after autopsy on calf carcasses/organs (Figure 1). Enteritis was the most common pathological finding (68/91), followed by hepatosplenomegaly (29/93) and pneumonia (23/91). The proportion of positive follow-up samples are listed in Table 1.

Discussion and Conclusion
The result of *Salmonella* serovars most frequently isolated is consistent with the data reported in Europe (1). The identification of

Salmonella fecal shedders was helpful in improving calving area management. *Salmonella* was isolated in the environment, both in collective pens for calves (22/61) and in maternity housing (12/33). The detection of *Salmonella* from various sources is evidence of its wide distribution on the farm. Therefore, during a *Salmonella* outbreak, control strategies should be rapidly implemented, including implementation of proper hygiene measures and isolation of infected animals to decrease the risk of disease transmission.

References

1. EFSA and ECDC. The European Union One Health 2022 Zoonoses Report. EFSA J 2023, 21, e8442, DOI 10.2903/ j.efsa.2023.8442

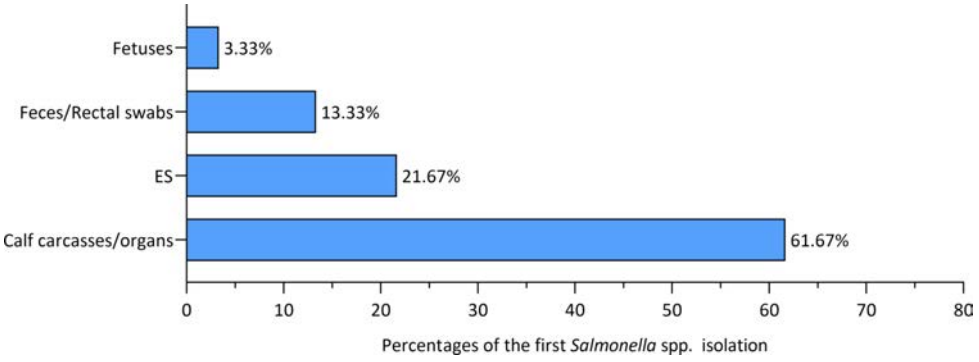


Figure 1. percentages of the first *Salmonella* isolations in the farm according to their source. ES: emergency slaughter samples.

Samples analyzed	N° farms with positive samples/N° total farms tested	N° positive samples/N° total samples
Feces/Rectal swabs	26/50	206/2145
Bulk milk	3/32	3/93
Calf carcasses/organs	15/21	21/36
Environmental overshoes	7/16	34/100
Environmental swabs/Sponges	4/7	9/27
Fetuses	2/3	3/4

Table 1. Analyses performed in the dairy cattle herds after first *Salmonella* detection.

.

P036

Animal health

INVESTIGATING THE ROLE OF *HELCOCOCCUS OVIS* AS PATHOGEN IN PNEUMONIA IN CALVES

F. Grégoire[2], C. De Meeûs D'Argenteuil[1], P. Cuvelier[2], M. Garigliany[1], F. Smeets[2], T. Petitjean[1], Z. Blockx[2]

[1]*FARAH, Faculty of Veterinary Medecine, University of Liège, Liège, Belgium*
[2]*Regional association for Animal Identification and Health, Ciney, Belgium*

Introduction

Helcococcus ovis is a facultative anaerobic, gram-positive coccus first described by Collins et al. in 1999. Since, it has been isolated from various pathological cases in different species such as valvular endocarditis, mastitis and abortions in cows, pulmonary abscess in horses and bronchopneumonia in sheep. Preliminary studies led in ARSIA showed that *H. ovis* is one of the most frequent taxon identified with 16S rRNA metagenetics in bovine pneumonia, this bacterium being almost absent in healthy lungs (1).

Materials and Methods

In order to confirm this observation, a real-time PCR was developed, targeting a gene coding for a putative chaperonin protein. The PCR was then applied to a set of lungs from calves (1-6 months) showing macroscopic lesions of pneumonia (n = 108) and to another set of macroscopically healthy lungs (n = 38). The lungs were sampled during routine necropsies. Alongside the *H. ovis* PCR, samples were subjected to bacteriological culture, PCR for common respiratory pathogens and histopathological examination.

Results
Four of the 38 healthy lungs gave a positive PCR result but with Ct over 36. Microscopical examination of these lungs showed congestion or a mild interstitial infiltrate. By contrast, 24 of the 118 lungs with lesions were positive on PCR, with an average Ct of 31,7 (min: 26,9 – max: 36,6). On histopathological examination, most lungs exhibited a severe diffuse necrotic, mostly acute, bronchopneumonia, with presence of small coccobacilli. In some cases, *H. ovis* was the only bacterium identified throughout the battery of tests performed. Analysis of the 16S rRNA metagenomics results showed that the detection of *H. ovis* in lungs with pneumonia was frequently associated with the presence of anaerobic or facultative anaerobic bacteria such as *Trueperella pyogenes, Bacteroides pyogenes* or *Fusobacterium necrophorum.*

Discussion and Conclusion
These results strongly suggest a link between the presence of *Helcococcus ovis* and the observation of lesions of bronchopneumonia in calves. Nevertheless, its pathogeny and its role as primary agent remains unclear.

References
1. Grégoire F. et al. 16S metagenomics by high-throughput sequencing : a tool for the etiological diagnosis of pneumonia in cattle?. Sixth Congress of the European Association of Veterinary Laboratory Diagnosticians - October 2022 – Sevilla.

P037

Animal health

STUDY ON *NEOSPORA CANINUM* DETECTION USING DIFFERENTIATED ELISA SEROLOGY

F. Gregoire[2], K. Lambert[1], K. Renier[1], L. Delooz[2], J. Evrard[2], P. Coppe[1], F. Bernard[1]

[1]*Bio-X Diagnostics, Rochefort, Belgium*
[2]*Regional association for Animal Identification and Health, Ciney, Belgium*

Introduction
Neosporosis due to *Neospora caninum* is an important disease that affects a large proportion of cattle in southern Belgium. It is a major cause of abortion, with about 10% of the cases imputed to the parasite. The Regional Association for Animal Health and Identification (ARSIA) has set up a control plan to eradicate the disease in herds on a voluntary basis. Since distinguishing between transient and permanent infection in adult animals is difficult, the diagnosis of vertically infected calves at birth is fundamental. Using an IgG2 detection test would allow to detect them without interference of the colostral immunity. The IgG2 test showed its reliability for the detection of vertically infected calves (0-1 month) in a previous investigation [1]; the aim of this study is to evaluate its use in adults and fetuses.

Materials and Methods
In a first part, an IgG2 ELISA kit was used for testing adult cattle, for which at least two blood samples (with a +/- 5 weeks interval) were taken before they were placed on the market. A total of 65 pairs of serum samples were analyzed using both ELISA analysis kits (IgG1/IgG2) in parallel. In a second part, 120 pairs of dam/aborted calf sera were tested with the two kits. The serum of aborted fetuses was collected on blotting paper.

Results
In the first part of the study, comparing the IgG2 results vs IgG1 for the 65 animals, the relative sensitivity was 94 % and the relative specificity 100 %, with a Kappa of 0,96.
In the second part of the study, aiming at comparing both tests in abortion syndrome for 120 pairs of dam/fetus, the study showed that both IgG2 and IgG1 tests can be applied on fetal samples. Nevertheless, a positive result in the dam doesn't imply systematically a positive result in its fetus.

Discussion and Conclusion
This study assesses the relevance of ELISA kits from Bio-X Diagnostics, implemented within the ARSIA laboratory and in collaboration with Walloon breeders.
The high concordance between both tests is in favor of using an unique IgG2 test in order to test cattle, adults as new- born calves.

References
1. Evrard J, Delooz L, Gregoire F. Neosporosis : how IgG2 ELISA could contribute in the diagnosis of vertically infected calves? abstract in World Buiatrics Congress 2022, Madrid

P038

Animal health

ENZOOTIC BOVINE LEUKOSIS: UPDATE OF ITALIAN LEGISLATION TO DISEASE CONTROL

C. Iscaro[2], R. Lomolino[3], D. Palma[1], L. Possenti[1], L. Ruocco[3], F. Feliziani[2]

[1]*Istituto Zooprofilattico Sperimentale Abruzzo e Molise "Giuseppe Caporale", Animal Identification and Registration Database (CSN), Teramo, Italy*
[2]*Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", National Reference Laboratory for Ruminants Retroviruses (CEREL), Perugia, Italy*
[3]*Ministry of Health, ex Direzione Generale della Sanità Animale e del Farmaco Veterinario, Ufficio III, Sanità Animale e Gestione Operativa del Centro Nazionale di Lotta ed Emergenza Contro le Malattie Animali e Unità Centrale di Crisi, Rome, Italy*

Introduction
Enzootic Bovine Leukosis (EBL) is listed as C disease according to the Regulation EU 2016/429 (1). Italy was declared EBL officially free in 2017 (2). Territorial infection clusters insist in Latium, Apulia, Campania regions. A surveillance programme must be carry out to the maintenance of EBL free status according to the Regulation EU 2020/689 (3), whereas EBL clusters are subject to specific eradication measures.

Materials and Methods
Ministry of Health, supported by CEREL and CSN, updated previous EBL guidelines 2018-2023 (4) according to the novel EU legislation. The working group refers mainly to Regulation EU 2016/429 and Regulation EU 2020/689. Information systems into VETINFO portal are required to be used for the following purposes (5): SANAN for data from surveillance activities; SIMAN for suspect and confirmed outbreaks notifications; CRUSCOTTI for activities monitoring.

Results
A new document was drafted, that contains modalities for official surveillance controls, management of EBL clusters, use and scope of official diagnostic tests, measures to be implemented in case of suspected and confirmed case, use of informative systems, tools for epidemiological surveys, and indications about disinfection of affected establishments.

Discussion and Conclusion
The novel EU legislation requires a new approach for diseases control. The risk-based approach was applied to the EBL surveillance national system. In EBL clusters more severe measures are required to eradicate the disease. Diagnostic indications are supplied to support the competent authorities, according to the novel definitions of suspected and confirmed cases. Practical information are also provided in case of EBL detection in an establishment. The new guidelines are needed to standardize the control activities for EBL at national level and the use of informative systems for data collection and analysis.

References
1. https://eur-lex.europa.eu/legal-content/IT/TXT/?qid=1717052675612&uri=CELEX%3A02016R0429-20210421
2. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017D1910&qid=1717078271803
3. https://eur-lex.europa.eu/legal-content/IT/TXT/?qid=1717052122536&uri=CELEX%3A02020R0689-20231011
4. https://www.izsum.it/area_letturaMalattia/25/pagsistema.html
5. https://www.vetinfo.it/

P039

Animal health

IDIOPATHIC IMMUNOMEDIATED HEMOLYTIC ANEMIA IN A HORSE

K. Satué[1], M. Velasco-Martínez[1], D. Lafauci[2], E. Fazio[2], P. Medica[2]

[1]*CEU Cardenal Herrera University*
[2]*University of Messina*

Introduction
The erythrocyte regeneration in anemic conditions in horses is complex (in Satué et al., 2023). Serial blood counts in which the increase in MCV and erythrocyte distribution width (RDW) persists for days or weeks help to retrospectively evaluate the regenerative response in this species. Both VCM and RDW are sensitive in detecting erythrocyte size heterogeneity under anemic conditions. Average RDW values that in healthy animals' range between 15.2% to 19.8%, in intense hemolysis reach a value of 26.4% (Radin et al., 1986).

Materials and Methods
A 5-year-old Hanoverian stallion is referred with loss of physical performance, weakness, apathy and jaundice.

Results
Serial blood counts over 5 days revealed average values of PCV, MCV, RDW of 21.2%, 79 fL, 25%, respectively. In the blood smear, intense macrocytosis and increase in Howell-Jolly bodies, without changes in leukocytes was showed. PCR was performed for the detection of B. caballi, T. equi, A. phagocytophilum and AIE with negative results. Neoplastic processes were ruled out by abdominal ultrasound and thoracic x-rays. Neither Heinz bodies, schistocytes nor intraerythrocytic parasites were visualized, ruling out oxidative or microangiopathic causes of hemolysis. Biochemical analysis revealed a slight increase in GGT and FAL without changes in GLDH, total bilirubin, bile acids, fibrinogen, total proteins and albumin, glucose, BUN, creatinine and coagulation times, ruling out possible liver or renal disease or hemolytic uremic syndrome.

Discussion and Conclusion
Although IMHA in adult horses is a rare disorder, certain infectious diseases such as *B. caballi* or *T. equi*, Anaplasma and Equine Infectious Anemia, the administration of certain drugs and neoplasms have been related (In: Satué et al., 2023). After ruling out these possible causes, and based on the laboratory results, AHIM of idiopathic origin was diagnosed.

References
Radin, M.J., Eubank, M.C., Weiser, M.G. (1986): Eletronic measurement of erythrocyte volume and volume heterogeneity in horses during erythrocyte regeneration associate with experimental anemias. Vet. Pathol. 23(6): 656-660.

Satué, K., Fazio, E., Gardón, J.C., Medica P. (2023): Contribution of Hemogram Plan in the Horse's Clinical Evaluation. J Equine Vet Sci. 126: 104292.

P040

Animal health

ASSESSMENT OF GLYCEMIA, CHOLESTEROLEMIA AND TRIGLYCERIDEMIA AS MARKERS OF INSULIN RESISTANCE IN SPANISH PUREBREED MARES DURING LACTATION

K. Satué[1], M. Velasco-Martínez[1], D. Lafauci[2], E. Fazio[2], P. Medica[2]

[1]*CEU Cardenal Herrera University, Spain*
[2]*University of Messina, Italy, Italy*

Introduction
In mares of various breeds, such as Thoroughbred, Standardbred, Arabians and ponies, mainly in obese animals, lactation leads to a decrease in glycemia (GLU), triglyceridemia and cholesterolemia (Harvey et al., 2005; Aoki to Ishii, 2012; Satué et al., 2022). This response is attributed to the excretion of these metabolites for the formation of dairy components at the mammary gland, leading at the same time to a peripheral state of insulin resistance (IR) (Rasmussen, 2007). IR decreases the absorption of GLU and favors lipogenic action in the liver, muscle and adipose tissue, with production of triglycerides (TRIG), release of other cholesterol particles (CHOL) and, consequently, the development of dyslipidemia.

Materials and Methods
The objective of this study was to analyze the levels of GLU, TRIG and CHOL during the first 4 months of lactation in 24 Spanish Purebred mares. Plasma concentrations of GLU, TRIG and CHOL were determined by spectrophotometry (Spin 120E).

Results
The comparative study between the months of lactation did not reveal variations in the plasma levels of these metabolites, detecting mean GLU values of 90.06±10.91, 93.53±10.77, 95.60±17.01 and 95.22±16.03 mg/dl, TRIG
values of 21.12±26.27, 17.15±31.66, 12.71±15.34 and 12.09±14.84 mg/dl and COL values of 94 .20±21.60, 99.76±12.16, 94.36±17.54 and 100.8±12.61 mg/dl, respectively.

Discussion and Conclusion
The results of this study demonstrate, unlike other studies, that in Spanish Purebred mares of normal body condition (Henneke 5/9) lactation does not guarantee a physiological mechanism of insulin resistance.

References
Aoki, T. and Ishii, M. (2012): Hematological and biochemical profiles in peripartum mares and neonatal foals (heavy draft horse). J. Equine Vet. Sci. 32, 170–176.

Harvey, J. W., Pate, M. G., Kivipelto, J., Asquith, R. L. (2005): Clinical biochemistry of pregnant and nursing mares. Vet. Clin. Pathol. 34, 248–254.

Rasmussen, K.M. (2007): Association of Maternal Obesity before Conception with poor lactation performance. Annu. Rev. Nutr. 27:103–21.
Satué, K., Fazio, E., Muñoz, A., Medica, P. (2022): Successful Pregnancy Outcome in Mares: The Potential Role of Body Conditional Score, Age and Biochemical Parameter's Adjustments, J. Equine Vet. Sci. 115, 104023.

P041

Animal health

COMPLETE GENOMIC SEQUENCE OF A PORCINE RESPIROVIRUS 1 (PRV1) ISOLATED IN ITALY

G. Leo[1], C. Bertasio[1], V. Carta[1], D. Lelli[1], C. Tolini[1], M. Carrera[1], T. Trogu[1], A. Moreno[1], A. Lavazza[1], E. Sozzi[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "Bruno Ubertini" (IZSLER), Brescia, Italy*

Introduction

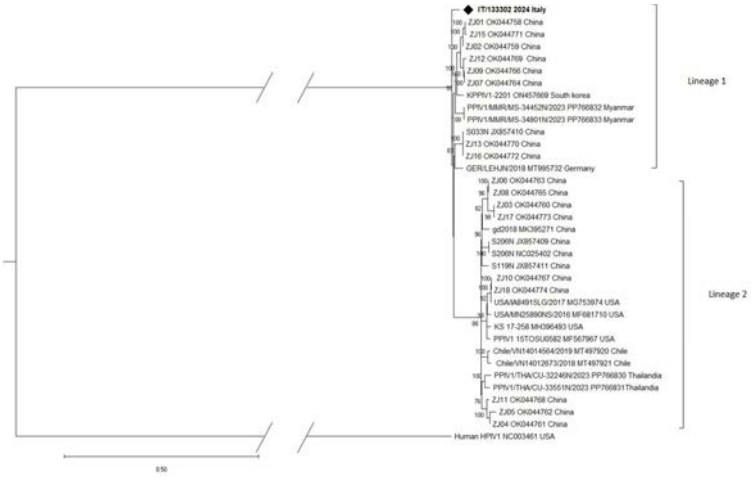
Porcine respirovirus type 1 (PRV1), a member of the genus *Respirovirus* in the family Paramixoviridae, was first detected from rectal and nasopharyngeal swabs of slaughtered pigs in Hong Kong in 2013[1]. PRV1 has been subsequently identified in America, South Korea and several European countries, including Northern Italy. The consequence of PRV1 infection on pig health is largely unknown. However, the virus could play a role as a co-factor with other respiratory viruses, such as swine influenza virus (SIV) and porcine reproductive and respiratory syndrome virus (PRRSV), in causing the Porcine Respiratory Disease Complex. Our study aimed to achieve the full-length genomic sequence of Porcine respirovirus 1, strain 133302/2024, identified by real-time RT-PCR in a deceased pig from a farm in North Italy.

Materials and Methods

The presence of Porcine respirovirus 1 has been determined in a pig with respiratory symptoms using a real-time RT-PCR gene-HN screening method. The cycle threshold (Ct) value in the sample was 24,96. The complete genome was obtained using the MiniSeq platform (Illumina, San Diego, CA, USA). Sequencing libraries were made with an Illumina Stranded Total RNA Prep with Ribo-Zero Plus kit according to the manufacturer's instructions. Reads of 133302_2024 were mapped vs strain S206N using Geneious Prime v. 2022.2.1. To create the PRV1 phylogenetic tree, the assembled PRV1 genome from this study and PRV1 genomes from GenBank were aligned with MAFFT. Phylogenetic trees were inferred with the Maximum Likelihood approach implemented in under the General Time Reversible (GTR+G+I) substitution model and bootstrap of 1000 replicates.

Results

The complete genome of 133302/2024 was 15348 (nt) in length. The whole genome showed 95.7% nucleotide identity with the ZJ16 (OK044772) strain identified in China (2022), within the European lineage 1 (Figure 1).



1. https://eur-lex.europa.eu/legal-content/IT/TXT/?qid=1717052675612&uri=CELEX%3A02016R0429-20210421
2. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32017D1910&qid=1717078271803
3.https://eur-lex.europa.eu/legal-content/IT/TXT/?qid=1717052122536&uri=CELEX%3A02020R0689-20231011
4. https://www.izsum.it/area_letturaMalattia/25/pagsistema.html
5. https://www.vetinfo.it/

Figure 1. Phylogenetic analysis of Italian complete genomes PRV1 strain (black rhombus) with global PRV1 strains available in GenBank. Human Respirovirus 1 was used as an outgroup.

Discussion and Conclusion
This is, to our knowledge, the first complete genomic sequence of a PRV1 strain isolated in Italy. Its availability will allow future comparative genomic studies of PRV1, extending our knowledge of its diversity and evolution.

References
1. Lau SKP et al. Identification and characterization of a novel paramyxovirus, porcine parainfluenza virus 1, from deceased pigs. J Gen Virol. 2013 Oct;94(Pt 10):2184-2190.

P042

Animal health

CANINE DISTEMPER VIRUS IN WILDLIFE, ITALY (2022-2024)

M.G. Lucibelli[1], N. D'Alessio[1], S. Rea[1], C. Auriemma[1], M.S. Lucente[2], F. Pellegrini[2], G. Diakoudi[2], E. De Carlo[1], N. Decaro[2], G. Lanave[2], V. Martella[2], G. Fusco[1], F. Alfano[1]

[1]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy*
[2]*Università degli Studi di Bari, Dipartimento di Medicina Veterinaria, Italy*

Introduction
Canine distemper virus (CDV) is responsible for a fatal disease in domestic and wild animals and also represents a high health risk because of the facility to cross the species barriers (1, 2). This study aimed to investigate the presence of canine distemper virus (CDV) infection in wildlife from southern Italy (2022-2024).

Materials and Methods
A total of 129 wild animals (stone martens, porcupines, otters, wolves, martens, badgers, foxes) were tested for CDV over 3 years (2022-2024). The sampled animals included: 4 stone martens, 12 porcupines, 6 otters, 10 wolves, 3 martens, 30 badgers, 64 foxes. Biological matrices (lung, liver, heart, spleen, kidney, intestine, brain) were extracted using an automatic extractor (QIAsymphony, Qiagen) and the CDV RNA was detected by real-time RT-PCR (1). Positive samples were sequenced using the nanopore technology (3). Phylogenetic analysis was done with maximum likelihood methods (GTR model with gamma distribution with invariable sites and 1000 replicates for statistical support).

Results
Out of the 129 wild animals analyzed, 14 (10.8%) tested positive for CDV (2 wolves, 1 marten, 6 badgers and 5 foxes). For a badger CDV strain, we obtained the whole genome sequence (canine distemper virus strain ITA/2024badger/33340). The sequence was aligned with cognate sequences available in GenBank and a phylogenetic tree was obtained (Figure 1). The sequence segregated within the Arctic-like cluster with other badger CDV strains.

Discussion and Conclusion
CDV was detected in nearly 11% of the animals tested in this study. The complete genome of a CDV strain was obtained and characterized as an Arctic-like. This lineage was first reported in animals of the Arctic ecosystem but seems common in European territories in both domestic and wildlife carnivores. Interestingly, the badger CDV strain clustered in the phylogenetic tree (Figure 1) with other badger and dog strains from Italy. The data from our work suggests the need for continuous surveillance, to understand better the ecology of CDV in domestic and wildlife animals.

This study was funded by the Italian Ministry of Health: Ricerca Corrente 2023, IZS ME 08/23 RC (recipient Flora Alfano)

References
1. Alfano et al. 2022.
2. Di Sabatino et al. 2016
3. Lanszki et al. 2022.

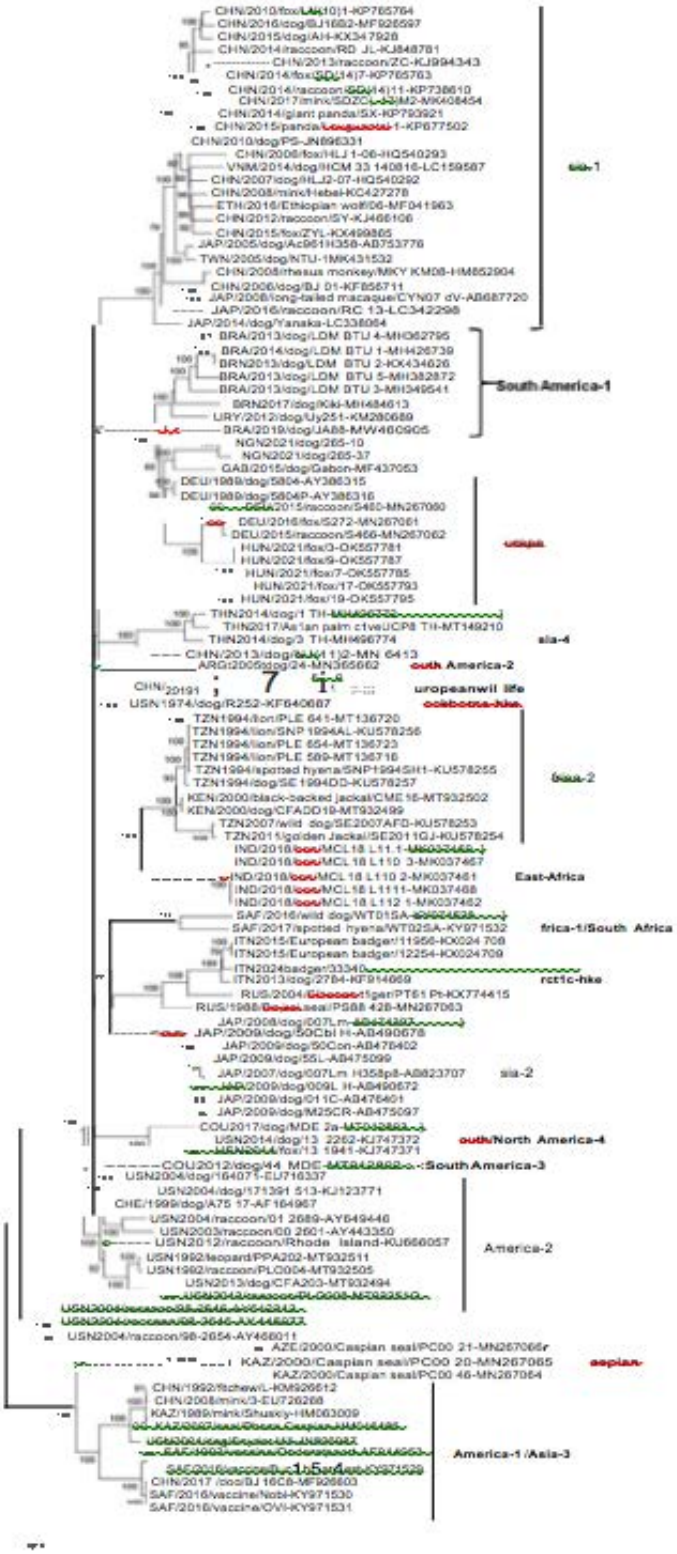

CHN/2010/fox/LH/10/1-KP765764
CHN/2016/dog/BJ1602-MF926597
CHN/2015/dog/AH-KX347528
CHN/2014/raccoon/RD JL-KJ848781
CHN/2013/raccoon/ZC-KJ994343
CHN/2014/fox/SDJ14/7-KP765763
CHN/2014/raccoon/SDJ14/11-KP738610
CHN/2017/mink/SDZC-L-2/M2-MK408454
CHN/2014/giant panda/SX-KP793921
CHN/2015/panda/Longyou/1-KP677502
CHN/2010/dog/PS-JN896331
CHN/2008/fox/HLJ 1-06-HQ540293
VNM/2014/dog/HCM 33 140816-LC159587
CHN/2007/dog/HLJ2-07-HQ540292
CHN/2008/mink/Hebei-KC427278
ETH/2016/Ethiopian wolf/06-MF041963
CHN/2012/raccoon/SY-KJ466106
CHN/2015/fox/ZYL-KX499805
JAP/2005/dog/Ac861H358-AB753776
TWN/2005/dog/NTU-1/MK431532
CHN/2008/rhesus monkey/MKY KM08-HM852904
CHN/2006/dog/BJ 01-KF856711
JAP/2008/long-tailed macaque/CYN07 dV-AB687720
JAP/2016/raccoon/RC 13-LC342298
JAP/2014/dog/Yanaka-LC338084
BRA/2013/dog/LDM BTU 4-MH062795
BRA/2014/dog/LDM BTU 1-MH426739
BRN2013/dog/LDM BTU 2-KX434626
BRA/2013/dog/LDM BTU 5-MH382872
BRA/2013/dog/LDM BTU 3-MH349541
BRN2017/dog/Kiki-MH484613
URY/2012/dog/Uy251-KM280689
BRA/2018/dog/JAB8-MW460905
NGN2021/dog/265-10
NGN2021/dog/265-37
GAB/2015/dog/Gabon-MF437053
DEU/1988/dog/5804-AY386315
DEU/1988/dog/5804P-AY386316
DEU/2015/raccoon/S460-MN267060
DEU/2016/fox/S272-MN267061
DEU/2015/raccoon/S466-MN267062
HUN/2021/fox/3-OK557781
HUN/2021/fox/9-OK557787
HUN/2021/fox/7-OK557785
HUN/2021/fox/17-OK557793
HUN/2021/fox/19-OK557795
THN2014/dog/1 TH-MH460768
THN2017/Asian palm civet/CP8 TH-MT149210
THN2014/dog/3 TH-MH460774
CHN/2013/dog/BJ/411/2-MN 6413
ARG/2005/dog/24-MN365662
CHN/20191
USN/1974/dog/R252-KF640687
TZN1994/lion/PLE 641-MT136720
TZN1994/lion/SNP 1994AL-KU578256
TZN1994/lion/PLE 654-MT136723
TZN1994/lion/PLE 589-MT136716
TZN1994/spotted hyena/SNP1994SH1-KU578255
TZN1994/dog/SE1994DD-KU578257
KEN/2000/black-backed jackal/CME16-MT932502
KEN/2000/dog/CFADD19-MT932499
TZN2007/wild dog/SE2007AFD-KU578253
TZN2011/golden Jackal/SE2011GJ-KU578254
IND/2018/goa/MCL18 L11 1-MK037458
IND/2018/goa/MCL18 L110 3-MK037467
IND/2018/goa/MCL18 L110 2-MK037461
IND/2018/goa/MCL18 L111-MK037468
IND/2018/goa/MCL18 L112 1-MK037462
SAF/2016/wild dog/WT01SA-KP994638
SAF/2017/spotted hyena/WT02SA-KY971532
ITN2015/European badger/11956-KX024708
ITN2015/European badger/12254-KX024709
ITN2024/badger/3334
ITN2013/dog/2784-KF914669
RUS/2004/Caucasus/1per/PT61 Pt-KX774415
RUS/1988/Baikal seal/PS88 428-MN267063
JAP/2008/dog/007Lm-AB623707
JAP/2009/dog/50Cbl H-AB490678
JAP/2009/dog/50Con-AB475402
JAP/2009/dog/55L-AB475099
JAP/2007/dog/007Lm H358p8-AB623707
JAP/2009/dog/009L H-AB490672
JAP/2009/dog/01C-AB476401
JAP/2009/dog/M25CR-AB475097
COU2017/dog/MDE 2a-MT943883
USN2014/dog/13 2262-KJ747372
USN2014/fox/13 1941-KJ747371
COU2012/dog/44 MDE-MT943882
USN2004/dog/164071-EU716337
USN2004/dog/171391 513-KJ123771
CHE/1999/dog/A75 17-AF164967
USN2004/raccoon/01 2689-AY649446
USN2003/raccoon/00 2601-AY443350
USN2012/raccoon/Rhode Island-KU666057
USN1992/leopard/PPA202-MT932511
USN1992/raccoon/PLC004-MT932505
USN2013/dog/CFA203-MT932494
USN2004/raccoon/98-2654-AY468011
AZE/2000/Caspian seal/PC00 21-MN267066
KAZ/2000/Caspian seal/PC00 20-MN267065
KAZ/2000/Caspian seal/PC00 46-MN267064
CHN/1992/Ilchew/L-KM926612
CHN/2008/mink/3-EU726268
KAZ/1989/mink/Shusky-HM063009
CHN/2017 /dog/BJ 16C8-MF926603
SAF/2016/vaccine/Nobi-KY971530
SAF/2016/vaccine/OVI-KY971531
South America-1
uropean
sia-4
outh America-2
uropeanwil life
olebooma-like
boua-2
East-Africa
frica-1/South Africa
rct1c-like
sia-2
outh/North America-4
-South America-3
America-2
caspian
America-1 /Asia-3

Figure 1.

P043

Animal health

OCCURRENCE OF AFLATOXIN B1 IN FEED COMMERCIALIZED IN SICILY (SOUTHERN ITALY)

A. Macaluso[2], G. Cammilleri[2], A. Cicero[2], L. Pantano[2], P. Riolo[2], E.M.D. Messina[2], C. Alfano[2], M.B. Drussilla[2], F.G. Galluzzo[1], V. Ferrantelli[1], G.M. Lo Dico[1]

[1] *Istituto Zooprofilattico Sperimentale della Sicilia A. Mirri, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Sicilia, Italy*

Introduction
Aflatoxin B1 (AFB1), produced by *Aspergillus* species, poses significant health risks to animals and humans. When present in animal feed, AFB1 can be metabolized by dairy animals into aflatoxin M1 (AFM1), which is then excreted into milk, extending the health risk to consumers. To mitigate these risks, the European Union has established maximum residue levels (MRLs) for AFB1 in food and feed, specified in Directive 2002/32/EC and Commission Regulation (EU) No 574/2011 [1]. These limits are 0.005 mg/kg for dairy animals, 0.02 mg/kg for feed materials and compound feed for cattle, and 0.01 mg/kg for complementary and complete feed.

Materials and Methods
In this study, sixty samples were analysed using High-Performance Liquid Chromatography coupled with Fluorescence Detection (HPLC-FLD), including twenty samples each from cattle, cow, and poultry feed. The method's LOD and LOQ were 0.00035 mg/kg and 0.00116 mg/kg, respectively.

Results
Resukts showed that 15% of cattle feed samples, 10% of cow feed samples, and 5% of poultry feed samples had detectable levels of AFB1. The mean AFB1 concentrations were 0.0044 mg/kg for cattle feed, 0.0049 mg/kg for cow feed, and 0.0056 mg/kg for poultry feed. None of the samples exceeded the EU regulatory limits.

Discussion and Conclusion
These findings highlight that while detectable levels of AFB1 were present in some samples, all were within the permissible limits, underscoring the importance of ongoing monitoring and regulatory compliance to ensure food safety. However, the sample size must be increased to improve the reliability and robustness of the results, emphasizing the need for ongoing monitoring and regulatory compliance to ensure food safety.

References
1. European Commission Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on Undesirable Substances in Animal Feed. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02002L0032-20191128 (accessed on June 1).

P044

Animal health

FATAL INFECTION ASSOCIATED WITH *ERYSIPELOTHRIX* SP. IN THREE FREE-RANGING BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*) STRANDED ALONG THE TUSCANY COAST OF ITALY

M. Maestrini[4], C. Cocumelli[5], A. Franco[5], F. Bellagamba[4], E. Bovi[5], E.L. Diaconu[5], A. Ianzano[5], T. Palmerini[5], R. Parmigiani[5], F. Stravino[5], C. Mancusi[1], L. Marsili[2], G. Di Guardo[3], G. Terracciano[4]

[1]*ARPAT-Agenzia Regionale Protezione ambientale Toscana, Via Marradi 114, 57126 Livorno, Italy*
[2]*Dipartimento di Scienze Fisiche, della Terra e dell'Ambiente, Università degli Studi di Siena, Siena, Italy*
[3]*Former Professor of General Pathology and Veterinary Pathophysiology at the Veterinary Medical Faculty of the University of Teramo, Italy*
[4]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Pisa, Italy*
[5]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Roma, Italy*

Introduction
Erysipelothrix sp. is a primary bacterial pathogen in mammals, birds, reptiles, fishes and humans, being of uncommon detection but potentially lethal to free-ranging and captive cetaceans.

Materials and Methods
Between March and May 2024, three free-ranging bottlenose dolphins (*Tursiops truncatus*) were found stranded along the coast of Tuscany. During necropsies, samples for bacteriological, virological and histopathological analyses were collected. After bacterial cultures, isolates were identified through biochemical (API® Coryne, bioMérieux) and 16s rRNA sequencing.

Results
At necropsy, haemorrhage and/or congestion of visceral organs, meninges and brain, along with sero-fibrinous and sero-haemorrhagic effusions in the pericardial and peritoneal cavities were observed in all three animals. Prescapular and mesenteric lymphadenomegaly was also present. Most relevant microscopic lesions were represented by intravascular thrombi, admixed with filamentous bacteria, particularly evident in lung capillaries and renal glomerular tufts; a moderate to severe, diffuse, lymphocytic meningoencephalitis with perivascular haemorrhages were observed. Rod-shaped, Gram-positive or Gram-variable bacterium, consistent with Erysipelothrix sp., were isolated from several tissues of the three cetaceans, including endocardial thrombus, brain, liver, lung, spleen and lymph nodes. Other cetacean major pathogens were not identified. Biochemical tests and 16s rRNA sequence analysis allowed to classify the isolates within the Erysipelothrix genus.

Discussion and Conclusion
This study highlights the importance of a constant monitoring of *Erysipelothrix* sp. circulation in the marine environment, assessing potential cetacean disease outbreaks and protecting human health, with special emphasis on occupational exposure.

References
Ceccolini M.E. et al. (2021). Dis Aquat Org 145:173-184. Lee K. et al. (2022). Front Vet Sci.
Martino L. et al. (2024). Dis Aquat Organ. 157:31-43.
Pomaranski EK et al. (2020). Int J Syst Evol Microbiol. 2020 Feb;70(2):857-867.

P045

Animal health

ENHANCED ARBOVIRUS SURVEILLANCE: TWO-YEAR MONITORING DATA FROM PIEMONTE, LIGURIA AND VALLE D'AOSTA (2022-2023)

C. Nogarol[5], O. Portanti[6], E. Trabunella[2], S.M. Di Trani[1], V. Listorti[4], S. Robetto[3], I.N. Miceli[5], S. Brusadore[5], E. Ciarrocchi[6], R. Irelli[6], G. Savini[6], M.L. Mandola[5]

[1]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, S.S. Cuneo, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, S.S. Patologia Animale, Italy*
[3]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, S.S. Patologia della Fauna Selvatica, Centro di Referenza Nazionale Malattie Animali Selvatici (CeRMAS), Italy*
[4]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, S.S. Sezione di Genova e Portualità Marittima, Italy*
[5]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, S.S. Virologia Specialistica, Italy*
[6]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Laboratorio di Referenza WOAH e Centro di Referenza , Italy*

Introduction
Usutu (USUV) and West Nile viruses (WNV) are mosquito-borne flaviviruses that have significant impacts on public health globally. Climate change significantly influences vector-borne diseases: warmer temperatures and altered precipitation patterns can expand the geographical range and seasonal activity of mosquito. The risk of virus transmission may also be increased by the arrival of migratory birds, reservoirs for these viruses. In the past 25 years, Italy has experienced numerous incursions of WNV and USUV, becoming the European country with the highest number of cases. This study aims to present our findings from 2 years of surveillance for WN and USUTU viruses.

Materials and Methods
Passive surveillance was conducted on wild birds found dead and through syndromic surveillance of neurological cases in equids; active surveillance involved culling wild birds of target species. For equids, blood samples and tissues from both live and dead subjects were analyzed. Viral RNA was extracted and purified and then assayed by RT-PCR to detect WNV Lineage 1 and 2 and USUV. All positive samples were sent to the WOAH and NRL for confirmation.

Results
Across the three regions of competence, a total of 1026 animals underwent analysis in 2022: 717 from Piemonte, 118 from Liguria and 191 from Valle d'Aosta. Out of these, 20 animals tested positive for WNL2 with only one *Pica pica* positive for USUV. In 2023, a total of 1307 animals underwent testing: 1014 from Piemonte, 174 from Liguria and 119 from Valle d'Aosta. In this year, 24 animals tested positive for WNL2, 5 tested positive for USUV and 5 turned co- infected with WNL2 and USUV. All positive samples originated from Piemonte, except for 2 WNL2 and 2 USUV positive samples from Liguria, collected in 2023. Positive samples are listed in Table 1, 2 and 3.

Discussion and Conclusion
Our results show that positive samples were found during uncommon periods for these viruses. In 2022, positivity in October and

November highlights how warmer winter temperatures can influence WNV circulation levels, prolonging the active season for mosquitoes and increasing the window of time for viral transmission. Such knowledge is crucial to strengthen prevention measures in winter to mitigate the potential risks posed by WNV and USUV both for animal and human populations.

Sample ID	Specie	Positive Matrix	Date
1	*Accipiter gentilis*	SNC	February 2022
2	*Corvus corone*	SNC Heart Spleen Kidney	July 2022
3	*Equus ferus caballus*	SNC	August 2022
4	*Corvus corone*	SNC	August 2022
5	*Ardea cinerea*	SNC	August 2022
6	*Equus ferus caballus*	SNC Spleen	August 2022
7	*Garrulus glandarius*	SNC Heart Spleen Kidney	September 2022
8	*Turdus merula*	SNC Heart Spleen	September 2022
9	*Turdus merula*	SNC	September 2022
10	*Corvus corone*	SNC Heart Spleen Kidney	September 2022
11	*Columba palumbus*	SNC Heart Kidney	September 2022
12	*Falco*	SNC Heart Spleen Kidney	September 2022
13	*Accipiter gentilis*	SNC Heart Spleen Kidney	September 2022
14	*Pica pica*	Heart Spleen Kidney	October 2022
15	*Falco tinnunculus*	SNC Heart Spleen Kidney	October 2022
16	*Accipiter gentilis*	SNC Heart Kidney	October 2022
17	*Corvus corone*	SNC Heart Spleen Kidney	October 2022
18	*Pica pica*	SNC	November 2022
19	*Pica pica*	SNC Heart Spleen Kidney	November 2022
20	*Pica pica*	SNC Heart Spleen Kidney	November 2022

Table 1. Details of WNL2 positive samples (year 2022).

Sample ID	Specie	Positive Matrix	Date
1	*Corvus corone*	SNC	June 2023
2	*Accipiter gentilis*	SNC Heart Spleen Kidney	July 2023
3	*Columba palumbus*	SNC	July 2023
4	*Corvus corone*	SNC Heart Spleen Kidney	July 2023
5	*Pica pica*	SNC Heart Spleen Kidney	July 2023
6	*Corvus*	Heart Spleen Kidney	July 2023
7	*Corvus corone*	SNC Heart Spleen Kidney	July 2023
8	*Corvus*	SNC Heart Spleen Kidney	August 2023
9	*Pica pica*	SNC Heart Spleen Kidney	August 2023
10	*Garrulus glandarius*	SNC Heart Spleen Kidney	August 2023
11	*Garrulus glandarius*	SNC	August 2023
12	*Phalacrocorax carbo*	Spleen Kidney	August 2023
13	*Corvus corone*	SNC	August 2023
14	*Pica pica*	SNC pool	August 2023
15	*Larus michahellis*	SNC Heart Spleen Kidney	August 2023
16	*Garrulus glandarius*	SNC	August 2023
17	*Columba palumbus*	SNC	August 2023
18	*Turdus merula*	SNC	August 2023
19	*Passer italiae*	SNC pool	August 2023
20	*Columba palumbus*	SNC pool	August 2023
21	*Apus pallidus*	SNC	August 2023
22	*Athene noctua*	SNC	August 2023
23	*Corvus corone*	SNC	September 2023
24	*Corvus corone*	SNC	September 2023
25	*Corvus corone*	SNC Heart Spleen Kidney	September 2023
26	*Corvus corone*	SNC	September 2023
27	*Columba palumbus*	Spleen	October 2023
28	*Garrulus glandarius*	SNC	October 2023
29	*Corvus corone*	SNC Heart Spleen	September 2023

Table 2. Details of WNL2 positive samples (year 2023).

Sample ID	Specie	Positive Matrix	Date
1/2022	*Pica pica*	SNC	October 2022
1	*Turdus merula*	SNC pool	July 2023
2	*Bubulcus ibis*	SNC	July 2023
3	*Turdus merula*	SNC pool	August 2023
*4	*Columba palumbus*	SNC	August 2023
*5	*Turdus merula*	SNC pool	August 2023
*6	*Passer italiae*	SNC pool	August 2023
*7	*Columba palumbus*	pool	August 2023
*8	*Athene noctua*	SNC	August 2023
9	*Turdus merula*	SNC Heart	July 2023
10	*Passeriformes*	SNC Heart Kidney	July 2023

*co-infection WNL2 and USUTUV

Table 3. Details of USUTUV positive samples (years 2022-2023).

P046

Animal health

PREVALENCE STUDY OF *BRUCELLA CANIS* IN DOGS RESIDING IN UMBRIA: THE COMPLEXITY OF CHOOSING APPROPRIATE DIAGNOSTIC TESTS

M.L. Marenzoni[5], S. Attura[5], B. Favi[4], M.T. Antognoni[5], M.B. Conti[5], A. Felici[2], C. Maresca[2], E. Scoccia[2], M.R. Bonci[4], A. Pistolesi[4], S. Zanghì[4], A. Confaloni[3], D. Marini[5], L. Sylla[5], F. De Massis[1], F. Sacchini[1], M. Tittarelli[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy*
[2]*Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", Perugia, Italy*
[3]*Private Practitioner, Perugia, Italy*
[4]*Servizio di Sanità Animale, USLUMBRIA[1], Perugia, Italy*
[5]*University of Perugia, Italy*

Introduction
Brucella canis is an emerging infection in Europe, with significant zoonotic potential. This study aims to determine the prevalence of *B. canis* infection in a canine population in Umbria, Italy. This is the first national study on this subject, which could be used as a model for future regional studies.

Materials and Methods
Blood samples (serum, plasma, and complete blood) were collected from various groups of dogs in Umbria: 1) dogs from three sanitary kennels; 2) breeding dogs; 3) blood donor dogs; 4) dogs accompanying refugees from Ukraine; 5) dogs from a large *B. canis* outbreak. Samples were tested using direct (bacteriological isolation and real-time PCR) and indirect methods (serum agglutination and complement fixation test).

Results
The study population consisted of diverse groups of dogs, varying in age, sex, and breed, representative of the regional population. Age ranged from 2 months to 17 years (mean and median age of 3 years). The gender distribution was nearly equal, with 64 males, 62 females, and 2 unknown due to anonymization. These dogs represented over 21 breeds, with mixed breeds comprising 25.78% of the population, followed by Golden Retrievers (19.53%) and Yorkshire Terriers (13.28%). Based on sample categories, not all tests could be conducted on every sample, necessitating the selection of the most appropriate tests depending on the biological sample type and possible infection phase. None of the 128 dogs tested positive for *B. canis*, suggesting a potential maximum prevalence of up to 3.5% (CI 95%).

Discussion and Conclusion
The complexity of diagnostic systems must be considered for reliable *B. canis* infection results. Despite no positive cases, the study underscores the need for continuous surveillance of *B. canis* in canine populations due to its zoonotic potential, risk factors for its introduction, and economic impact on breeding.

References
De Massis, F., et al. "First Isolation of *Brucella* canis from a breeding kennel in Italy." Veterinaria italiana 57.3 (2021). Djokic, V., et al. "The emergence of *Brucella* canis as a public health threat in Europe: what we know and what we need to learn." Emerging microbes & infections 12.2 (2023): 2249126.

P047

Animal health

LEPTOSPIROSIS IN CENTRAL-ITALY: A RETROSPECTIVE CONTEST ANALYSIS ON DOMESTIC ANIMALS AND HUMANS IN YEARS 2018-2022

S. Costarelli[2], S. Crotti[2], C. Panzieri[2], V. Piscioneri[2], A. Fiorucci[2], E. Scoccia[2], C. Maresca[2], P. Cristini[1], M.L. Marenzoni[1]

[1]*Dipartimento di Medicina Veterinaria, Università degli Studi di Perugia, Italy*
[2]*Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", IZSUM, Italy*

Introduction
The aim of this work is to obtain epidemiological information related to leptospirosis in the Umbria and Marche regions (Central Italy) using data from the laboratory of IZSUM.

Materials and Methods
The results were collected over the period 2018-2022 in non-wild animals from the Marche and Umbria regions, sampled because of clin-

ical or anatomical-pathological suspicion, human included.
The sample tested positive by qPCR and/or MAT was considered as "positive case".

Results

The obtained data was divided into 4 groups:

1) Livestock: 528 animals (bovine, ovine, caprine, equine, swine) were tested (Table 1). The number of tested farms, the positive results and the relative percentage are reported in Table 2. The most frequent serovars were: Pomona, Hardjo and Icterohaemorrhagiae in cattle herds; Bratislava, Icterohaemorrhagiae, Grippotyphosa in equidae and Hardjo in sheep.
2) Pets: 275 dogs and 11 cats (286) were tested (Table 3). Positivity was found in 55 dogs; the most frequent serovar was Icterohaemorrhagiae.
3) Human: 40 samples were tested, and positivity was found in 5 people. The serovars obtained were Ballum, Icterohaemorrhagiae and Grippotyphosa.
4) Bulls of the Genetic Centre: the animals were subjected to a monthly serological check (682 samples/5 years) as part of a surveillance system, in absence of clinical manifestations. 8 animals were positive (6 buffalo and 2 cattle). Hardjo, Pomona and Tarassovi were the serovars found, with antibody titers range between 1:100 and 1:400

Discussion and Conclusion

The results showed that: 1) Leptospira is widespread in the Umbria and Marche regions; 2) monitoring and application of preventive measures, especially vaccination, are relevant; 3) detection of leptospirosis appears sporadic in humans, linked more not to recreational than occupational exposure. This study, moreover, identified the serotypes of pathogenic *Leptospira* present in the considered territory, useful for diagnosis and vaccine update.

References

Tagliabue et al., 2016. Serological surveillance of Leptospirosis in Italy: two-year national data (2010-2011). Vet Italiana, 52: 129–138
Balboni et al., 2023. Identification of the most effective serovars to be included in the MAT antigen panel to optimize the serodiagnosis of Leptospira infection in Northern Italy. Vet Res Comm., 47: 2185–2192

Year	Bovine	Caprine	Equine	Ovine	Swine	Total
2018	8	21	6	4	4	43
2019	28	7	4	13	53	105
2020	15	-	5	-	2	22
2021	39	4	6	6	57	112
2022	86	13	7	128	12	246

Table 1. Productive animals tested: distribution by species and year

Year	Bovine		Equine		Swine		Ovine		Caprine	
	T	P (%)	T	P (%)	T	P (%)	T	P (%)	T	P (%)
2018	6	-	6	2 (33%)	2	-	4	-	1	-
2019	15	-	4	-	7	-	8	-	1	-
2020	10	-	5	2 (40%)	2	-	-	-	-	-
2021	13	1 (8%)	6	-	6	-	5	-	1	-
2022	17	2 (12%)	7	-	7	-	16	1 (6%)	4	-

Table 2. Tested farms (T), positive farms (P) and relative percentage (%)

Year	Dog			Cats
	Tested	Positive	Morbidity rate	Tested
2018	77	22	28%	2
2019	49	11	22%	1
2020	46	7	15%	2
2021	68	9	13%	4
2022	35	6	16%	2

Table 3. Distribution of pets by results and year

P048

Animal health

A CASE OF A FATAL SEPSIS CAUSED BY *ACTINOBACILLUS EQUULI* INFECTION IN A 3 DAYS OLD DONKEY FOAL

L. Marino[1], D.A. Raele[1], E. Macolino[1], G. Siragusa[1], M.A.R. Ferrantino[1], M.L. Prencipe[1], L. Pace[1], V. Rondinone[1], A. Bianco[1], L. Addante[1], C. Trisolini[1], A. Petrella[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*

Introduction

Neonatal septicemia in foals represents an important problem in equine neonatology and are supported, in most cases, by Gram negative germs [1]. The most serious form is caused by *Actinobacillus equuli*, a member of the *Pasteurellaceae* family. Due to its dual nature as both commensal and pathogen agent, the bacterium is commonly found in the oral and alimentary tracts of healthy horses but is also known to cause fatal septicemia, termed "sleepy foal disease" (SFD) [2]. Here we report the case of a fatal sepsis caused by *A. equuli* in a 3 days old donkey foal.

Materials and Methods

During the necropsy procedure, the spleen, liver, kidney, brain, heart and lung were collected and then submitted for histological examination, the molecular procedures for the detection of equine viral arteritis and equine herpesvirus and seed on Blood Agar, Tryptic Soy Agar and Tryptic Soy Broth for bacteriological testing at 37°C in aerobic and microaerophilic conditions. Bacterial colonies, previously screened using the CAMP test, oxidase test and MALDI-TOF, were Gram stained and observed using an optical microscope.

Results

Macroscopic and microscopic lesions were suggestive of septicemia (jaundice, DIC, purulent interstitial nephritis, purulent hepatitis, purulent interstitial pneumonia, purulent plexochoroiditis and vascular thrombosis associated with bacterial emboli) (Figure 1 and Figure 2). From all sample's pure cultures of α-hemolytic Gram negative coccobacilli, oxidase positive and CAMP test negative were obtained. The MALDI-TOF examination identified the colonies as *A. equuli*. None viral agent has been detected.

Discussion and Conclusion

Anatomopathological and histological lesions linked to the pure culture isolations of the bacteria from all samples confirm *A. equuli* as the causative agent of fatal sepsis in the foal. Due to the ambiguous nature of the pathogen, proper management of the foal, the brood and the environment are recommended to reduce the incidence of the SFD.

References

1. Taylor S. A review of equine sepsis. Equine Vet Educ. 2015 Feb;27(2):99-109. doi: 10.1111/eve.12290. Epub 2015 Jan 14. PMID: 32313390; PMCID: PMC7163761.
2. Adu-Addai B, Smedley RC, Mullaney TP. Pathology in practice. A equuli septicemia in a neonatal foal. J Am Vet Med Assoc. 2014 Dec 15;245(12):1339-41.

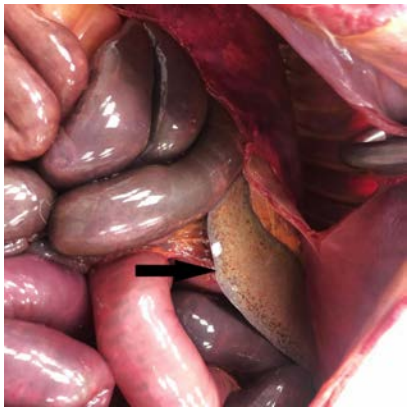


Figure 1. Macroscopic appearance: DIC, splenic hemorrhagic petechiae (arrow).

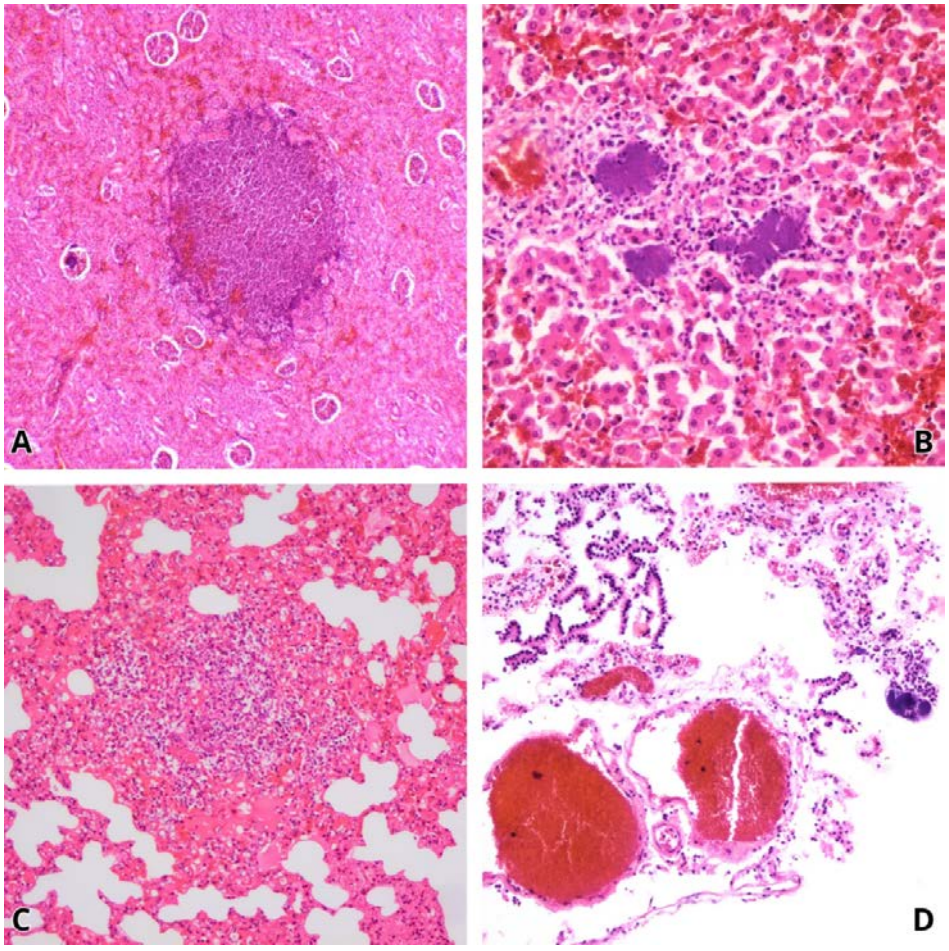


Figure 2. Microscropic view: A interstitial nephritis with a follicular pattern. Presence of one focus of neutrophils with toxic granulation; B purulent necrotic hepatitis associated with bacterial colonies; C purulent interstitial pneumonia; D purulent plexochoroiditis associated with bacterial colonies.

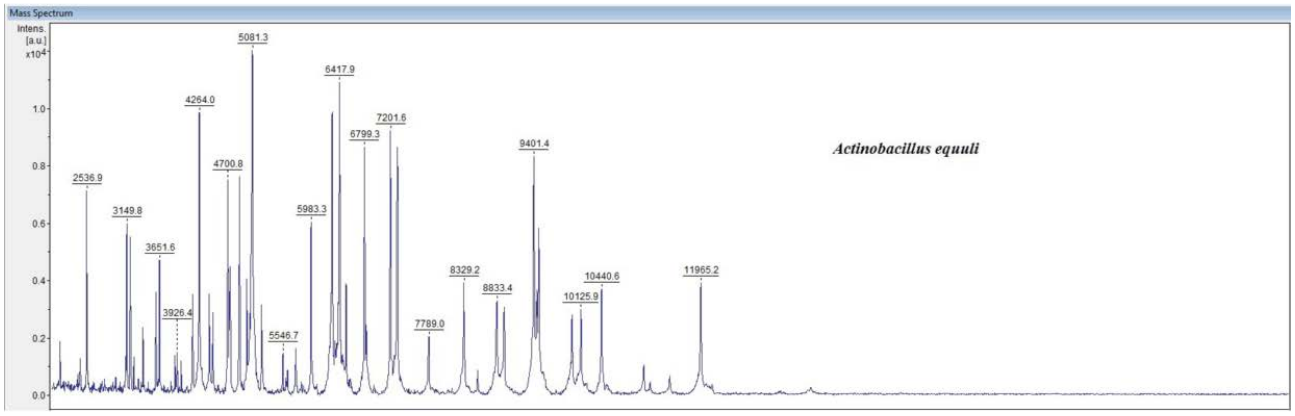


Figure 3. MALDI TOF spectrum of *Actinobacillus equuli* strain.

P049

Animal health

BIOMARKERS FOR DETECTING MYCOBACTERIAL DISEASE IN CATTLE: A COMPARATIVE STUDY

P. Mazzone[2], G. Franzoni[1], M. Torricelli[2], A. Fratto[2], A. Donniacuo[3], L. Schiavo[3], L. Petrucci[2], L. Madeo[2], S. Zinellu[1], S. Dei Giudici[1], E. De Carlo[3], G. Galiero[3], A. Martucciello[3]

[1]*Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*
[2]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy*
[3]*National Reference Centre for Hygiene and Technologies of Water Buffalo Farming and Productions, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Salerno, Italy*

Introduction

In vivo diagnosis of Bovine tuberculosis (TB) due to mycobacteria of *Mycobacterium tuberculosis* complex (MTBC), and Paratuberculosis (PTB) due to *M. avium* ssp. paratuberculosis (MAP), relies on dated methods such as skin tests or culture. The interferon-gamma release assay (IGRA) adopted for official TB diagnosis is experimentally used also for PTB (1). Here we describe the use of other cytokines as biomarkers to detect mycobacterial infections.

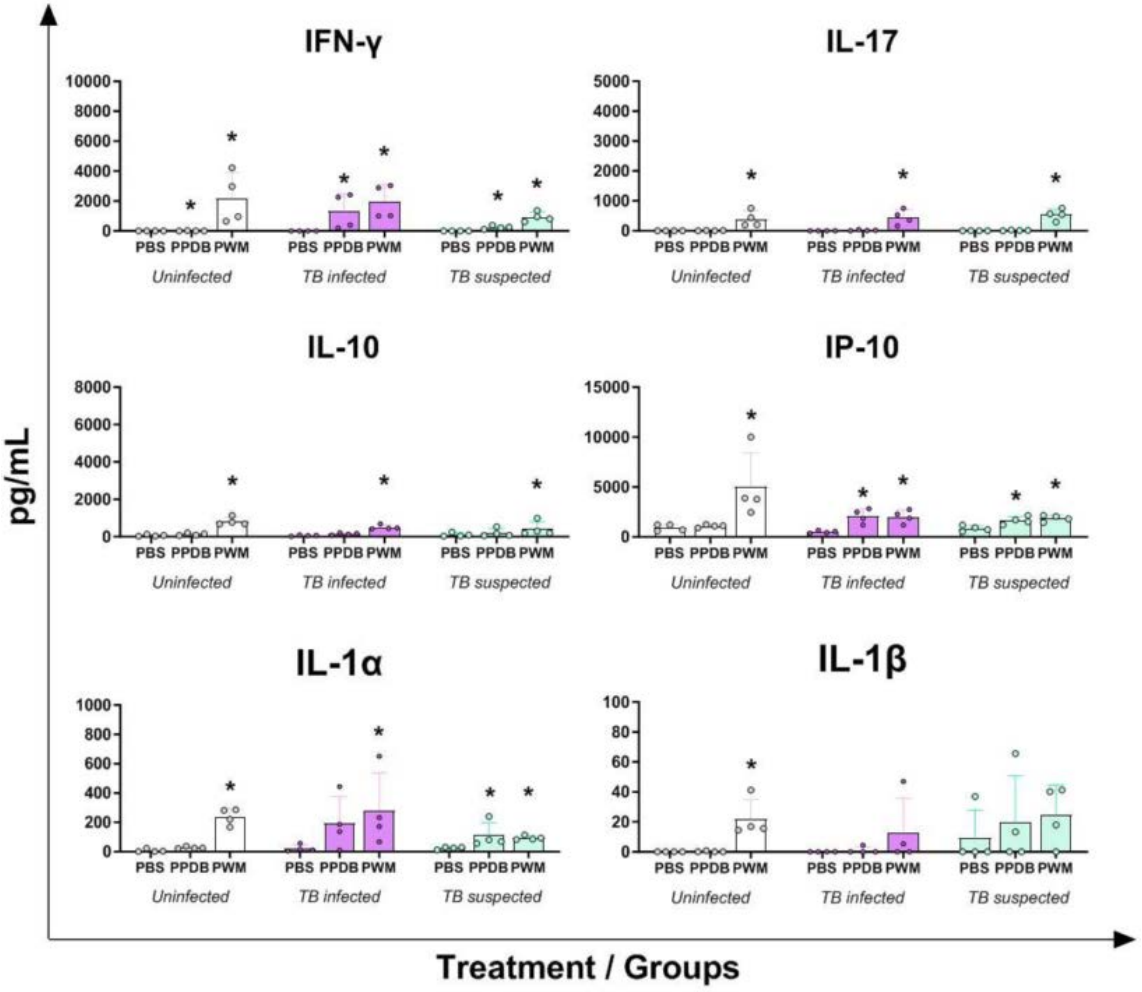


Figure 1. IFN-, IL-17, IL-10, IP-10, IL-1alpha and IL-1beta production in whole blood from cattle. Heparin blood from TB infected (n=4), TB suspected (n=4), and uninfected (n=4) cattle, were collected. Whole blood was stimulated for 16-24h with PBS, PPDB, Mitogen (PWM). Plasma levels of IFN- IL-17, IL-10, IP-10, IL-1alpha and IL-1beta were evaluated with MILLIPLEX® kit ELISA. Differences between treatments (PBS vs PPDB; PBS vs PWM) were assessed using a Mann- Whitney test and p value < 0.05 were considered statistically significant; * $p < 0.05$.

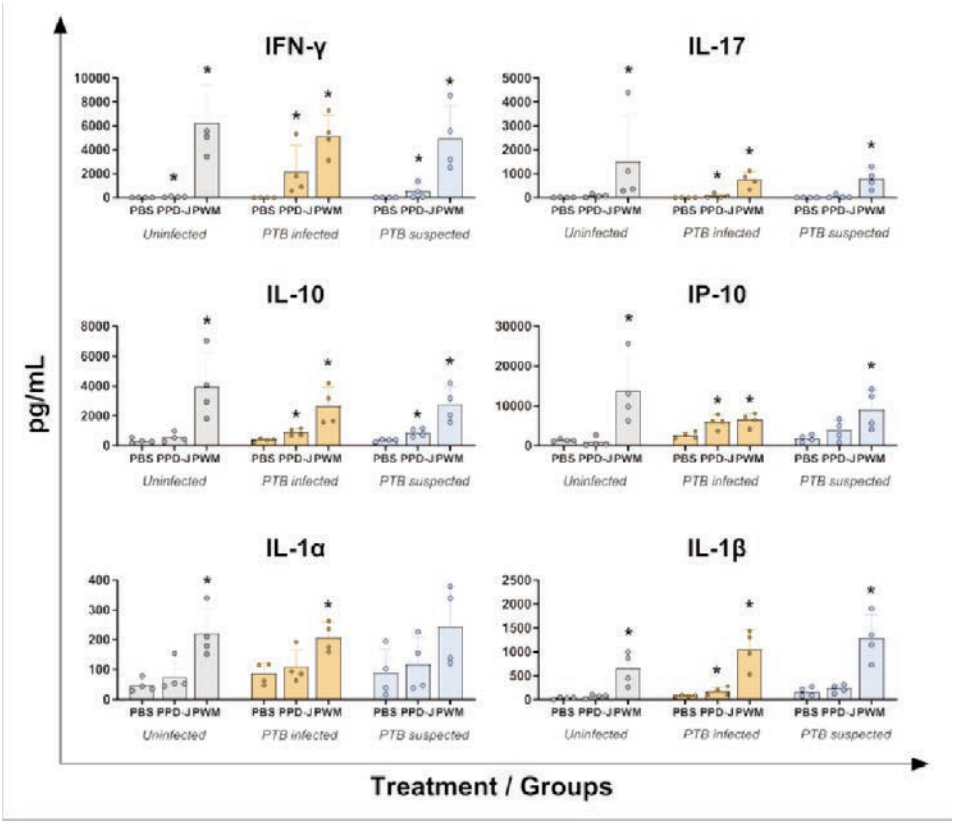


Figure 2. IFN-, IL-17, IL-10, IP-10, IL-1alpha and IL-1beta production in whole blood from cattle. Heparin blood from PTB infected (n=4), PTB suspected (n=4), and uninfected (n=4) cattle, were collected. For all the groups, whole blood was stimulated for 16-24h with PBS, Johnin PPD (PPDJ), Mitogen (PWM). Plasma levels of IFN- IL-17, IL-10, IP-10, IL-1alpha and IL-1beta were evaluated with MILLIPLEX® ELISA kit. Differences between treatments (PBS vs PPDJ; PBS vs PWM) were assessed using a Mann-Whitney test and p value < 0.05 were considered statistically significant; * $p < 0.05$.

Materials and Methods

Twenty-four cattle were enrolled: 8 from a TB outbreak, 8 from a PTB affected herd, 4 from a TB-Free herd and 4 from a PTB negative herd. Animals were divided into 6 groups of 4 cattle, based on tests outcomes: TB infected (IGRA, PCR, Culture Positive), TB suspected (IGRA positive only), TB uninfected; PTB infected (ELISA/qPCR positive), PTB suspected (IGRA positive only), PTB uninfected. Blood samples of each animal were dispensed in aliquots, stimulated with PBS (Nil Antigen), Bovine (B) or Johnin (J) PPD, Pokeweed Mitogen, respectively. Levels of IFN-γ, IL-1α, IL-1β, IL-4, IL-6, IL-10, IL17, MIP-1α, IL-36Ra, IP-10, MCP1, TNF, VEGF were measured on plasma 16-24 h post-stimulation using MILLIPLEX® kit (2). Cytokines' data were compared with the Mann-Whitney test.

Results

As described in Figures 1 and 2, in TB infected/suspected and PTB infected cattle, IP-10 release was significantly higher after PPDB and PPDJ stimulation, respectively, compared to PBS. TB suspected and PTB affected animals realeased IL- 1α in response to PPDB stimulation, and IL-1β in response to PPDJ stimulation with statistically significant differences between treated and PBS samples. TB infected/suspected and PTB affected cattle released low levels of IL-17 in response to specific stimulation, with no significant differences between PPDB-treated and PBS samples and with significant differences between PPDJ-treated and PBS samples. In PTB affected/suspected animals IL-10 release was significantly higher, after PPDJ stimulation compared to PBS. These differences were not observed in TB groups.

Discussion and Conclusion

These preliminary findings suggest IP-10 as an excellent candidate biomarker, both in TB and PTB. All data should be validated on a larger number of cattle.

Research funded by Italian Ministry of Health RC IZSUM 06/21, RC IZSM 14/22

References

Mazzone at al.,2021 Franzoni et al., 2022

P050

Animal health

MOLECULAR MARKERS MODULATION BY EFFECT OF PORCINE EPIDEMIC DIARRHEA VIRUS INFECTION

D. Masiuk[1], V. Nedzvetsky[1]

[1]*Dnipro State Agrarian and Economic University, Dnipro, Ukraine*

Introduction
Adhesion proteins are confirmed to be important component for the intestinal barrier maintenance and the restriction the pathogens invasion. The weaned piglets extremely susceptible to enteric infections including porcine epidemic diarrhea virus (PEDV) which affects epithelial cells. Molecular markers could be a prospective approach to detect intestinal injury including enteric virus progress. extracellular matrix (ECM) are the crucial players to provide structural framework and to convey signals to surrounding enterocytes as well as to regulate programmed cell death

Materials and Methods
Present study was aimed on adherens junctions protein E-cadherin, (ECM) protein fibronectin and apoptotic marker cleaved caspase-3. We also evaluated abnormalities in the intestinal morphology between PEDV-infected and normal piglets. Sixty piglets12-day-old were inoculated with PEDV (1000 virions/ animal). Control group was inoculated with PBS. Both groups were euthanized on 5 and 14 and PID and intestine tissue was sampled to assess intestinal morphology and aforementioned molecular markers content. All infected piglets exhibited severe watery diarrhea and/ or vomiting and severe atrophic enteritis.

Results
EDV antigens were detected in the intestine tissue by both PCR and western blot all infected piglets. The content of E-cadherin and fibronectin in PEDV group was statistically reduced in compare to control group ($P<0,001$) on both 5 and 14 days. Besides, there was observed the increase in the intact fibronectin fragmentation in PEDV group compared with control group ($P<0,01$) while this increase was most high on 14th day in compare with 5th day. The upregulation of caspase-3 content was observed in PEDV that evidence the progress of apoptosis.

Discussion and Conclusion
Observed disturbances in morphology of enterocytes lining the villous epithelium evidence that these abnormalities correlate with detected molecular markers changes. Taking together, our results suggest that adherence junction protein E-cadherin and ECM protein fibronectin are included in the progress of epidemic diarrhea symptoms. Obtained data suggest that the downregulation of these molecular markers could be applied as promised diagnostic tool to assess farming animal health.

P051

Animal health

A CASE OF FOOD-BORNE SALMONELLOSIS IN A CORN SNAKE (PANTHEROPHIS GUTTATUS) AFTER A FEEDER MOUSE MEAL

Meletiadis[2], A. Romano[2], B. Moroni[2], M.R. Di Nicola[1], V. Montemurro[2], M. Pitti[2], M. Pezzolato[2], E. Bozzetta[2], S. Sciuto[2], P.L. Acutis[2]

[1]*Faculty of Veterinary Medicine, Department of Pathobiology, Pharmacology and Zoological Medicine,Ghent University*
[2]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy*

Introduction
Reptiles are well-known *Salmonella* reservoirs, harbouring multiple species of *Salmonella* in their gastrointestinal tract. In reptiles, *Salmonella* becomes the primary pathogen in individuals that are elderly, kept in poor condi-tions or experiencing a depressed immune response. None of the report in literature regarding fatal salmonellosis in various species of snakes investigated the source of infection 1.

Materials and Methods
An adult male *Pantherophis guttatus*, with no pathological conditions, died after having been fed a frozen and thawed mouse from a batch of feeder mice purchased online. Gross examination was performed and samples was taken for histological and microbiological analysis from both snake and mouse. *Salmonella* spp. was isolated from livers and faeces of the snake and the mouse and were subjected to whole-genome sequencing.

Results
At necroscopy gastric mucosa was hyperaemic. Histological analysis of small intestine revealed a severe diffuse necrosis of mucosa resulting in erosion of the epithelium with degenerate and necrotic heterophils. No pathogens other than salmonella were isolated. Both the assembled genomes belonged to the strain type ST-357 for the classic MLST scheme and to the strain type ST-171322 for the cgMLST scheme. The in-silico identification of the serotype revealed the occurrence of *S. enterica* subsp. enterica ser. Midway.

Discussion and Conclusion
This report describes a case of food-borne salmonellosis in an adult corn snake associated with the ingestion of an infected feeder mouse dead for hyperacute septic shock. The sequence of events and the isolation of the pathogen from both the snake and mouse, suggest that *Salmonella* was likely the causative agent of the pathology. The sero-type implicated was *S. enterica* subsp. enterica ser. Midway, which has not yet been linked to disease in animals or humans. The role of the feeder mouse as a possible source of *Salmonella* infection for the snake was confirmed through a whole- genome analysis of the isolates, demonstrating that they belonged to the same strain. This case report high-lights how feeder mice should be regarded as potential sources of salmonellosis, posing a direct threat not only to carnivorous reptiles fed with frozen–thawed prey.

References
Bertolini M. et al., 2021

P052

Animal health

PRECLINICAL DIAGNOSIS OF CLASSICAL SCRAPIE PRPSC IN ARQ/ARQ EXPERIMENTAL SHEEP

D. Meloni[1], M.C. Cavarretta[1], A. D'Angelo[3], F. Ingravalle[1], G. Perrotta[2], E. Bozzetta[1], P. Acutis[1], M. Mazza[1], C. Corona[1], B. Iulini[1], S. Ravetta[1], L. Pite[1]

[1]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Italy*
[2]*Ministero della Salute, Roma, Italy*
[3]*Università degli studi di Torino, Italy*

Introduction
Classical Scrapie is a chronic, neurodegenerative, fatal disease of sheep and goats caused by prion protein Scrapie (PRPSc). The study aimed to provide an understanding of the preclinical diagnosis of classical Scrapie in ARQ/ARQ experimental sheep, which could contribute to the control of the disease spread and the potential zoonotic risk, acquiring strategic importance of animal and public health.

Materials and Methods
Fourteen ovine aged 14 days of ARQ/ARQ genotypes were included in a two-year pilot study. Twelve animals were challenged orally with ovine brain tissue homogenate positive for classical Scrapie by Western Blot technique. Two animals were kept as a control group. Serum was tested with the ultra-sensitive Single Molecule Array (SiMoA). Cerebrospinal fluid (CSF) was tested with the ultra-sensitive Single Molecule Array (SiMoA) and the Real-Time Quaking- Induced Conversion (RT-QuIC). Medulla oblongata was tested with IDEXX HerdChek BSE-Scrapie Rapid Test, TeSeE™ Western Blot (TeSeE™ WB) and Immunohistochemical method (IHC).

Results
Multilevel regression models explored the relationship between the concentration of Neurofilament Light protein (NfL) in the serum and CSF detected by SiMoA since the challenge time. The NfL concentration in CSF in the Scrapie group was statistically significant, with an increasing trend up to 21 months. The Receiver Operating Characteristic Curves identified an adequate NfL concentration cut-off value for CSF. The correctly classified samples are 91.67% for the NfL concentration $\geq$ 318 pg/mL with Se=100% and Sp=50%, AUC=55%. The CSF of two out of six animals in the Scrapie group tested positive using RT-QuIC at a late incubation stage before the clinical disease endpoint. The eight animals that reached the clinical disease endpoint were positive by IDEXX HerdChek BSE-Scrapie Rapid Test, TeSeE™ WB, and IHC. After the challenge, they developed Scrapie typical clinical signs at a mean of 729 days (N = 8, range 655–793 days). The six animals who died of other causes at different times after the challenge (mean 144 days, range 34 – 290 days) were negative by all tests.

Discussion and Conclusion
The preclinical diagnosis of PrPSc remains to be further explored in order to contribute to the disease surveillance and control.

Funded by The Ministry of Health of Italy. Internal code 15M03.

P053

Animal health

INTRODUCTION OF A DIVERGENT CANINE PARVOVIRUS TYPE 2 STRAIN IN SICILY, SOUTHERN ITALY, THROUGH THE MEDITERRANEAN SEA ROUTE TO EUROPE

F. Mira[4], G. Schirò[4], D. Vicari[4], G. Franzo[2], G. Purpari[4], E. Giudice[3], M. Trapani[1], A. Carrozzo[4], G. Spene[4], V. Talarico[4], A. Guercio[4]

[1]Azienda Sanitaria Provinciale di Trapani, Dipartimento di Prevenzione Veterinaria, Trapani, Italy [2]Department of Animal Medicine, Production and Health (MAPS), University of Padua, Legnaro (PD), Italy [3]Department of Veterinary Science, University of Messina, Messina, Italy
[4]Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy

Introduction
Canine parvovirus type 2 (CPV-2) is a leading cause of viral gastroenteric infection in canine species. CPV-2 variants (CPV-2a/-2b/-2c) are worldwide distributed, with different local prevalences. To date, available data on the potential drivers of the emergence and spread of different CPV-2 variants to long-distant areas, in the so fast-evolving international scenario, are still limited. This study reports the detection and genetic characterization of a CPV-2 strain from a dog introduced in Italy through the mediterranean sea route to Europe.

Materials and Methods
Samples were collected from a dog arrived in the mediterranean coasts of Sicily along with the owner, crossing the Mediterranean Sea by boats. Soon after their rescue, it showed severe gastroenteric signs and, despite medical cares, died 24 hours later. A preliminary immunofluorescence examination to detect rabies virus antigen and a Real Time RT- PCR for the detection of Lyssavirus RNA were performed. Samples were then tested to detect CPV-2 and the canine coronavirus (CCoV). The near complete CPV-2 sequence was obtained; sequence, phylogenetic and phylogeographic analyses were then performed.

Results
A CPV-2b strain was detected in all tested samples. Negative results were obtained for rabies, Lyssavirus, and canine coronavirus. Both CPV-2 VP2 and NS1 gene sequences showed high nucleotide identities (99.83-99.72% and 99.70- 99.40%, respectively) with CPV-2b strains detected in dogs and cats in Egypt and Turkey in 2019-2021. Sequence analysis revealed an amino acid pattern never observed before in European CPV strains. Phylogenetic and phylogeographic analyses showed a close relationship with strains detected in Middle East and Asian countries.

Discussion and Conclusion
The detection of a divergent CPV-2 strain in an insular Mediterranean area of southern Italy underlines how rapid and long-distance transport of dogs can impact virus spreading dynamics. Indeed, as for other viruses, transport of live animals can potentially favor the introduction of novel or re-emerging viral strains. To the best of our knowledge, this is the first report of a CPV-2 strain, previously never reported in Europe, transported during the Mediterranean maritime refugee crisis.

P054

Animal health

DETECTOR DOGS AS A TOOL TO MITIGATE RISK OF ASF INTRODUCTION IN SARDINIA (ITALY): PRELIMINARY RESULTS

B. Mossa[2], P. Uras[2], G. Usai[2], P. Desini[2], N. Spissu[2], G. Campus[2], G. Bitti[2], S. Canu[2], F. Dettori[2], C. Pinna[2], A. Tempesta[1], E. Caione[1], M. Merlini[1], F. Sgarangella[2]

[1]Azienda sanitaria Locale Gallura, Italy
[2]Azienda Sanitaria Locale Sassari, Italy

Introduction
African swine fever (ASF) is a fatal infectious disease of wild and domesticated suids. In Sardinia, historically affected by genotype I and in the final stage of eradication, in 2023, it was detected the first case of ASF genotype II in a domestic pig farm, most likely as a consequence of infected meat products brought from Italy (outbreak declared officially resolved by European Commission).Movements of pork meat and products play a key role in ASF introduction and spread,so the priority is to prevent introduction of genotype II in the Island. In 2023 Sardinian Government approved a surveillance plan that, among other risk mitigation measures, foresees strengthening controls in ports with sniffer dogs. Dogs have an extremely sensitive olfactory system.They have been reported to identify distinct volatile organic compounds (VOCs) released by their hosts' metabolic processes in various conditions.The aim of the work is to describe prevention activities with sniffer dogs on passengers arriving in the sea ports of north Sardinia to search meat products possibly contaminated with ASF.

Materials and Methods
Controls took place from November 2023 in Porto Torres and Olbia sea ports respectively 1 time and 2 times a week on a sample of disembarked vehicles, by a team composed of 2 veterinarians, one Prevention technician and 2 dyads (dog and handler, affiliated with Progetto Serena a.p.s.). Dogs were trained to distinguish between pork meat and other meat with an operant conditioning protocol based on previous research. The screening of vehicles and lugagges lasted around 5 minutes and once the dog smelled the target odor, it sat to alert its handler. Products considered at risk were then sampled and
sent to the laboratory for ASFV testing.

Results
The results are shown in Table 1.

Discussion and Conclusion
Data evidence that approximately 30% of the examined vehicles carried pork products, highlighting the risk of introduction and spread associated with swine products carried in vehicles and baggage specially from countries affected by ASF. Detector dogs are a quick and reliable method to intercept meat products, making them an effective tool in mitigating risk of introduction. In addition to the checks, it's advisable to raise travelers'awareness with public campaigns. In phase 2 dogs will be trained to find ASF in meat

PORT	DAYS	VEHICLES	MEAT OR PDCTS FOUND	% MEAT OR PDCTS
PORTO TORRES	26	420	98	23,33
OLBIA	32	354	115	32,49
TOTAL	58	774	213	27,52

Table 1.

P055

Animal health

INFECTION BY *MYCOBACTERIUM CAPRAE* IN A HERD IN NORTHERN SARDINIA (ITALY)

B. Mossa[1], S. Canu[1], N. Spissu[1], G. Campus[1], G. Bitti[1], M. Lasia[1], T. Piseddu[2], A. Satta[1], F. Balia[1], S. Dore[2], P. Desini[1], F. Sgarangella[1]

[1]*Azienda Sanitaria Locale Sassari, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*

Introduction
Bovine tuberculosis (bTB) is a contagious chronic disease. Symptoms generally have a slow progression. In addition to its economic importance to the livestock industry, its zoonotic nature deserves the greatest attention as public health risk. *M. bovis* and *M. caprae* are both members of the *Mycobacterium tuberculosis* complex (MTC). *M. bovis* is acknowledged as associated with bTB and *M. Caprae* has recently been classified as a separate species.

Materials and Methods
In 2023, 22 cows coming from an establishment in the north of Sardinia (Italy) were subjected to pre-moving intradermal tuberculin test (IDT); 4 of them were inconclusive for the test. The local veterinary services started an epidemiological investigation at the time of the outbreak's suspect. Therefore the entire herd (n=24) and an epidemiologically-related herd (n=9) were submitted to both IDT test and to ELISA gamma-interferon test. Based on the results, other 2 cows were inconclusive. A total of 6 inconclusive plus 15 negative cows were sent to the slaughterhouse where blood samples were taken in order to perform ELISA gamma-interferon test. Post mortem examination was performed on of all the slaughtered cows, 9 of which showed gross lesions, therefore samples were taken and sent to Istituto Zooprofilattico Sperimentale for anatomopathological, histological, and coltural exams.

Results
the results are shown in Tables 1, 2 and 3.

Discussion and Conclusion
The epidemiological investigation indicated that 4 cows with gross tuberculosis lesions at slaughter and positive at coltural exam for M. Caprae, were transferred in 2022 from Orgosolo (NU), an area were *M. caprae* was isolated from TB outbreaks in 2009. Movement of animals is the main risk factor for a new introduction of bTB in a herd. It has been suggested that *M. caprae*, which has already overtaken *M. bovis* in certain countries, plays an emerging role as the pathogen responsible for animal TB. As observed in the present bTB outbreak, infected cattle frequently give negative or inconclusive results at IDT test, therefore pre- or post-movement testing, due to the low sensitivity of test, do not completely eliminate the risk of transmission of *Mycobacteria* among herds. In addition, our findings highlight the importance of Official Veterinary post mortem inspection at slaughterhouse.

Establishment	RP	Result	n.
IT078SSXXX (n.=24)	42123/2023	Negative	15
		Aviarian reaction /aspecific	2
		Not discriminant reaction	2
		Not adequate sample	5
IT078SSXXX (n.=9)	42124/2023	Negative	8
		Reazione aviaria/aspecifica	1

Table 1. ELISA gamma-interferon test of 2 establishments

Bovine ID	IDT	(Gamma-interferon)	Gross lesions	Coltural exam+PCR
IT09199	Negative	Not performed	+	**TB Complex +; *M. caprae***
IT09199	inconclusive	Bovine reaction	+	**TB Complex +; *M. caprae***
IT09199	inconclusive	Campione non idoneo	-	**TB Complex +; *M. caprae***
IT09199	inconclusive	Bovine reaction	+	**TB Complex +; *M. caprae***
IT09199	inconclusive	Bovine reaction	+	Genere +
IT09199	Negative	Not performed	+	Genere +
IT09199	Negative	Not performed	+	PCR negative.
IT09199	inconclusive	Not discriminating	-	PCR negative.
IT09199	inconclusive	Aviarian reaction/aspecific	-	PCR negative.

Table 2. Test and Gross findings of slaughtered bovines.

CODICE CAPO	Est. 1	Moved to est.2	Reazione IDT	Lesioni AP	Esame colturale
IT09199022	062NUXXX (Orgosolo)	07/10/2022	Negative	+	*M. caprae*
IT09199037	062NUXXX (Orgosolo)	07/10/2022	inconclusive	+	*M. caprae*
IT09199032	062NUXXX (Orgosolo)	07/10/2022	inconclusive	-	*M. caprae*
IT09199021	062NUXXX (Orgosolo)	07/10/2022	inconclusive	+	Negative
IT09199032	062NUXXX (Orgosolo)	07/10/2022	inconclusive	+	*M. caprae*
IT09199019	077NUXXX (Sarule)	07/10/2022	inconclusive	+	Negative
IT09199021	077NU004 (Sarule)	07/10/2022	Negative	+	*M. caprae*
IT09199035	077NUXXX (Sarule)	07/10/2022	inconclusive	-	Negative
IT09199032	077NUXXX (Sarule)	07/10/2022	inconclusive	-	Negative

Table 3. Results for moved cattle.

P056

Animal health

SEROLOGICAL CROSS-REACTIVITY BETWEEN BOVINE GAMMAHERPESVIRUS 4 (BOGHV-4) AND BOVINE ALPHAHERPESVIRUS 1 (BOAHV-1) IN VIRUS-NEUTRALIZATION TESTS

C. Righi[3], E. Scoccia[3], S. Mrabet[3], A. Martucciello[2], G. Cappelli[2], C. Casciari[3], C. Grassi[2], C. Pellegrini[3], E. De Carlo[2], G. Costantino[3], M. Maria Luisa[1], F. Feliziani[3], S. Petrini[3]

[1]*Department of Veterinary Medicine, University of Perugia, Perugia, Italy*
[2]*National Reference Centre for Hygiene and Technology of Breeding and Buffalo Production, Istituto Zooprofilattico Sperimentale Mezzogiorno, Salerno, Italy*
[3]*National Reference Centre for Infectious Bovine Rhinotracheitis (IBR), Istituto Zooprofilattico Sperimentale Umbria- Marche "Togo Rosati", Perugia (PG), Italy*

Introduction

Bovine alphaherpesvirus 1 (BoAHV-1) can cause different clinical syndromes (respiratory: IBR; reproductive: IPV, IPB) that determine a significant economic impact on control/eradication programs. In this context, to correctly diagnose infected from false-positives animals determined by serological cross-reactions with other herpesviruses, Petrini et al. (1) demonstrated the serological cross-reactivity between Bovine alphaherpesvirus 2 (BoAHV-2) and BoAHV-1. For this reason, in this study, we hypothesized that Bovine gammaherpesvirus 4 (BoGHV-4) experimental positive sera could cross-react with serological tests against BoAHV-1. The aim of this study was to test BoGHV-4 positive sera in virus neutralization (VN) assays.

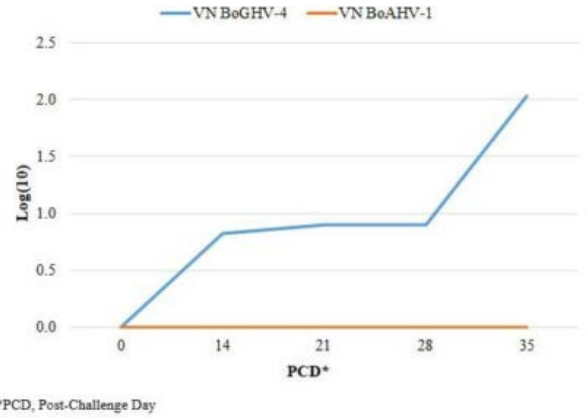


Figure 1. Serum samples of calves experimental infected with BoGHV-4 tested, in different work sessions, by VN assays against BoGHV-4 and BoAHV-1.

Group	Virus	PCD Δ				
		0	14	21	28	35
A	BoGHV-4	0	0.828	0.903	0.903	2.032
B	BoAHV-1	0	0	0	0	0
	p-value	-	**0.0114***	**0.0082***	**0.0082***	**0.0114***

Δ PCD, post challenge day; * Significant statistical differences, $p \leq 0.05$.

Table 1. Comparison of NAs titres between the A (VN BoGHV-4) and B (VN BoAHV-1) groups.

Materials and Methods

Four three-month-old calves, devoid of BoGHV-4 and BoAHV-1, neutralizing antibodies (NAs) were experimentally infected using the virulent strain 85/16 TV of BoGHV-4. One calf was used as a negative control. Serum samples were collected from all calves on 0, 14, 21, 28 and 35 post-challenge days (PCDs). The sera were tested in VN using BoGHV-4 and BoAHV-1 in different work sessions. The VN protocols used were described in [2]. The Wilcoxon Mann–Whitney non- parametric test was used to evaluate the statistically meaningful differences in NAs between BoGHV-4 and BoAHV-1 VN tests. Differences were considered significant at $p \leq 0.05$.

Results
The control remained negative in VN to BoGHV-4 and BoAHV-1 until the end of the experiment. In contrast, infected calves showed neutralizing antibodies (NAs) against BoGHV-4 on 14 PCD, and these increased progressively to 35 PCD, otherwise, no NAs were detected against BoAHV-1.

Discussion and Conclusion
In this study, we demonstrated that there is no serological cross-reactivity between BoGHV-4 and BoAHV-1 in the VN test against BoAHV-1 (Figure 1). Moreover, from a comparison of NAs titres against BoGHV-4 and BoAHV-1, in all experimental times, a statistically significant difference between BoGHV-4 and BoAHV-1 was observed (Table 1). Furthermore, these findings confirm that the VN tests as the gold standard and are a valuable tool in the eradication and control programs for IBR.

References
1. Petrini S., et al. Front. Vet. Sci. 2020. 7:587885.
2. WOAH,2018.http://www.oie.int/fileadmin/Home/eng/Health_ standards/tahm/3.04.11_IBR_IPV.pdf.

P057

Animal health

INVESTIGATION ON THE CIRCULATION OF CORONAVIRUSES IN WILD BOARS IN BASILICATA REGION

C. Ortello[4], P. Lorenzo[2], A. Bianco[1], L. Palazzo[3], D. Raele[2], N. Cavaliere[2], A. Parisi[1], A.C. Romano[3], M. Toce[3], S. Castellana[1], A.M. D'Antuono[4], D. Galante[2]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Contrada San Pietro Piturno, Putignano (BA), Italy*
[2]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy*
[3]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Zona Industriale Tito Scalo, Tito (PZ), Italy*
[4]*PhD National Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy*

Introduction
In recent years, the presence of wild boars has increased in Southern Italy. Wild boar is one of the most problematic species from the sanitary point of view because it represents a dangerous reservoir of pathogens, such as *B.suis* and *M.bovis*, responsible for serious diseases in both humans and animals.
The objectives of the project are the monitoring of the circulation and the genetic characterization of Coronaviruses in this species, a virus known to be subject to spillover between different species.

Materials and Methods
Following the slaughter plan established by Basilicata region relating to this species, 189 animals were analysed. Lungs and nasal swabs were collected at the slaughterhouse. A Pan-Coronaviruses PCR was performed using a Nested PCR, targeting the conserved RNA-dependent RNA polymerase (RdRp) genome region. Positive samples have later been sequenced by Sanger method for confirming and typing the detected Coronaviruses.

Results
Out of the 189 analyzed wild boars, three (2 adult females and 1 male) resulted positive for Pan-Coronavirus PCR/ Nested PCR. The sequencing of the obtained amplicons by Sanger method and the blast with the deposited sequences in GenBank allowed the identification of the porcine Coronavirus, the porcine hemagglutinating encephalomyelitis virus (PHEV) with an identity percentage of 96% in all of the three samples.

Discussion and Conclusion
Only the 1.6% of the analysed wild boars resulted positive for Coronavirus, in particular to PHEV, that is a Betacoronavirus able to cause neurological disorders, vomiting and wasting disease and death. The ecology of PHEV in commercial pig farms is unknown and a surveillance strategy could be considered to reduce contact between wild and farm animals in order to avoid inter-species transmission and reduce possible economic losses. The future strategy is the sequencing of positive samples by Next Generation Sequencing with Illumina platform, using a Pan-Coronavirus Panel. This panel supports the characterization and genomic surveillance of 225 known and novel coronaviruses from humans and animal reservoirs.

References
Wang X, Xiu L, Binder RA, Toh TH, Lee JS, Ting J, Than ST, Qi W, Coleman KK, Perera D, Ma M, Gray GC. A pan-coronavirus RT-PCR assay for rapid viral screening of animal, human, and environmental specimens. One Health. 2021 Dec; 13:100274.

P058

Animal health

BLOOD CULTURE AS A DIAGNOSTIC METHOD FOR PNEUMONIA IN DAIRY CALVES: A FIELD INVESTIGATION

M. Ossola[1], S. Raineri[1], A. Gazzola[1], L. Filippone Pavesi[3], V. Ferrulli[3], A. Boccardo[3], D. Pravettoni[3], S. Catania[2], D. Prataviera[2], M. Cornaggia[1], C.F. Magistrali[1], A.M. Maisano[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna "Bruno Ubertini" – Sede Territoriale di Lodi*
[2]*Istituto Zooprofilattico Sperimentale delle Venezie – Struttura Complessa Territoriale 1 Verona*
[3]*Università degli Studi di Milano - Dipartimento di Medicina Veterinaria e Scienze Animali, Italy*

Introduction

Blood culture (BC) tested on hospitalized critically ill calves revealed the potential to detect respiratory pathogens up to 23.1% prevalence[1]. Our study assessed the effectiveness of BC as a diagnostic method (DM) for pneumonia in non- critical calves, compared to Bronchoalveolar lavage (BAL), evaluating its utility as an early diagnostic tool in field.

Materials and Methods

Eighty-eight Friesian dairy calves (20-90 days of age) with at least one lung lesion ≥ 1cm in diameter after ultrasound examination were enrolled from 12 farms for BC and BAL. BAL were performed according to Van Driessche et al. (2017)[2]. Two blood samples per calf were collected AM and PM according to Fecteau et al. (1997)[3] for BC, which was performed using Oxoid Signal® Blood Culture System following product instructions. Pathogens were identified with MALDI-TOF (VITEK MS, Biomerieux®).

Results

Overall, bacterial pathogens were detected in 62.5% (55/88) of BALs, whereas only 5.7% of BCs tested positive, exclusively for *Salmonella* genus. Bacteriological and statistical results for single pathogen are show in Table 1.

Bacterial species	Calf prevalence from BALs	I.C. 95%	Calf prevalence from BCs	I.C. 95%	Cohen's K	Sample prevalence from BCs	I.C. 95%
Pasteurella multocida	36.4% (32/88)	28.9-43.9	0.0% (0/88)	-	0	0.0% (0/176)	-
Mannheimia haemolytica	17.1% (15/88)	13.6-20.6	0.0% (0/88)	-	0	0.0% (0/176)	-
Trueperella pyogenes	8.0% (7/88)	6.4-9.6	0.0% (0/88)	-	0	0.0% (0/176)	-
Moraxella sp.	5.7% (5/88)	4.6-6.8	0.0% (0/88)	-	0	0.0% (0/176)	-
Mycoplasma bovis	23.9% (21/88)	19.0-28.8	0.0% (0/88)	-	0	0.0% (0/176)	-
Salmonella enterica subsp. *enterica* serovar Dublin	2.3% (2/88)	1.9-2.7	5.7% (5/88)	4.6-6.8	0.56	4.0% (7/176)	3.5-4.5
Other bacterial species (likely contaminants)	10.2% (9/88)	8.2-12.2	29.5% (26/88)	23.4-35.6	0	15.9% (28/176)	13.6-18.2

Table 1. Bacteriological and statistical results of Bronchoalveolar lavage (BAL) and Blood culture (BC).

Discussion and Conclusion

The high contamination rate of field samples is comparable to data reported in veterinary hospitals (14.9%)[1], suggesting that the main challenge is to maintaining sample integrity. BC and BAL differ significantly in terms of number of detected pathogen species, with the latter showing a higher sensitivity, except for *Salmonella* genus. In conclusion, although potentially useful to detect *Salmonella* septicemia, BC is not an effective DM for the early detection of pathogens in pneumonia cases in dairy calves.

References
1. M. L. Pas, F. Boyen, …, B. Pardon and J. Bokma, "Bayesian evaluation of sensitivity and specificity of blood culture media and hypoglycemia in sepsis-suspected calves," J. Vet. Int. Med.,2:1906–1916, 2024
2. L. Van Driessche, B. R. Valgaeren, …, B. Pardon, "A Deep Nasopharyngeal Swab Versus Nonendoscopic Bronchoalveolar Lavage for Isolation of Bacterial Pathogens from Preweaned Calves With Respiratory Disease", J. Vet Int. Med., 31/3:946-953, 2017
3. G. Fecteau, D. C. Van Metre, …, W. Guterbock, "Bacteriological culture of blood from critically ill neonatal calves". Can Vet J., vol. 38/2:95-100, 1997

P059

Animal health

THE HUNT FOR THE ONE BSE POSITIVE SAMPLE IN OVER 500.000 COLLECTED SAMPLES.

C. Overbeek[1], L. Van Keulen[1], M. Mozol[1]

[1]*Wageningen Bioveterinary Research, the Netherlands*

Introduction
Bovine Spongiform Encephalopathy (BSE) also known as "mad cow" disease was first discovered in 1986 in Great Britain from where it spread to several other countries. This infectious prion disease affects the nervous system of cattle. In the Netherlands, 88 cases of BSE have been found from 1997 until 2022, the last positive case was found in 2011. BSE can be distinguished in two forms, classical BSE caused by C-type BSE and atypical BSE caused by L-type and H- type BSE. Classical BSE occurs in bovines after ingesting prion contaminated food, and atypical BSE is believed to occur spontaneously in elderly bovine populations. Classical BSE is the only form that can be transmitted to humans through contaminated meat causing the deadly variant Creutzfeldt-Jakob disease.

Materials and Methods
The diagnostic department of Wageningen Bioveterinary Research (WBVR) is the Dutch national reference laboratory for BSE. WBVR still conducts over 50,000 BSE tests (cattle older than 48 months) each year as part of the compulsory active surveillance on BSE in the EU. The 50.000+ cattle tested are fallen stock from Dutch farms collected by a destruction company (RENDAC). Testing is done on brainstem material (obex region) using the Idexx ELISA which is recommended and approved by the European Reference Laboratory. Confirmation and discrimination between classical and atypical BSE is done using various western blot methods and immunohistochemistry. Only when all tests indicate a positive BSE result the final result will be BSE positive.

Results
Since the last case of BSE in 2011 WBVR conducted over 500.000 tests for BSE. These tests all came back negative which led to the discussion whether or not costs outweigh the benefits. This discussion paused when the BSE-lab got a positive sample in the BSE -test at the beginning of 2023. After confirming this result in the confirmatory western blot, WBVR started discrimination western blots and immunohistochemistry tests. The results of the combined methods led to of the diagnosis of atypical BSE (L-type), the only case in the last 12 years of testing.

Discussion and Conclusion
Since this case was a form of atypical BSE and not the zoonotic classical form, the discussion whether or not the costs outweigh the benefits will most likely be continued.

P060

Animal health

DEVELOPMENT OF INNOVATIVE DIAGNOSTIC TECHNOLOGIES TO SUPPORT FIELD CONTROL ACTIVITIES OF *AETHINA TUMIDA* (THE SMALL HIVE BEETLE - SHB) IN ITALY, UPDATE 2023

S. Ambrogio[1], P. Palermo[1], M. Garzi Cosentino[1], F. Allevato[1], L. De Marco[1], V. Bonina[2], G. Federico[2], L. Barca[1]

[1]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Sezione diagnostica di Cosenza, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Sezione diagnostica di Reggio Calabria, Italy*

Introduction
Aethina tumida, a small beetle native to South Africa, was first found in Portugal, from where it was eradicated. Since September 2014, it has appeared in Calabria (IT), where it remains present (Figure1). SHB has evolved its biological cycle preferably within bee colonies. To contain its spread, SHB has been listed among pathogens of the WOAH in the Italian Veterinary Police Regulation. Projects under the supervision of the CRN of the Experimental Zooprophylactic Institute of Venezie (IZS-Ve) included:
- Experimentation of SHB breeding by the IZS of Campania and Calabria seat of Reggio Calabria for monitoring and control of the beetle
- Application of PCR methodologies by IZS of Lazio and Tuscany (IZS-LT) and IZS of Campania and Calabria seat of Cosenza (IZSM-CS)

- Design and improvement of traps and good beekeeping practices
- Verification of the method's performance in breeding samples and evaluation from field samples by IZS-LT

Materials and Methods

PCR methodologies for the detection of SHB involved blinded tests on different matrices. Design/improvement of traps.

Results

Samples of hive bottom debris, frame swab, and hive bottom swab were taken from apiaries with several levels of infestation (Figure2). Total of 230 nucleic acid samples were analyzed from homogenized bee carcasses; 9 tested positive in the PCR-RT test. Among 77 samples of hive bottom debris, all tested negative in Real Time PCR (Figure3). Good practices for managing SHB in the field have been identified and published in the manual “Good Beekeeping Practices”. The traps and good beekeeping practices were tested in a field study in two apiaries in Calabria, with updates on the “Movable Wall” (MW).

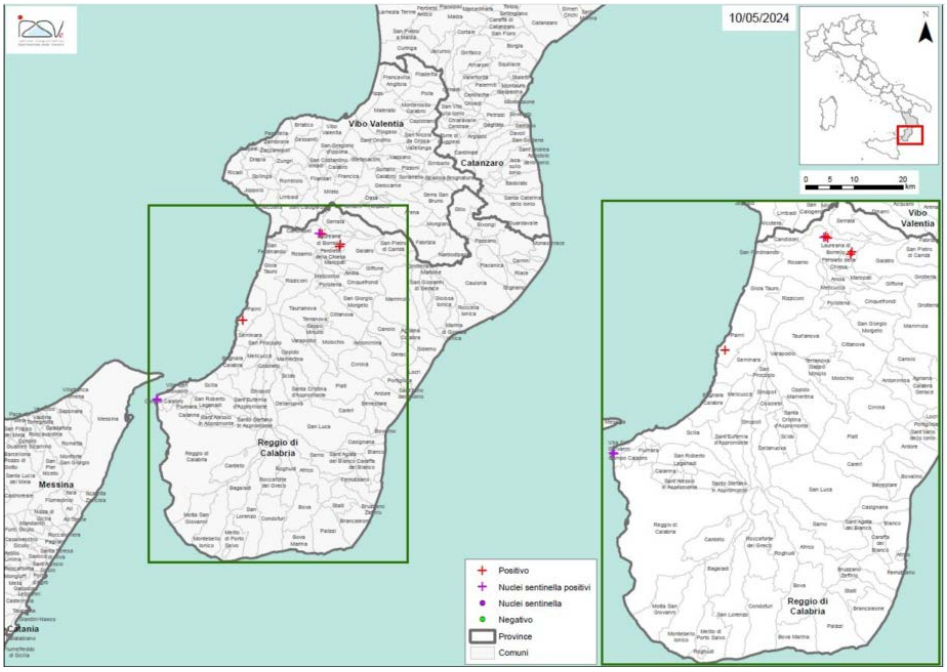


Figure 1. Protection zone of the Calabria_IZSVE

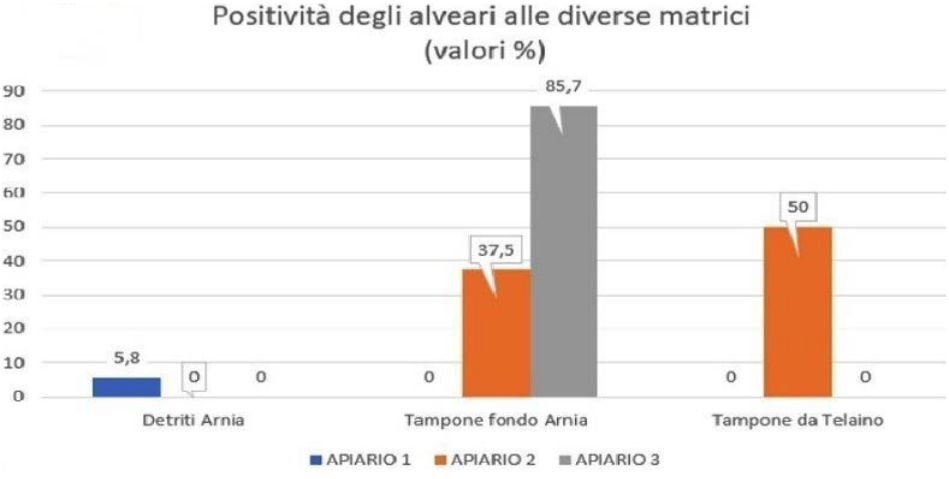
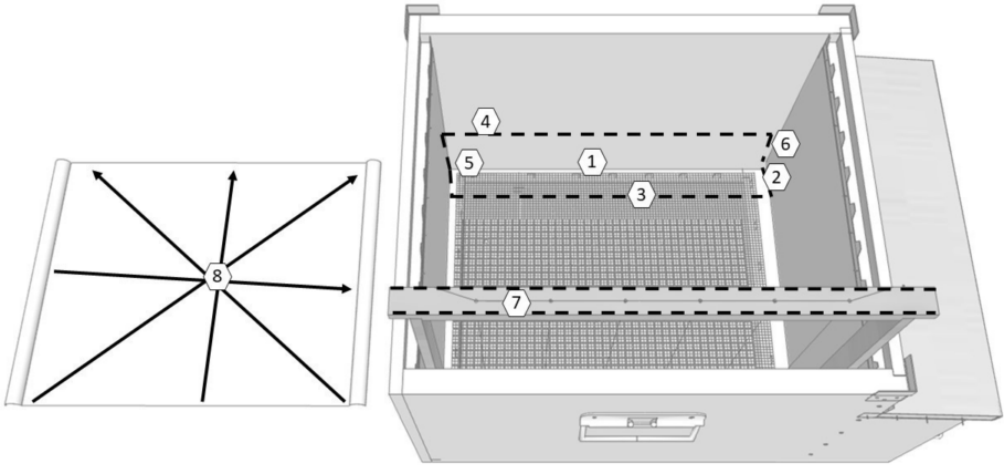


Figure 2. Positive effect of hives in different matrices (values expressed as %).



Area prescelta per i prelievi detriti e tamponi: angolo laterale (1), primi 3 cm dell'angolo posteriore (2), superficie della parte laterale del fondo dell'alveare (3), metà inferiore della laterale parete (4), angoli della parete laterale con le pareti anteriore e posteriore (5 e 6).

Figure 3. Selected area for debris and swabs.

Discussion and Conclusion

Debris sample analysis can be useful for early surveillance in high-risk areas, acting as a pre-warning indicator when SHB presence is not massive. Further studies are needed to confirm the effectiveness of frame and hive bottom swabs. The MW method was found to be more effective than the official method, applicable year-round, combining routine beekeeper visits and SHB searches. The biotechnology laboratory of IZSM-CS will continue routine diagnostics, thanks to the approved research project, studying molecular protocols for the existing soil matrix.

References

Neumann, Pettis, Schäfer, (2016). Quo vadis Aethina tumida. Apidologie.

P061

Animal health

DISTRIBUTION OF TOXIC AND POTENTIALLY TOXIC ELEMENTS IN ALBUMEN AND YOLK OF SEA TURTLE *CARETTA CARETTA* EGGS: ANALYSIS AND CORRELATION STUDY

A. Pepe[2], M. Esposito[2], F. Maffucci[3], R. Miniero[1], S. Hochscheid[3], P. Gallo[2], S. Canzanella[2]

[1]*Istituto Superiore di Sanità, Dipartimento Ambiente e Salute, Roma, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Centro di Referenza Nazionale per l'Analisi e Studio di Correlazione tra Ambiente, Animale e Uomo, Portici, Italy*
[3]*Marine Turtle Research Group, Stazione Zoologica Anton Dohrn, Portici, Italy*

Introduction

Trace elements transferred by nesting loggerhead turtles (*Caretta caretta*) to their eggs may impact embryonic development and hatching success. This study examines 17 trace elements in the yolk and albumen of *C. caretta* eggs from southern Italy to understand bioaccumulation patterns, providing insights into the exposure of developing embryos and adult females.

Materials and Methods

During the 2022 nesting season, 20 whole egg samples were collected from nests along the coasts of the Campania region. From each nest, three to five whole unhatched eggs were collected and separated into yolk and albumen. Then, 0.75 g of each sample was subjected to acid mineralization and analyzed using ICP-MS.

Results

Element concentrations in the yolk are higher than in the albumen, particularly for As, Co, Cu, Fe, Hg, Mn, Pb, Se, Sr, and Zn (Table 1). The albumen has lower concentrations but strong associations between elements, such as Zn-Pb (rs=0.95), Pb-V (rs=0.99), and Mn-Co

(rs=0.90). The yolk shows higher concentrations with fewer, weaker associations, including Sr-Zn (rs=0.75) and Cr-Ni (rs=0.99) (Figure 1). No correlation was found between trace element levels and hatching success.

Discussion and Conclusion

Trace elements are higher in the yolk than in the albumen of *C. caretta* eggs, likely due to the elements' chemical forms and formation processes. This pattern may be attributed to the binding of elements to vitellogenin, the primary egg yolk protein synthesized in the liver. Vitellogenins transport metal ions (e.g., Ca, Mg, Fe, Zn, Cu), minerals, and vitamins to the oocyte, which are essential for embryonic development. In contrast, the albumen produced in the oviduct, has a different composition affecting trace element accumulation. The metal levels in both the yolk and albumen originate from the maternal body burden and are not influenced by contamination at the nesting site. This research was funded by the Italian Ministry of Health, project TurtHea IZSME RC 07/22.

References

1. Muñoz C. et al. 2023. Sea turtle egg yolk and albumen as biomonitoring matrices for maternal burdens of organic pollutants, Mar Pollut Bull. 194 (Pt A), 115280.
2. Baskaralingam, V. and Vanichviriyakit, R. (Eds.) 2023. Vitellogenin in fishes: Diversification, biological properties, and future perspectives. Springer Nature Singapore Pte Ltd.

Element	Yolk *(n= 20)*		Albumen *(n= 19)*	
	Mean	Std. Dev.	Mean	Std. Dev.
As	0.904	0.606	0.220	0.125
Bi	0.086	0.012	0.013	0.0046
Cd	0.005	0.003	< LOQ	
Co	0.013	0.002	0.007	0.002
Cr	0.311	0.202	0.297	0.254
Cu	1.46	0.546	0.974	0.961
Fe	20.2	5.5	3.56	1.78
Ga	0.081	0.034	0.013	0.0040
Hg	0.022	0.014	< LOQ	
Mn	0.530	0.283	0.077	0.044
Ni	0.172	0.113	0.175	0.145
Pb	0.040	0.012	0.016	0.008
Rb	0.833	0.138	0.623	0.130
Se	1.15	0.40	0.282	0.183
Sr	38.6	10.9	5.06	2.41
V	0.045	0.018	0.015	0.010
Zn	31.1	26.2	3.93	3.93

Table 1. Trace element concentrations (mg/kg) in the yolk and albumen of unhatched *Caretta caretta* eggs from southern Italy (mean ± standard deviation).

	Ga	As	Sr	Pb	Rb	Bi	Mn	Zn	Se	V	Co	Ni	Fe	Cr
Ga	1													
As	0.4035	1												
	0.9999													
Sr	0.7635	0.5397	1											
	0.0149	0.8361												
Pb	0.6851	-0.1856	0.2336	1										
	0.1191	1.000	1.000											
Rb	0.7847	0.3352	0.8263	0.5262	1									
	0.0073	1.000	0.0014	0.8881										
Bi	-0.0769	0.3592	-0.151	-0.2162	-0.3095	1								
	1.000	1.000	1.000	1.000	1.000									
Mn	0.7489	0.0659	0.3989	0.7285	0.6292	-0.1617	1							
	0.0233	1.000	1.000	0.0416	0.3366	1.000								
Zn	0.737	-0.0509	0.3491	0.952	0.5877	-0.2491	0.768	1						
	0.0329	1.000	1.000	0.000	0.576	1.000	0.0128							
Se	0.5561	0.6889	0.5544	0.0855	0.4895	0.1576	0.3954	0.1561	1					
	0.7582	0.1097	0.7668	1.000	0.9718	1.000	1.000	1.000						
V	0.7013	-0.1599	0.2363	0.9854	0.5507	-0.2499	0.7743	0.9258	0.1238	1.000				
	0.0826	1.000	1.000	0.000	0.7852	1.000	0.0104	0.000	1.000					
Co	0.6914	0.2149	0.4037	0.7	0.6289	-0.1023	0.9032	0.7616	0.4378	0.7298	1			
	0.1037	1.000	0.9999	0.0852	0.3382	1.000	0.000	0.0158	0.9986	0.0401				
Ni	0.6258	0.1439	0.2789	0.6893	0.5246	-0.0576	0.8963	0.7509	0.3509	0.7202	0.9635	1		
	0.3544	1.000	1.000	0.1088	0.8937	1.000	0.000	0.022	1.000	0.0517	0.000			
Fe	0.8429	0.2378	0.6561	0.6752	0.7491	-0.1198	0.884	0.7351	0.5246	0.6992	0.8845	0.8175	1	
	0.0006	1.000	0.2133	0.1471	0.0232	1.000	0.0001	0.0347	0.8937	0.0869	0.0001	0.002		
Cr	0.6011	0.1808	0.2667	0.6523	0.5018	-0.033	0.877	0.7193	0.3561	0.6851	0.9635	0.9965	0.8105	1
	0.4953	1.000	1.000	0.2289	0.9525	1.000	0.0001	0.053	1.000	0.1192	0.000	0.000	0.0027	

Figure 1. Spearman correlation coefficients (rs) and p-values between pairs of elements within the albumen of C. caretta eggs from Campania nesting sites. Significant correlations are highlighted in yellow.

P062

Animal health

OCCURRENCE OF *SALMONELLA ENTERICA* SUBSP. DIARIZONAE IN FINNISH SHEEP

T. Pohjanvirta[1], T. Autio[1], S. Heinikainen[1], T. Pitkänen[2], H. Kuronen[1]

[1]*Animal Health Diagnostic Unit, Finnish Food Authority, Kuopio*
[2]*Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki*

Introduction
All serovars of *Salmonella* (S.) *enterica* are monitored in cattle, swine and poultry in Finland, according to the Finnish *Salmonella* Control Program approved by EU. Sheep are not included in the control program. *S. enterica* subsp diarizonae 61:(k):1,5,(7) is considered to be particularly adapted to sheep. It can be found in the intestine and nose of clinically healthy sheep, but it has also been described in clinical disorders in sheep. It can be occasionally isolated from human clinical samples, but generally its significance for public health is considered low. In Finland *S. diarizonae* has been sporadically found in sheep sent for autopsy. When sheep are sent for slaughter it is obligatory to inform in the food chain information if salmonella has been detected on the farm during last year. These sporadic *S. diarizonae* findings have caused problems for sheep farms as some slaughterhouses considers these farms to be truly infected with salmonella. The aim for this study was to find out what is the prevalence of salmonella in sheep fecal samples collected during slaughter.

Materials and Methods
Fecal samples were collected in three slaughterhouses during a 9-month period in 2023. One fecal sample per farm was taken. The detection method was ISO 6579:2017, amendment 1/2020. The isolates were serotyped by slide- agglutination test ISO 6579-3: 2014 in the national reference laboratory.

Results
Out of the 187 fecal samples analyzed for salmonella, *S. diarizonae* was detected in 37 samples (20 %). All isolates were serotyped as S. ssp. diarizonae 61: -:1,5. Other *Salmonella* serotypes were not detected.

Results show that *S. diarizonae* 61: -:1,5 is common in Finnish sheep. Public health records show that yearly zero to three domestic *S. diarizonae* (unknown serotype) infections are detected in humans. The findings suggest the need for a risk assessment in Finland regarding the mandatory reporting of this serotype in the food chain information when sheep are sent for slaughter.

References
Sören K et al. Changes in the risk management of *Salmonella* enterica subspecies diarizonae serovar 61:(k):1,5,(7) in Swedish sheep herds and sheep meat due the results of a prevalence study 2012. Acta Vet Scand (2015) 57:6.

P063

Animal health

DIVERSITY OF BOVINE S*TAPHYLOCOCCUS AUREUS* ISOLATED FROM ALPINE DAIRY FARMS IN AUSTRIA

M. Ehling-Schulz[1], T. Grunert[1], I. Loncaric[1], N. Ramezani[1], P. Mester[2], J.L. Khol[3]

[1]*Institute of Microbiology, Center of Pathobiology, University of Veterinary Medicine, Vienna, Austria*
[2]*Units of Food Microbiology, Department for Food Safety, Food Technology and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria*
[3]*University Clinic for Ruminants, Tyrol Extension, University of Veterinary Medicine, Vienna, Austria*

Introduction
Staphylococcus aureus is one of theatre predominant pathogens causing responsible bovine mastitis, leading to substantial economic losses in the dairy industry worldwide. Due to the potential induction of foodborne intoxications in humans, *S. aureus* also constitutes a significant public health risk. Our preliminary work indicates that a highly contagious variant of *S. aureus* (bovine ST8, CC8) has spread among cattle in western Alpine Austria (1,2). In the current project, we aim to evaluate the prevalence of *S. aureus*, particularly of the highly contagious variant bovine ST8 (CC8) in Alpine dairy farms in Tirol (western Austria).

Materials and Methods
We collected 464 bulk-tank milk (BTM) samples from 154 seasonal communal dairy herds of the alpine season 2023 at four sampling times. *S. aureus* isolates were identified using a culture-based approach, including MALDI-TOF MS, confirmed by nuc-gene PCR and subtyped by molecular genotyping (incl. spa-typing, MLST).

Results
The prevalence of *S. aureus* in the 1st (June), 2nd (July), 3rd (August), and 4th (September) sampling points were 34%, 37%, 36%, and 20%, respectively. Of 154 farms tested 91 (59 %) were tested at least once positive for *S. aureus* during the sampling period (June – September). From 149 strains isolated and analyzed, 33 different spa types were detected, with the most frequent being t2953 (30%), t024 (10%), and t529(9%). Isolates can be assigned to 7 clonal complexes (CCs), including bovine CC8 (53%), CC97 (19%), and CC151 (9%).

Discussion and Conclusion
Preliminary investigations suggest that approx. half of the tested alpine farms are *S. aureus*-positive. Most isolates (approximately 50%) were assigned to the bovine CC8 variant, which confirms its widespread distribution in the central Alpine region. These results serve as a basis for further investigations into infection dynamics at the herd level, as well as subsequent examination, treatment, and prophylactic measures for herds.

References
1. Sears et al., 2003, Vet. Clin. North Am. Food Anim. Pract.19(1):171-185
2. Grunert et al., 2018, Sci Rep. 8:15968
3. Kümmel et al., 2016, Front Microbiol 7:1603
4. Cosandey et al., 2016, J Dairy Sci. 99(1):529-40.

P064

Animal health

SEROLOGICAL INVESTIGATIONS OF NON-HUMAN PRIMATES (NHP) MAJOR INFECTIONS IN CAPTIVE NPH COLONIES

I. Ricci[3], G. Barlozzari[3], F. Rosone[3], G. Pacchiarotti[3], C. Eleni[3], V. Galietta[3], G. Marafini[3], K.G. Friedrich[2], P. Di Cerbo[2], M. Aloisi[1], M. Scarpulla[3], M.T. Scicluna[3]

[1]*Animanatura Wild Sanctuary, Semproniano (GR), Italy*
[2]*Fondazione Bioparco, Roma, Italy*
[3]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Italy*

Introduction
As part of a study conducted by our Institute for the development of diagnostic panel, to monitor the presence of major non-human primate (NHP) viral and parasitic infections, serological tests were employed to analyse blood samples of some of the NHP housed in Italian animal centres.

Materials and Methods
40 sera were collected from live NHPs to verify their health status by serological analysis, using in house and commercial kits (XpressBio Life Science Products) for the following pathogens: Simian Immunodeficiency virus, Simian retrovirus type D, Herpes B virus, Simian Foamy virus, Lymphocytic choriomeningitis virus, Simian T-cell leukemia virus, Hepatitis B Virus, Toxoplasma gondii and *Leishmania infantum*[1, 2, 3].

Results
A high percentage (70%) of positivity for Simian Foamy virus was detected. This virus can affect all NHP species even if with subclinical infections, and is very widespread in primate colonies[4]. Serological analyses for parasitic agents did not reveal any positivity, however, further analysis of the samples using molecular diagnosis detected *Leishmania infantum* in five Old World NHPs.

Discussion and Conclusion
The setup of diagnostic panels can support the development of sanitary monitoring protocols for NHP centres to verify the circulation of infectious agents, especially those with zoonotic potential providing a valuable tool for the health management of animals as well as to protect the safety of operators in contact with these animals.

References
1. Jiang, X.; Fan, Z.; Li, S.; Yin, H. A Review on Zoonotic Pathogens Associated with Non-Human Primates: Understanding the Potential Threats to Humans. Microorganisms 2023, 11, 246. https://doi.org/10.3390/microorganisms11020246
2. Dubey JP, Murata FHA, Cerqueira-Cézar CK, Kwok OCH, Yang Y, Su C. Recent epidemiologic, clinical, and genetic diversity of Toxoplasma gondii infections in non-human primates. Res Vet Sci. 2021 May;136:631-641. doi: 10.1016/ j.rvsc.2021.04.017. Epub 2021 Apr 16. PMID: 33957546.
3. Azami-Conesa, I.; Gómez-Muñoz, M.T.; Martínez-Díaz, R.A. A Systematic Review (1990–2021) of Wild Animals Infected with Zoonotic *Leishmania*. Microorganisms 2021, 9, 1101. https://doi.org/10.3390/ microorganisms9051101
4. Murray, S.M.; Linial, M.L. Simian Foamy Virus Co-Infections. Viruses 2019, 11, 902. https://doi.org/10.3390/ v11100902

P065

Animal health

CONCENTRATIONS OF TRACE ELEMENTS IN STRIPED DOLPHIN (*S.COERULEOALBA*) TISSUES FROM THE TYRRHENIAN AND IONIAN COASTLINES (CALABRIA, ITALY)

D. Ricupero[1], A. Priolo[1], G. Lucifora[1], P. Gallo[1], M. Esposito[1], E. De Carlo[1], L. Curcio[1]

[1]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy*

Introduction

Metals pose the greatest toxicological threat due to their persistence,bioaccumulation capacity and biomagnification[1], with different effects in marine mammals [2,3]. The study aim was to evaluate concentrations of toxic elements in striped dolphin tissues by integrating our data with model outputs for coherent environmental assessments in the Mediterranea Sea. It should be emphasized that the results have a broad strategic in the One Health perspective, highlighting the importance of the cetaceans as bio- indicator.

Materials and Methods

From 2015 to 2022, 57 specimens of striped dolphin (*S.coeruleoalba*) stranded along the coast of Calabria region (Figures 1-2). The biometric parameters were recorded during the necropsy examination, at the same time liver, kidney and muscle tissues were removed and stored at -20°C until analysis. Atomic absorption spectrophotometer with a graphite furnace (GF-AAS) for determination of Cd e Pb (wavelength: Cd= 228.80 nm; Pb=283.31 nm) was used (PinAAcle 900Z, Perkin Elmer). Mercury was detected as total Hg by atomic absorption at 253.65 nm using a Direct Mercury Analyzer (DMA 80 evo, Milestone).

Results

A total of 57 specimens of *S.coeruleoalba* were analyzed for assessing heavy metal concentrations in their tissues. Concentrations of Cd, Pb and Hg in liver, kidney, and muscle of striped dolphin tissues are shown in Figure 3. The accumulation trend of Cd was kidney>liver>muscle. The distribution pattern of Hg followed this general order: liver>kidney>muscle. No significant difference in Pb was found in the sample analyzed.

Figure 1.

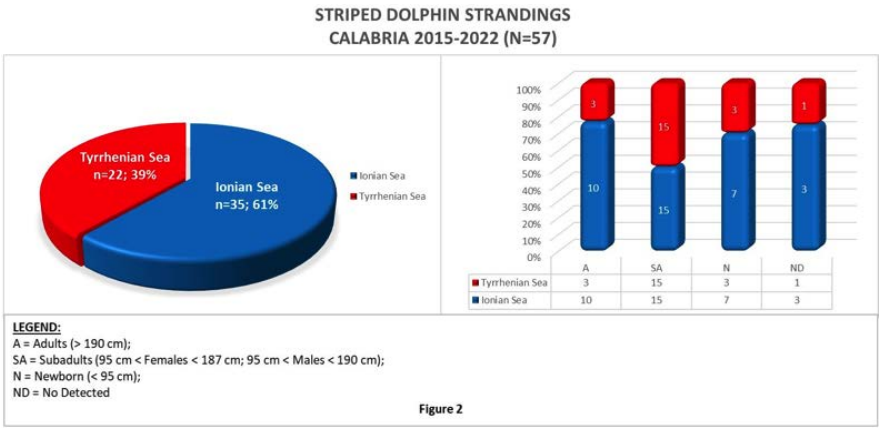


Figure 2.

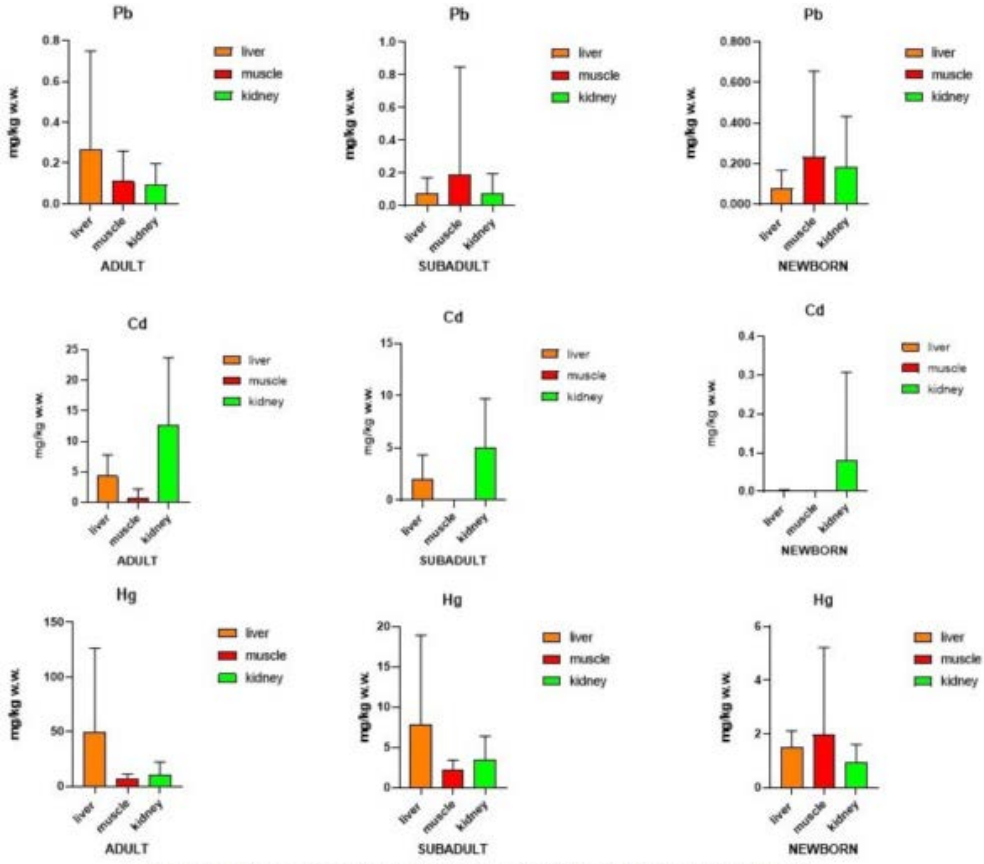


Figure 3. Mean ± SD metals concentration in target organs of adult, subadult and newborn specimens

Figure 3.

Discussion and Conclusion

To the best of our knowledge, this is the first paper reporting levels and distribution of toxic elements in the striped dolphins tissues stranded along the coast of the Calabria region in southern of Italy. Our results showed a greater accumulation of Hg in the liver, according to data reported for the same species from other locations, while Cd was more widely distributed in renal tissue. Finally, Pb levels were similar in the three target organs.

References

1. Sujitha, S.B. et al. 2019.Health 41, 1075–1089.
2. Jakimska, A.,et al. 2011a. Pol. J. Environ. Stud. 20, 1117–1125.
3. Jakimska, A., et al. 2011. Pol. J. Environ. Stud. 20 (5), 1127–1126.

P066

Animal health

SUBCLINICAL INFECTION WITH *ANAPLASMA PHAGOCYTOPHILUM* IN LAMBS AND IMPLICATIONS FOR LAMB PERFORMANCE

M. Rocchi[1], A. Stephen[1], A. Hayward[1], M. Maley[1], H. Mcdougall[1], S. Brown[1], J. Duncan[1], F. Kenyon[1], G. Mitchell[1]

[1]*Moredun Research Institute, Pentlands Science Park, Bush Loan, Penicuik, EH26 0PZ, Scotland, UK*

Introduction

Anaplasma phagocytophilum (AP) is a tick-transmitted pathogen causing disease in many species. In sheep, AP is the causative agent of Tick-Borne Fever (TBF). TBF symptoms can differ between animals and vary in severity from asymptomatic to fatal and the disease manifests generally as immunosuppression. Here we describe the effects of a naturally-occurring AP subclinical infection on lamb performance, including body weight, dag score and faecal egg counts (FEC), with relevance to the UK farming management system.

Materials and Methods

TBF was diagnosed in 2022 in a group of lambs undergoing parasitological field studies. As planned by the initial study design, lamb performance metrics were collected fortnightly over 21 weeks, and blood was collected at the end of the study for AP qPCR. Of 101 lambs, 4 presented with clinical symptoms, 12 were subclinical, 1 was equivocal and 84 were negative by AP qPCR. Mean total weight gain across the study was compared between groups using linear models with birthweight and strongyle FEC added into the model as continuous covariates.

Results

Comparing weight gain among animals, the subclinically infected group showed a 2.5 kg reduction over 21 weeks. There was no significant differences in strongyle FEC between infected/uninfected lambs, however increasing strongyle egg count was associated with lower weight in TBF-positive animals, but not in TBF negative animals. Other clinical parameters were not correlated with AP infection.

Discussion and Conclusion

Despite historical recognition of the presence of AP in the UK tick and livestock population, effects of infection on sheep performance specific to the UK management systems are just beginning to be investigated. The 2.5 kg decrease in total weight gain in infected lambs is similar to, though less than, the reduction of 3.8 kg seen in a group of lambs in Norway (1), and this effect seems to be accentuated in animals with higher strongyle egg counts, suggesting an interplay between the two infections. Further research is needed into the long-term effects of AP infection on and its association with lamb performance in this specific management system.

References

1. Stuen, Bergstrom, and Palmer (2022). J Clin Microbiol. 2002 Sep; 40(9): 3192–3197.

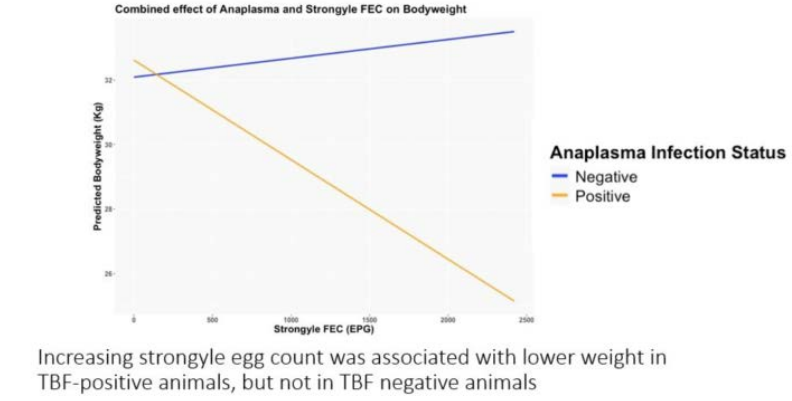


Figure 1. Interaction between TBF status and Fecal Egg Count (FEC)

P067

Animal health

VAIRIMORPHA CERANAE AND *VAIRIMORPHA APIS* IN BEES (*APIS MELLIFERA*), POLLEN AND HONEY IN SOUTHERN ITALY.

G. Sgroi[2], A. Mancusi[2], G. Rofrano[1], D. Signorelli[1], M.G. Lucibelli[2], M. Esposito[1], Y.T.R. Proroga[2], N. D'Alessio[2], A. Gallo[1], A. Siciliano[1], L.J. D'Auria[1]

[1]*Centro di Referenza Nazionale per l'analisi e studio di correlazione tra ambiente, animale e uomo, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy*

Introduction
Populations of bees have undergone significant decline partly due to pathogens and environmental pressure, threatening their health and immune response [1]. In this scenario, investigating the occurrence of agents such as *Vairimorpha* spp. in bees, honey and pollen is crucial [2].

Materials and Methods
Several sampling sites in Campania region (southern Italy) were selected based on environmental conditions [3]. DNA was extracted from bee, honey and pollen samples and tested for *Vairimorpha* spp. targeting a partial fragment of the 16S rRNA gene. Amplicons obtained were sequenced and sequences compared with those on GenBank.

Results
Regarding bees, *V. ceranae* and *V. apis* were found in 2 out of 10 and 4 out of 10 sites, respectively. In pollens, only V. ceranae was found in a single samples, whereas in honeys a single *V. apis* infection was detected.

Discussion and Conclusion
The genetic variability observed in *V. ceranae* suggests genetic diversity in the population of this pathogen, as also highlighted by previous data in the Piemonte region. These findings highlight the importance of further investigation of parasite transmission between bees and other environmental matrices. Future research will aim to evaluate the presence of *V. ceranae* and *V. apis* in other sites and farms, deepening the understanding of their distribution and its implications for bee health and the ecosystem in general. Research funded by the italian Ministry of Health, grant number IZS ME 08-22 RC.

References
1. World Health Organization. Antimicrobial resistance: global report on surveillance. Geneva, Switzerland 2014.
2. Martín-Hernández R, Meana A, Prieto L, Salvador AM, Garrido-Bailón E, Higes M. Outcome of colonization of Apis mellifera by Nosema ceranae. Appl Environ Microbiol. 2007;73:6331-8.
3. Signorelli D, D'Auria LJ, Di Stasio A, Gallo A, Siciliano A, Esposito M, De Felice A, Rofrano G. Application of a Quality- Specific Environmental Risk Index for the Location of Hives in Areas with Different Pollution Impacts. Agriculture 2023;13:998.

P068

Animal health

GAINED EXPERIENCE, CHALLENGES AND LESSONS LEARNED FROM A RECENT DOG BOTULISM OUTBREAK IN ITALY

C. Scalfaro[2], G. Cillo[4], L. Marino[3], A. Lanza[1], S. Taglieri[2], F. Anniballi[2]

[1]*Azienda Ospedaliera di Rilievo Nazionale A. Cardarelli, Poison Centre, Napoli, Italy*
[2]*Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health, National Reference Centre for Botulism, Rome, Italy*
[3]*Istituto Zooprofilattico Sperimentale dell'Abbruzzo e del Molise, Campobasso, Italy*
[4]*Practitioner Veterinarian, Colle Sannita, Benevento, Italy*

Introduction
Dogs are considered relatively resistant to botulinum neurotoxins; however, some clinical cases have also been reported. Animals show paresis affecting all four limbs, with normal mentation and satisfactory cranial nerve function. Reflexes are usually absent, while pain perception is intact. Urinary retention has been observed in several cases. In this study, we describe the largest outbreak of dog botulism in Italy, which occurred upon the consumption of poultry carrions in litter spread in the agricultural land as fertiliser.

Materials and Methods
The outbreak occurred in a poorly anthropised area between Benevento and Campobasso provinces. In this area, each farm has some dogs

that, during the night, are free to wander and eat. On the 7th of February morning, a 4.5-year- old Caucasian shepherd returned to the farm with an overstretched abdomen and held a poultry carcass between its jaws. In a few hours, the dog was unable to stand, showing quadriplegia, lateral recumbency, persistent mydriasis and tachypnoea. Two other dogs of the same owner showed similar symptoms. Botulism was suspected, biological samples and poultry litter were collected for laboratory tests, and an epidemiological investigation was conducted.

Results
The outbreak, consisting of 8 cases, was laboratory-confirmed by detecting botulinum toxin in dropping material (9000 MDL/g) and detecting *Clostridium botulinum* in faecal samples of 3 animals and poultry litter. Multiple-Locus of Variable number tandem-repeat Analysis conducted on enrichment faecal and litter broths showed that neuro-toxigenic strains were indistinguishable.

Discussion and Conclusion
The outbreak reported here occurred because several incorrect behaviours and practices were carried out at different levels. Considering the amount of botulinum neurotoxin recovered, the farm should have been interested in a botulism outbreak. In this situation, the farmer should have removed carcasses from the litter to prevent the disease from perpetuating and stabilise the manure before spreading on the land, as requested by national and European legislation. Veterinary public health authorities should collaborate with the reference centre to increase farmers' and veterinarians' awareness of animal botulism and include it among the notifiable diseases.

References
Uriarte A. et al. Can Vet J 2020;51:1139-42..

P069

Animal health

PRESENTATION OF THE GERMAN CONSILIARY LABORATORY FOR *CORYNEBACTERIUM PSEUDOTUBERCULOSIS*

R. Sting[1], B. Polley [1], E. Hiller[1], L. Schneider-Bühl[1]

[1]*Chemical and Veterinary Analysis Agency Stuttgart (CVUAS); Fellbach, Germany*

Introduction
In addition to National Reference Laboratories for listed animal diseases, in Germany there are Consiliary Laboratories for some animal diseases that are not notifiable but very important. We are reporting on our work as Consiliary Laboratory for *Corynebacterium* (C.) *pseudotuberculosis*, the causative agent of contagious lymphadenitis, CLA. Following the successful control of CLA in goat farms in Baden-Wuerttemberg (Germany), this infectious disease is increasingly becoming a problem in camelids (alpacas, llamas, dromedaries, camels). As in small ruminants, CLA manifests in abscesses in lymphnodes and internal organs, particularly the lungs. CLA in camelids can lead to considerable losses. The pathogen is mainly transmitted through animal contact. The risk of introducing CLA into a herd is particularly high if infected animals are brought in for breeding purposes or by purchase. As with small ruminants, the spread of CLA in camelids can only be prevented by consistent quarantine measures prior to the introduction of foreign animals into a herd and by regular testing. These measures are particularly important as effective treatment options for curing CLA are currently not available.

Materials and Methods
In our laboratory, we offer serological tests using commercial ELISA, in-house ELISA and Western blot. Furthermore, we carry out classical bacteriological tests as well as the detection of pathogen DNA by qPCR. We also use Next Generation Sequencing (NGS) for the genetic characterization of selected isolates and for molecular epidemiology. Furthermore, we offer ring trials for the direct and/or indirect detection of *C. pseudotuberculosis*. Next step is the development of partial sequencing of selected and suitable gens for simple and cost-effective isolate characterization.

Results
Laboratory tests in combination with clinical examinations of the animals make it possible to determine the status of farms. Animals should only be exchanged if they have the same status. This is an important basis for preventing the spread of CLA and for taking targeted measures to combat this infectious disease in affected herds.

Discussion and Conclusion
Although the pathogen is well-known and widespread, we were able to gain new insights through the use of NGS, which are briefly presented in the poster.

P070

Animal health

PREVALENCE OF SOME RESPIRATORY VIRUSES AND EVALUATION OF BACTERIAL COINFECTIONS IN SWINE FROM SOUTHERN ITALY

M. Levante[1], F. Serra[1], N. Riccone[1], G. Desio[1], A.M. Pugliese[1], S. Brandi[1], F. Del Piano[1], A. Cacace[2], E. Di Lecce[2], G. De Luca[1], E. De Carlo[1], G. Fusco[1], M.G. Amoroso[1]

[1]*Experimental Zooprophylactic Institute of Southern Italy, Department of Animal Health, Portici, Italy*
[2]*University of Naples Federico II, Naples, Italy*

Introduction

Respiratory diseases, caused by pathogenic viral agents, represent one of the main causes of economic losses in pig farms. Viruses mainly involved are: porcine circovirus (PCV-2, -3), porcine respiratory and reproductive syndrome virus (PRRSV) and Aujeszky's disease virus (SHV-1), which might induce respiratory, enteric, immunosuppressive and reproductive systemic syndromes (1-3). The present study was carried out on 95 animals, collected in Southern Italy from 2021 to 2023. Specifically, all the samples were analysed for SHV-1, 50 were investigated for PCV2-3, and 50 for PRRSV.

Materials and Methods

Nucleic acids extraction was performed on 200 µl of homogenized organ by QIAsymphony extraction system. Detection of the viruses was investigated by Real-Time PCR, using specific protocols (1,2) or kits (VetMAX PRRSV). Positive organs underwent bacterial isolation. Selective and non selective media were used (no salt Mac Conkey Agar and Tryptone soy Agar +5% Blood), incubated in both aerobiosis and anaerobiosis.

Results

The results showed that 28/95 (29.5%) animals were positive to at least one virus. Specifically, 20/50 samples were positive to PCV2-3 (in details 12 to PCV2, 6 to PCV3, 2 to both the PCVs), with an overall prevalence of 40%; 12/50 (24%) resulted infected by PRRSV. All specimens were negative to SHV-1. Among the animals positive to at least one viral agent, 25% showed viral coinfection. Moreover, bacterial analysis, performed on animals with viral infection, resulted positive for 60.7% of the analyzed samples (Table 1), with presence of bacteria among which: *T. pyogenes , P. multocida, S. suis.*

YEAR	VIRAL PRESENCE			BACTERIAL POSITIVITY
	PCV-2	PCV-3	PRRSV	
2021	N	**P**	N	*Escherichia coli; Enterococcus hirae*
	N	N	**P**	*Escherichia coli; Streptococcus suis*
	P	N	**P**	*Escherichia coli; Clostridium perfringens*
	N	N	N	*Escherichia coli*
	P	N	N	*Aeromonas spp*
	P	N	N	*Streptococcus intermedius; Klebsiella pneumoniae*
	P	N	N	*Trueperella pyogenes*
2022	**P**	**P**	N	*Clostridium perfrigens;Streptococcus dysgalactiae;Streptococcus equisimilis*
	N	**P**	N	*Escherichia coli; Clostridium perfrigens*
	N	N	**P**	*Escherichia coli*
2023	**P**	N	**P**	*Proteus vulgaris*
	P	N	N	*Escherichia coli*
	N	**P**	N	*Escherichia coli*
	N	N	**P**	*Staphylococcus chromogenes*
	P	N	**P**	*Pasteurella multocida; Escherichia coli*
	P	N	**P**	*Streptococcus spp*
	N	N	**P**	*Escherichia coli*

Table 1. Bacterial co-infections in viral positive samples.

Discussion and Conclusion

In this study, viral prevalence was significant, with the exception of SHV-1, never found due to a vaccination plan for eradication. Infections by PCV2-3 and PRRSV might increase the susceptibility of pigs to bacterial contagion, as shown by the presence of both viruses and bacteria in more than 50% of the cases. Interestingly some animals showed the simultaneous presence of 2 viruses and 3 bacteria. On this regard, attention must be posed on co-infections which might raise the severity of the diseases also increasing mortality rates.

References

1. Amoroso MG. et al.Vet. Med. and Sci.2020
2. Amoroso MG. et al.Animals.2021
3. Ferrara et al.Viruses.2023

P071

Animal health

RECOMBINANT N PROTEIN OF RIFT VALLEY FEVER EXPRESSION IN BACULOVIRUS SYSTEM AND ITS USE IN IMMUNOENZYMATIC ASSAY: PRELIMINARY DATA

A. Serroni[1], G. Armillotta[1], C. Laguardia[1], S. Ulisse[1], M. Iorio[1], L. Testa[1], F. Parolini[1], S. Capista[1], S. Traini[1], S. Fanì[1], C. Palucci[1], G.F. Ronchi[1], M. Di Ventura[1], M.T. Mercante[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Campo Boario, Teramo, Italy*

Introduction

Rift Valley Fever (RVF) is an arthropod-borne, zoonotic disease caused by the RVF virus (*Phlebovirus genus, Bunyavirales* order, and *Phenuiviridae* family); it affects domestic animals and humans. The genome consists of 3 single-strand RNA segments, encoding structural proteins: N, Gn, Gc and polymerase L; non-structural proteins: NSs and NSm; and a 78 KDa protein. The virus presence in Africa, in some islands in the Indian Ocean, in a French overseas department (Mayotte), increases the risk of RVF incursion into Europe [1]. The aim of this study is to optimise the RVF N protein production and purification protocol to use in the development of a diagnostic kit.

Materials and Methods

The His-tag recN protein was expressed in the baculovirus system. The optimal protocol was developed testing different conditions (MOI and harvest time). The production was performed infecting 1.5 L of Sf9 cells (1.5 ×10^6 cells/mL) at 0.001 MOI; the supernatant was collected at 120 h post infection, supplemented with 0.2M L-arginine hydrochloride was added and placed at 4°C overnight until purification by affinity chromatography. The protein was characterised by SDS-PAGE Coomassie, Western blot and indirect ELISA using different mAbs and a positive serum.

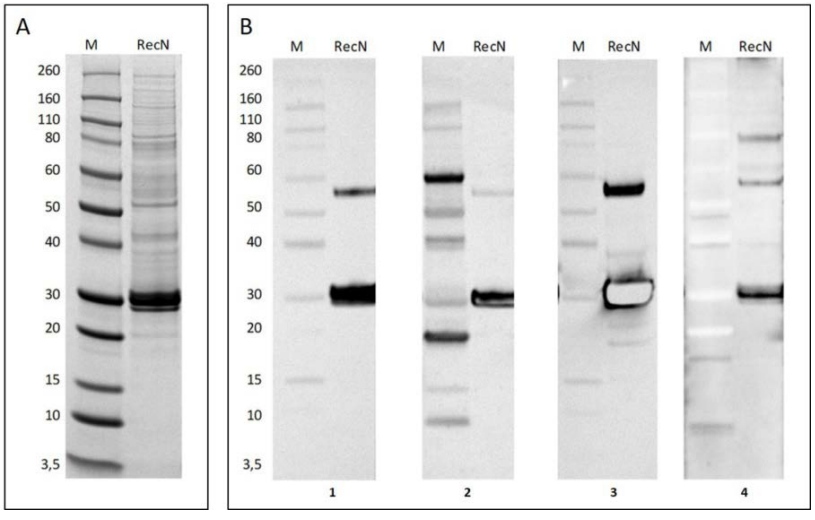


Figure 1. A) Coomassie Blue staining of purified RVF recombinant N protein. B) Western blot of purified RVF recN-protein using anti-Rift Valley Fever NP Monoclonal antibody (1); His-tag antibody anti 6xHis C-term (2); V5-tag antibody (3); positive serum (4).

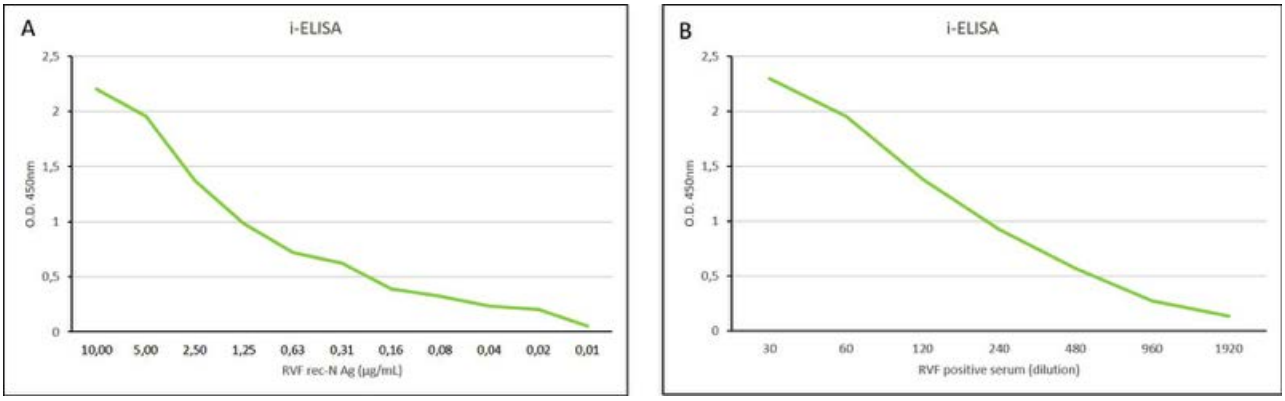


Figure 2. A) Indirect-ELISA using serial dilutions of RVF recN as antigen and RVF positive serum. B) Indirect-ELISA using RVF recN as antigen and RVF positive serum at serial dilutions.

Results

A good yield of recN with a high grade of purity was obtained (about 10 mg per $2x10^9$ Sf9 infected cells), and the Western blot performed using different mAbs and the positive serum showed an immunoreactive band at the attended molecular weight of 30 KDa (Figure 1). The ELISA results using the positive serum and the commercial mAb showed a specific Ag-Ab reaction (Figure 2).

Discussion and Conclusion

The increased risk of RVF incursion in Europe requires national and international health authorities to increase attention, so it is essential to implement surveillance. The immunogenic properties of the Gn, Gc and N proteins make them good candidates for both diagnostic tests and vaccine development. In this study, we produced a good amount of purified RVF recN protein and, as shown in ELISA, the protein retains its native antigenic characteristics. These results lay the foundations for further studies aimed to the development of diagnostic methods to support disease monitoring and useful tools in case of disease incursion into Europe.

References

1. WOAH Terrestrial Manual 2023, Chapter 3.1.19

P072

Animal health

COMPLETE GENOMIC SEQUENCE OF A PESTIVIRUS N (TUNISIAN SHEEP-LIKE PESTIVIRUS) ISOLATED IN ITALY

E. Sozzi[1], C. Chiapponi[2], G. Leo[1], L. Soliani[2], D. Lelli[1], C. Tolini[1], M. Carrera[1], T. Trogu[1], A. Moreno[1], M. Bazzucchi[1], A. Lavazza[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "Bruno Ubertini" (IZSLER), Brescia, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "Bruno Ubertini" (IZSLER), Parma, Italy*

Introduction

The Tunisian sheep-like pestivirus (TSV, *Pestivirus* N) was first isolated from batches of a contaminated sheep pox vaccine in Tunisia. Subsequently, it has been detected in sheep in France and goats and sheep in Italy [1]. In 2023, a non-cytopathogenic *Pestivirus* N was isolated using lamb kidney cells (LKi) from a sheep and its lamb held on pasture in northern Italy. Animals were immunotolerant and persistently viraemic, testing negative for antibodies against pestiviruses over the long term. Our study aimed to carry out the full-length sequence of the *Pestivirus* N, strain 26203/2_2024, isolated from the sheep's serum.

Materials and Methods

The complete genome was obtained using the MiSeq platform (Illumina, San Diego, CA, USA). Sequencing libraries were made with an Illumina TruSeq RNA Sample Preparation Kit v 2 according to the manufacturer's instructions. Reads of 26203/2_2024 were assembled de novo using CLC Genomic Workbench v.11 (QIAGEN, Milan, Italy) with an average coverage of 218X. A maximum likelihood phylogenetic tree was constructed using MEGAX by applying a generalised time-reversible nucleotide substitution model and modelling among-site rate heterogeneity through a discretised gamma distribution with invariant sites (GTR + G + I) with 1000 replicates, identified using ModelFinder selection.

Results

The analysis of the full-length genome of 26203/2_2024, 12288 nucleotides (nt) in length, confirmed the grouping of the new Italian sheep strain within the *Pestivirus* N cluster (Figure 1). The whole genome showed 83,78% nucleotide identity with the 92019/2007/AG strain, which was identified in a goat in Sicily, Italy.

Discussion and Conclusion

This is the first case reported from the north of Italy, and thus, further epidemiological investigation is required to understand its origin, the way of diffusion, and the clinical outcomes. Indeed, it would be interesting to better define its presence within sheep herds and real distribution in the area. The availability of the full sequence will be useful also for the development of specific diagnostic tools.

References

1. Meyer D. et al. Comparative Analysis of Tunisian Sheep-like Virus, Bungowannah Virus and Border Disease Virus Infection in the Porcine Host. Viruses. 2021 Aug 4;13(8):1539.

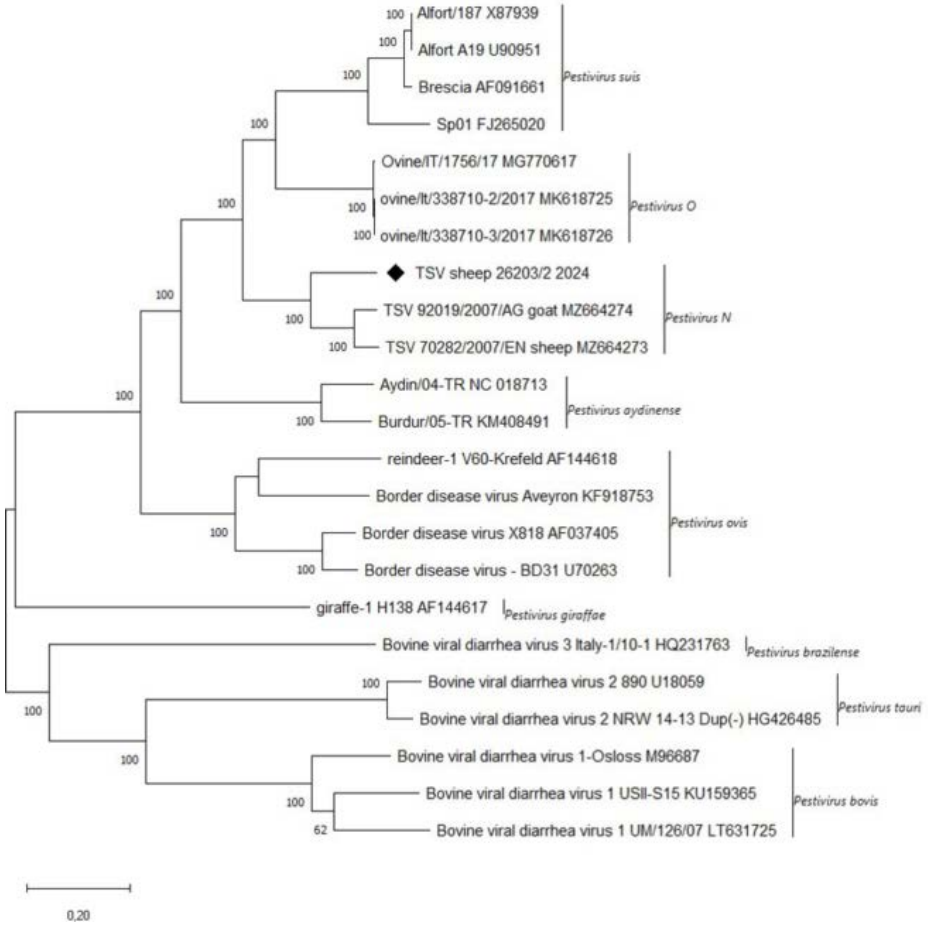


Figure 1. Maximum Likelihood phylogenetic tree, based on the complete genomes of the 26203/2_2024 *Pestivirus* N (black rhombus) and other pestivirus sequences deposited in GenBank.

P073

Animal health

USE OF DNA TESTING TO ASSESS TRACEABILITY OF KEPT ANIMALS

N. Spissu[1], G. Campus[1], B. Mossa[1], S. Canu[1], G. Bitti[1], M. Lasia[1], A. Satta[1], G. Cavedagna[1], S. Bionda[1], G.B. Petretto[1], C. Maestrale[2], F. Sgarangella[1], P. Desini[1]

[1]*Azienda Sanitaria Locale Sassari, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*

Introduction
Traceability of animals and products is an essential tool for public and animal health protection and food safety. Italian Legislative Decree 134/2022, imposes to identify and register all animals kept in establishments, with fixed terms and methods and to cull unidentified animals or to destine them for other uses than human consumption or to prove traceability submitting them to laboratory tests. Typing of DNA is used as an individual identification method. Short tandem repeat (STR) analysis is a common molecular biology method used to compare allele repeats at specific loci in DNA between two or more samples. By analyzing these markers we obtain the genetic profile from which it is possible to attribute or exclude paternity or maternity. The authors describe the use of DNA testing to confirm the parentage instead of culling in presence of unidentified animals.

Materials and Methods
Subject of the study is a swine establishment sited in Sardinia (Italy) submitted to an official control in which was recorded the presence of n. 10 animals without identification and registration. The pigs where submitted to genetic testing aimed at establishing compatibility with the 13 other regularly identified subjects present in the farm. Blood samples from 23 pigs were collected in EDTA tubes, and sent to the Istituto Zooprofilattico Sperimentale for the test's execution. The comparison was performed using STR markers and PCR.

Results
The results showed genetic compatibility for 16/16 analysed loci for 9 unidentified pigs with 6 of the identified pigs. Genotype was compatible for more than 50% of allels so maternity/paternity was highly proven. For 1 unidentified sow the test didn't evidence any genetic compatibility.

Discussion and Conclusion
Traceability is the ability to maintain the identification of animal, or animal products, all along the production chain. It represents an essential tool to safeguard public and animal health. Our result evidence that parental identification is a rapid, cost effective and ethical alternative to culling that is less morally problematic thus respecting what is prescribed by the law in case of unidentified animals. In fact DNA is unalterable, detectable in every cell, and allows for individual, breed or species identification.

P074

Animal health

SUBCLINICAL MASTITIS RELATED TO *STREPTOCOCCUS CANIS* INFECTION

A. Sposato[3], L. Del Sambro[2], S. Castellana[2], L. Capozzi[2], A. Bianco[2], G. Schino[2], E. Catalano[2], A. Miccolupo[2], M. Galgano[2], C. Trisolini[2], D. Pugliese[2], A. Milano[2], L. Addante[2], L. Pace[1], L. Marino[1]

[1]*Istituto Zooprofilattico Sperimentale di Puglia e Basilicata, Sede Centrale di Foggia (FG), Foggia, FG, Italy*
[2]*Istituto Zooprofilattico Sperimentale di Puglia e Basilicata, Sezione di Putignano (BA), Putignano, BA, Italy*
[3]*Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy*

Introduction
Streptococcus (S.) *canis* could represent a contagious pathogen causing subclinical mastitis (SM) in dairy cows (1). Potential sources of infection are cats and dogs with access to the barn (2). Poor udder health management during the milking procedure is the main risk factor. In the present study, we isolated *S. canis* from milk samples. These strains were characterized for their phenotypic and molecular properties.

Materials and Methods
A dairy farm located in Apulia was inspected for a suspect of SM. The hygienic conditions were moderate, free domestic carnivores were present. Eight milk samples tested + on CMT were collected aseptically from different cows for bacteriological and genomic analyses to find causative pathogen. The SCC were determined using FC equipment. A mutiplex qPCR assay was performed with the VetMAX™ MastiType Multi Kit to detect 15 possible pathogens. Microbiological procedures were performed, 3 strains were extracted, sequenced and submitted to bioinformatic analysis.

Results
Three milk samples showed SCC >200 x103 cell/ml. All samples tested - at the qPCR. Three samples were positive on CBA plates, showing β-hemolysis, and on ED plates. Bacteria were phenotypically referred to Streptococci. Strains were identified as *S. canis* by using MALDI-TOF methodology. The colonies were tested for the common ATB and the resistant for TE was evaluated. Genomic DNA from three *S. canis* isolates was sequenced on Illumina MiSeq® platform. Genetic relationships were investigated by 7 gene-MLST and revealed a unique sequence type (ST24); genomes were 99.99% similar to each other (ANIb). One relevant AMR gene, related with resistance to TE (tetM) was predicted. Swabs samples from the oral cavity of the carnivores are currently being performed, in order to identify who is the possible source of infection.

Discussion and Conclusion
Association between *S. canis* and mastitis is not routinely detected. Chances of recovery of the animals are linked to an early diagnosis of bacterial infection and to the study of the antimicrobial profile. Pets could act as a potential reservoir of bacteria. Improving hygienic conditions are needed to prevent new infections.

This research was supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT)

References
1. https://doi.org/10.3168/jds.2015-9454
2. https://doi.org/10.3168/jds.S0022-0302(05)72949-0

P075

Animal health

EVALUATION OF DIFFERENT MARKERS OF TRAINED INNATE IMMUNITY INDUCED BY MYCOBACTERIA IN A PORCINE MODEL

T. García-Seco[4], I. Moreno[3], C. Herranz[4], A. Pérez-Domingo[4], A. Díez-Guerrier[2], A. Buendía[4], M.Á. Risalde[1], F. Cruz[4], M. Domínguez[3], L. Domínguez[5], M. Pérez-Sancho[5]

[1]*Departamento de Anatomía y Anatomía Patológica Comparadas y Toxicología, Grupo de Investigación en Sanidad Animal y Zoonosis (GISAZ), UIC Zoonosis y Enfermedades Emergentes (ENZOEM), Córdoba University, Córdoba, Spain*
[2]*MAEVA SERVET S.L., 28749-Alameda del Valle, Spain*
[3]*Unidad de Inmunología Microbiana, Instituto de Salud Carlos III. -Majadahonda, Spain*
[4]*VISAVET Health Surveillance Center, Complutense University, Madrid, Spain*
[5]*VISAVET Health Surveillance Center, Complutense University, Madrid, Spain; Departamento de Sanidad Animal, Faculty of Veterinary Medicine, Complutense University, Madrid, Spain*

Introduction
Trained innate immunity (TII) is the ability of the innate immune system to enhance its response following an initial exposure to certain specific stimuli (such as *Mycobacterium bovis* BCG), where metabolic and epigenetic modifications in some innate cells lead to a more efficient response against heterologous agents. Thus, TII opens a new research line for the control of diseases for which there are still no effective and universal vaccines. In this context, the PID2020-112966RB-I00 project has evaluated the ability of BCG to stimulate TII in porcine model to induce an effective protection against other heterologous bacteria such as *Streptococcus suis* (*S. suis*).

Materials and Methods
Twenty piglets (n=4/group) were included: group A (live BCG intravenously, 1 dose), group B (inactivated BCG intravenously, 1 dose), group C (inactivated BCG intravenously, 2 doses-14 days apart), group D (inactivated BCG orally, 1 dose), and group E (uninoculated control). Blood was taken on 0, 14, 36, 57, 91, and 120 days post-inoculation (dpi) to assess the induced TII by the study of immune cell subpopulation profiles (measured by flow cytometry) and the phagocytic capacity of immune cells against *S. suis* (by oxidative burst and phagocytosis assays).

Results
On 14 dpi, an increase in the percentage of granulocytes was observed in group D (compared to group E), as well as in the phagocytic capacity in groups A, C, and D (compared to 0 dpi). On 57 dpi, an increase in oxidative burst was observed in groups B and C. No more differences were observed along the study.

Discussion and Conclusion
The complexity of evaluating the TII based on the response of peripheral blood cells is evidenced by the results obtained in porcine model. Although the potential usefulness of these approaches has been demonstrated, limitations are also revealed. This suggests the need to improve the available tools to characterize the association between TII markers (in different models using several candidates) and the immunomodulatory capacity of BCG for the development of an effective TII against *S. suis.*

Work funding: MCIN/ AEI /10.13039/501100011033

References
Kleinnijenhuis 2012. doi: 10.1073/pnas.1202870109 Sánchez-Ramón 2018. doi: 10.3389/fimmu.2018.02936 Vas-Rodrigues 2022. doi: 10.1186/s13567-022-01047-8

P076

Animal health

LAMP : NEW DNA/RNA AMPLIFICATION TECHNOLOGY AS A POINT OF CARE TOOL TO HELP DIAGNOSTIC OF CORYZA IN CATS

T. Thibault[1], L. Melo[1], L. Valot[1], A. Zocevic[1], M. Simonnet[1], W. Yakoub[1], D. Schieb[1], M. Gene[1], E. Schaeffer[1], C. Bettin[1], L. Thiery[1]

[1]*Enalees, France*

Introduction
Currently, the direct detection of pathogens contaminating animals can be performed by 1) PCR, giving highly sensitive results but this requires to send the samples to a specialized laboratory which can give the results only few days later. 2) Antigenic tests which can give a result quickly but with a lower sensibility than PCR. LAMP technology (Loop mediated isothermal AMPlification) (Notomi T et al., 2000) is a NAAT technology (Nucleic Acid Amplification test) which can occur at constant temperature, unlike PCR which need thermal cycles. This allow the use of this technology as a POC (point of care) test and allow the obtention of the result in only 30 min without the necessity to use a thermal cycler. This technology can be really useful to detect highly contagious pathogens causing diseases in animals and the possibility to administrate the appropriate medicine or isolate the animal immediately. In case of a pandemic, this technology can be useful to isolate quickly contaminated animals and avoid a propagation of the pathogen.

Materials and Methods
Specific LAMP primers for Feline Herpesvirus / Feline Calicivirus / Chlamydia felis were designed and LAMP reactions were performed on oropharyngeal swab samples extracted by our own specific quick extraction (Valot L et al, 2021). Performances of these tests (AC-HCC, Enalees) were evaluated and compared to PCR performed in an external clinical laboratory.

Results
LAMP tests have a limit of detection for the complete method (quick extraction and LAMP assay) similar or better than the limit of detection of the complete method (extraction on magnetic beads and PCR assay) obtained in a conventional analysis laboratory. LAMP test which was designed are really specific for the target (>95% specificity) and are really sensitive for the target (>93% sensitivity). This technology is really fast (<35 min) and can be used as a point of care test.

Discussion and Conclusion
LAMP tests are a new generation of DNA/RNA amplification test, really specific and sensitive, which can be performed near a sick animal and can give a result in only 30 min. These parameters allow the consideration to use this technology to analyse quickly potential pathogens holder and treat or isolate the animal immediately.

References
Notomi, T. et al. (2000)
Valot, L. et al. WO2021160849

P077

Animal health

SURVEY ON SEROLOGICAL ASSESSMENT OF *LEISHMANIA INFANTUM* IN CATS IN CAMPANIA AND CALABRIA REGIONS

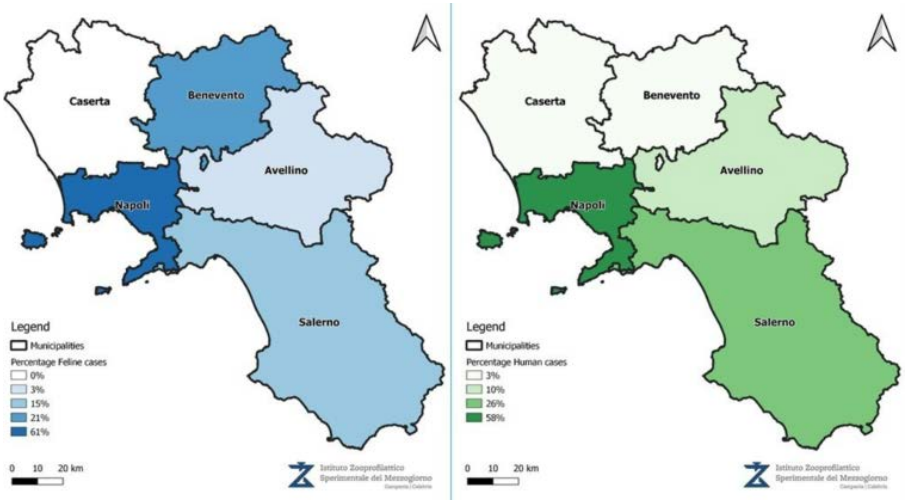
S. Cavallo[2], T. Toscano[2], R. D'Ambrosio[2], L. Barca[2], M. Garzi Cosentino[2], R. Brunetti[2], P. Palermo[2], F. Vitale[3], L. Spadari[2], V. Fogliamanzillo[4], L. Petrullo[1], M. Ascierto[1], A. Pucciarelli[2], P. Serpe[2], L. Baldi[2]

[1]*Azienda Ospedaliera dei Colli, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Mezzogiorno* [3]*Istituto Zooprofilattico Sperimentale della Sicilia* [4]*Università degli Studi di Napoli Federico II, Italy*

Introduction
The study's goal is to establish the role of infected cats as active reservoir of *Leishmania infantum* in Southern Italy using serological and direct testing (PCR) of both owned and free-roaming patients.

Materials and Methods

*Leishmania*sis was tested on 1053 cats between 2022 and June 2024. The patients were chosen from among the cats who had elective spay/neuter procedures performed at the Public Veterinary Hospitals of the Campania Region and the Veterinary Teaching Hospital of the Department of Veterinary Medicine and Animal Production, University of Naples. This study includes patients from private practices as well. The serological assessment (IFAT) for the feline *Leishmania* diagnosis was performed by the IZSM both in Portici (NA) and in Cosenza. IFAT was performed following the WOAH Terrestrial Manual-Leishmaniosis protocol and the Kit was supplied by National Reference Centre for *Leishmania*sis. The blood samples were stained with Giemsa's stain to differentiate cellular morphology from parasites. In some cases, some fo the blood and spleen samples underwent real time q-PCR. The location of *L. infantum* positive cats and human patients was geo-referenced using a geographical information system, according to the owner's province.



Cartography 1. Cases in Human (2017-2024) and Feline (2022-2024) based on Municipalities

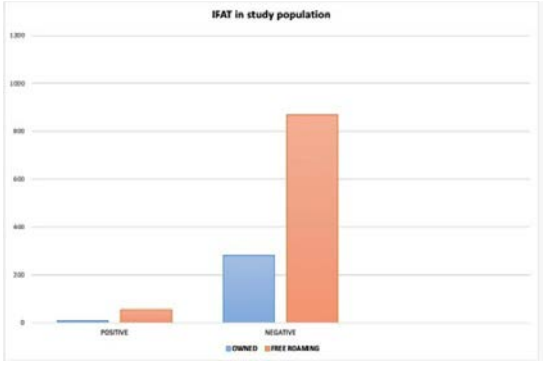


Figure 1. IFAT results between owned and free roaming cats.

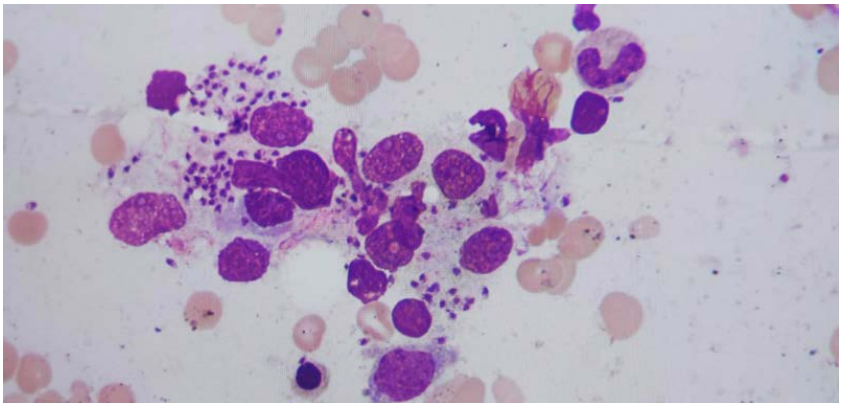


Figure 2. MGG Staining Method in Human Blood smear.

Results
The sample size was of 907 cats in Campania Region and 146 cats in Calabria Region. The results of the tests are in Figure 1. The territorial distribution of infected cats and human patients in Campania region was reported in Cartography 1.

Discussion and Conclusion
Most of the cats examined were stray cats and the data suggest that in Calabria and Campania regions, the infected cats are most free-roaming cats living in Naples Municipality like the human cases detected by Cotugno hospital. This may point to a correlation between human and feline cases. This study concerning FeL allows the hypothesis that cats could be considered as potential reservoirs. This research was funded by the Italian Ministry of Health (grant number: Research Project IZS ME 09-21 RC).

References
Persichetti MF, Solano-Gallego L, Vullo A, Masucci M, Marty P, Delaunay P, Vitale F, Pennisi MG. Diagnostic performance of ELISA, IFAT and Western blot for the detection of anti-*Leishmania infantum* antibodies in cats using a Bayesian analysis without a gold standard. Parasit Vectors. 2017

P078

Animal health

LAMP: NEW DNA/RNA AMPLIFICATION TECHNOLOGY AS A POINT OF CARE TOOL TO HELP DIAGNOSTIC OF PARVOVIRUS IN CATS AND DOGS

L. Valot[1], A. Zocevic[1], L. Melo[1], T. Thibault[1], M. Simonnet[1], W. Yakoub[1], D. Schieb[1], M. Gene[1], C. Bettin[1], E. Schaeffer[1], L. Thiery[1]

[1]*Enalees, France*

Introduction
Currently, the direct detection of pathogens contaminating animals can be performed by 1) PCR, giving high sensitive results but this require to send the samples to a specialized laboratory which can give the results only few days later. 2) Antigenic tests which can give a result quickly but with a lower sensibility than PCR. LAMP technology (Loop mediated isothermal AMPlification) (Notomi T et al., 2000) is a NAAT technology (Nucleic Acid Amplification test) which can occur at constant temperature, unlike PCR which need thermal cycles. This allows the use of this technology as a POC (point of care) test and allow the obtention of the result in only 30 min without the necessity to use a thermal cycler. This technology can be useful to detect highly contagious pathogens causing diseases in animals and the possibility to administrate medicine or isolate the animal immediately. In case of a pandemic, this technology can be useful to isolate quickly contaminated animals and avoid a propagation of the pathogen.

Materials and Methods
Specific LAMP primers for Feline parvovirus and Canine parovirus were designed and LAMP reactions were performed on blood samples or on faeces samples, both extracted by our own specific quick extraction (Valot L et al, 2021). Performances of these tests were evaluated in our lab and compared to PCR performed in an external lab.

Results
The limit of detection of all method (quick extraction and LAMP test) is similar or better of the limit of detection of all method (extraction on column and PCR test) obtained in a conventional analysis laboratory. LAMP test which was designed are specific for the target (>94% specificity) and are sensitive for the target (>97% sensibility). This technology is fast (<30 min) and can be used as a point of care test.

Discussion and Conclusion
LAMP tests are a new generation of DNA/RNA amplification test, specific, sensitive which can be performed near a sick animal and can give a result in only 30 min. These parameters allow the consideration to use this technology to analyse quickly potential pathogens holder and treat or isolate the animal immediately.

References
Notomi, T. et al. (2000).
Valot, L. et al. WO2021160849

P079

Animal health

BIOMARKERS OF OXIDATIVE STRESS IN CEREBROSPINAL FLUID IN DOGS WITH INTERVERTEBRAL DISC HERNIATION AND IDIOPATHIC EPILEPSY

M.G. Velasco-Martínez[1], A. Campos[3], Á.M. Hernández[1], D. Chicharro[1], A. Del Romero[1], L. Pardo[2], J.J. Cerón[2], K. Satué[1]

[1]Department of Animal Medicine and Surgery, Faculty of Veterinary Medicine, CEU-Cardenal Herrera University, Valencia, Spain
[2]Interdisciplinary Laboratory of Clinical Analysis (Interlab-UMU), University of Murcia, Murcia, Spain
3Les Alfàbegues Veterinary Hospital, Valencia, Spain

Introduction
Although cerebrospinal fluid (CSF) analysis is a common procedure in neurology (1,2), diagnosis cannot be reached on numerous occasions, highlighting the need for new biomarkers (3). Paraoxonase 1 (PON1) is an enzyme that belongs to the paraoxonase family, useful as a serum biomarker in different pathophysiological situations related to increased oxidative stress (4). Copper ion reducing antioxidant capacity (CUPRAC) is a method used to quantify total antioxidant activity (TAC) by means of the non-enzymatic antioxidant components of the sample (5). Although both parameters have been analyzed in serum in different inflammatory and/or infectious processes (6,7,8), their study in canine CSF is very limited (9). The aim of this work was to analyze the values of PON1 and CUPRAC in CSF from dogs with idiopathic epilepsy and acute and chronic disc herniations.

Materials and Methods
A total of 24 dogs were included. The animals were distributed into 3 groups according to their diagnosis: idiopathic epilepsy (IE; n=8), acute intervertebral disc herniations (AH; n=11) and chronic intervertebral disc herniation (CH; n=5). Samples were frozen at -18°C until biomarker quantification. Both PON1 activity and TAC analysis by CUPRAC were performed by previously validated procedures on canine serum (10,5) using the Olympus AU 600 automated analyzer (Minneapolis, MN).

Results
In dogs with chronic disc herniations, PON1 activity (41.69±9.51 IU/ml) was significantly higher than those with acute disc herniations (29.91±3.69 IU/ml) and idiopathic epilepsy (29.07±7.90 IU/ml). No differences were detected in CUPRAC values (mean value in the three groups: 0.08±0.02 mmol/l).

Discussion and Conclusion
The results show significantly higher PON1 activity in chronic disc herniations compared to the other groups. Due to the antioxidant power of this molecule (4), the increase in PON1 activity in chronic disc herniations detected could be explained as a compensation of the organism after the moment of maximum inflammation and oxidative stress. Perhaps, this antioxidant function provides a protective effect against oxidative modifications by reactive oxygen species with disease progression. However, future studies are needed to elucidate the role of both parameters in canine neurology.

References
All references sending an email to: gemma.velascomartinez@uchceu.es.

P080

Animal health

5 YEARS OF PHYLOGENETIC ANALYSIS OF PRRSV-1 STRAINS CIRCULATING IN ITALY

D. Vio[1], M. Ustulin[1], C. Zanon[1], L. Ferino[1], E. Floreani[1], C. Targhetta[1]

[1]Istituto Zooprofilattico Sperimentale delle Venezie, SCT[4], Swine Pathology and Welfare Laboratory, Italy

Introduction
Sequencing and analysis of ORF5 gene of PRRS virus is a useful resource for epidemiologic surveillance. Information obtained from public databases allows the evaluation of viral variability, the comparison and the correlation among strains of different geographical origins. This study presents the results of a phylogenetic analysis of PRRSV in Italy between 2019 and 2024.

Materials and Methods
521 PRRSV-1 ORF5 sequences were collected during routine monitoring between 2019 and 2024. Vaccine strains and 134 GenBank sequences of various geographical origins (labeled with GeneBank entry in Figure 1) were included for comparison. Sequences were aligned with MAFFT software and Maximum likelihood phylogenetic tree calculated using IQ-TREE v.1.6.6.
PRRSV-2 prototype strain VR2332 ORF5 was used as outgroup.

Results

The analysis of phylogenetic tree (Figure 1) shows that all sequences belong to subtype 1 of PRRSV-1, typical of Western Europe (1), and are divided in 6 cluster. Cluster 1 to 5 mainly includes Subtype 1 sequences from Italy correlated with lineage 3 (1) which appears to be prevalent in the country. Few sequences clustering with Lelystad virus (cluster 1) suggest circulation of prototype-like or vaccine-derived strains.

Discussion and Conclusion

The Province distribution suggests a correlation among strains of the same geographical origin; a more detailed evaluation could be achieved by adding animal transfer information. Phylogenetic analysis of PRRSV strains can be a useful epidemiological tool for monitoring and evaluating the variability and evolution of circulating strains. Moreover, it can be a tool for the assessment of biosecurity measures. Sequencing of ORF5 gives limited information, representing only 5% of PRRSV-1 genome. Development of standardized NGS protocols will provide more robust and informative data.

References

1. Balka G, Podgórska K, Brar MS, Bálint Á, Cadar D, Celer V, Dénes L, Dirbakova Z, Jedryczko A, Márton L, Novosel D, Petrović T, Sirakov I, Szalay D, Toplak I, Leung FC, Stadejek T. Genetic diversity of PRRSV 1 in Central Eastern Europe in 1994-2014: origin and evolution of the virus in the region. Sci Rep. 2018 May 17;8(1):7811. doi: 10.1038/ s41598-018-26036-w. PMID: 29773820; PMCID: PMC5958080.

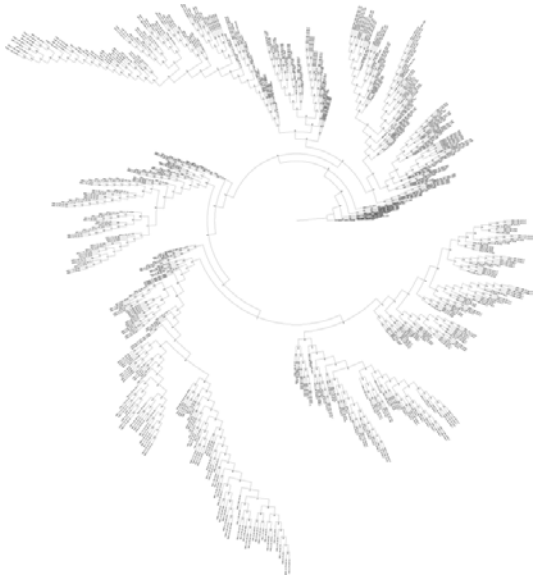


Figure 1.

P081

Animal health

LAMP: NEW DNA/RNA AMPLIFICATION TECHNOLOGY AS A POINT OF CARE TOOL TO HELP DIAGNOSTIC OF TICK DISEASES IN DOGS

A. Zocevic[1], L. Melo[1], T. Thibault[1], L. Valot[1], M. Simonnet[1], W. Yakoub[1], D. Schieb[1], M. Gene[1], E. Schaeffer[1], C. Bettin[1], L. Thiery[1]

[1]*Enalees, France*

Introduction

Currently, the direct detection of pathogens contaminating animals can be performed by 1) PCR, giving highly sensitive results but this requires e to send the samples to a specialized laboratory which can give the results only few days later.
2) Antigenic tests which can give a result quickly but with a lower sensibility than PCR.
LAMP technology (Loop mediated isothermal AMPlification) (Notomi) is a NAAT technology (Nucleic Acid Amplification test) which can occur at constant temperature, unlike PCR which need thermal cycles. This allow the use of this technology as a POC (point of care) test and allow the obtention of the result in only 30 min without the necessity to use a thermal cycler. This technology can be really useful to detect highly contagious pathogens causing diseases in animals and the possibility to administrate the appropriate medicine or isolate the animal immediately.

Materials and Methods
Specific LAMP primers for *Babesia canis, Babesia gibsoni, Anaplasma phagocytophilum/Anaplasma platys/ehrlichia canis* and *Borrelia* spp were designed and LAMP reactions were performed on genome and on blood samples extracted by our own specific quick extraction (Valot). Performances of these tests (AT-BBAB, Enalees) were evaluated and compared to PCR performed in an external clinical laboratory.

Results
LAMP tests have a limit of detection for amplification between 8-65 copies/reaction dependant of the test. The limit of detection of the complete method (quick extraction and LAMP assay) is similar or better of the limit of detection of the complete method (extraction on magnetic beads and PCR assay) obtained in a conventional analysis laboratory. LAMP tests which were designed are really specific for the target (>94% specificity) and really sensitive (>90% sensitivity). This technology is really fast (< 40 min) and can be used as a point of care test.

Discussion and Conclusion
LAMP tests are a new generation of DNA/RNA amplification test, really specific and sensitive, which can be performed near a sick animal and can give a result in only 35 min. These parameters allow the consideration to use this technology to analyse quickly potential pathogens holder and treat immediately.

References
Notomi (2000)
Valot WO2021160849

P082

Animal welfare

HEMATOLOGICAL AND BIOCHEMICAL PROFILES: AN AID IN WELFARE ASSESSMENT

G. Tedde[2], M. Roccaro[1], G. Pilo[2], A. Peli[1], G. Carta[2], L. Secchi[2], A. Simula[2], G. Argiolas[3], M.C. Deiana[3], P. Nicolussi[2]

[1]*Department for Life Quality Studies, Alma Mater Studiorum - University of Bologna, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*
[3]*Veterinary freelance – Sementusa Counsulting, Italy*

Introduction
This study was conducted to evaluate possible associations between the laboratory parameters used as indicators of health status (metabolic profile, electrophoresis and blood count) and animal welfare (AW) estimated through the use of the ClassyFarm self-control checklist in dairy sheep.

Materials and Methods
The present study was approved by Ethic Committee of Istituto Zooprofilattico della Sardegna "G. Pegreffi" on december 12, 2022. Welfare of dairy sheep was evaluated in 12 Sardinian farms by applying the ClassyFarm self-control checklist validated by the Italian National Centre of Reference for Animal Welfare. Complete blood count, hepatorenal profile and serum protein electrophoresis were performed in blood samples from the same sheep on which animal-based measures were assessed on each farm (about 20 per farm, n=237). Statistical analysis was carried out using STATA BE software (v17.0); the examined variables were described using summary and dispersion measures. The normal distribution of the variables was verified using the Shapiro-Wilk test. The association between the ClassyFarm AW score and the results of the blood analyses was explored by calculating Spearman's correlation coefficient. A significance value of $p<0.05$ was chosen.

Results
All the examined farms showed good animal welfare conditions (AW score range 74-95). All the analyzed laboratory profiles were within the reference values for the species. Weak correlation was found between the ClassyFarm AW score and most laboratory parameters; there was a significant moderate negative correlation between AW score and total bilirubin, phosphorus, MCHC (r= -0.31- -0.45).

Discussion and Conclusion
All the sampled animals were in good health and kept in farms with more than sufficient welfare levels. It appears to be no significant correlation between AW score and laboratory profiles, at least in healthy animals. Further studies aimed at exploring this correlation in animals with low welfare levels or poor health might allow to better understand the relationship between AW score and animal health status.

References
Loi F., Pilo G., Franzoni G., Re R., Fusi F., Bertocchi L., Santucci U., Lorenzi V., Rolesu S., Nicolussi P. "Welfare Assessment: Correspondence Analysis of Welfare Score and Hematological and Biochemical Profiles of Dairy Cows in Sardinia, Italy", Animals 2021, 11, 854

P083

Antimicrobial resistance / susceptibility testing

ROLE OF THE *C. COLI*'S PLASMIDS IN THE SPREAD OF THE ANTIMICROBIALRESISTANCE (AMR)

P. Di Matteo[1], D. Smedile[1], M. Iurescia[1], A. Ianzano[1], E. Dell'Aira[1], G. Cordaro[1], E.L. Diaconu[1], A. Giacomi[1], R. Onorati [1], A. Caprioli[1], V. Carfora[1], A. Franco[1], A. Battisti[1], P. Alba[1]

[1]*Laboratorio di Riferimento Nazionale (NRL-AR) e Centro di Referenza Nazionale per l'Antibioticoresistenza (CRN-AR). Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy*

Introduction

*Campylobacter*iosis is the most frequently reported food-borne zoonosis in Europe, caused mainly by *Campylobacter* jejuni (80% of cases) and *C. coli* (10%) (1). The prevalence of *C. jejuni* and *C. coli* in food-producing animals in Italy, particularly in broilers (main reservoir), is around 20% (2). *C. coli* and *C. jejuni* share the same habitat and their genomes are highly prone to acquire new genetic traits (3). *C. coli* may act as a reservoir of AMR genes for *C. jejuni*. In Italy (2022), the prevalences of phenotypic *Campylobacter* spp. resistance in broilers were 4.1% for gentamicin, 79.1% for ciprofloxacin, 11.0% for erythromycin and 69.8% for tetracycline (2). The objective of this work was to study the role of *C. coli* plasmids in the maintenance and spread of acquired AMR in *Campylobacter* spp.

SAMPLE ID	HOST SPECIE	CHROMOSOME	PLASMID	
			1	2
22024283	broiler	aadE-Cc, blaOXA-61_G-57T, blaOXA-489, gyrA_T86I, rplV_A103V	No resistance genes found	No resistance genes found
22029851	broiler	23S_A2074C*, blaOXA-61_G-57T, blaOXA-193, gyrA_T86I, tet(O)	NA	NA
22035429	broiler	23S_A2075G*, blaOXA-61_G-57T, blaOXA-193, gyrA_T86I, rplV_A103V, tet(O)	No resistance genes found	NA
22051200	broiler	aad9, aadE, aph(2'')-IIIa, aph(3')-IIIa, blaOXA-61_G-57T, blaOXA-193, erm(53), gyrA_T86I, tet(O)*, sat4, spw	No resistance genes found	NA
22054049	broiler	aadE-Cc, blaOXA-193, gyrA_T86I, rplV_A103V	aph(3')-IIIa, aph(2'')-Ii, tet(O)	NA
22067114B	broiler	23S_A2075G*, aph(2'')-If, aph(3')-IIIa, catA13, gyrA_T86I, lnu(C), tet(O), tet(O/M/O)	No resistance genes found	No resistance genes found
22089702	broiler	23S_A2075G, aadE-Cc, lnu(C)*	lnu(C)	tet(O/M/O), aph(2'')-Ii, aph(3')-
22102433	broiler	aadE-Cc, aadE, aph(3')-IIIa, aph(2'')-Ii, blaOXA-489, gyrA_T86I, tet(O), tet(O/M/O), rplV_A103V	No resistance genes found	NA

Table 1. summary of the AMR genetic determinants.

Materials and Methods

Eight selected *C. coli* strains isolated during the 2022 EU harmonised antimicrobial monitoring from broilers' caecal content were sequenced using both short and long reads technologies. After cleaning the reads, the hybrid assembly was obtained using Unicycler v0.4.8. Assembly was annotated using Bakta v1.9.1 and the resistance genes with AMRfinder v3.12.8.

Results

Chromosomal point mutations in housekeeping genes as gyrA and r23S, related to (fluoro)quinolone and macrolide resistance, respectively, and acquired AMR genes were detected. In 5/8 isolates, acquired AMR genes as tet(O) (tetracycline resistance), aadA, aph(3')-IIIa, aph(2'')-II (aminoglycoside resistance) or erm(53) (macrolide resistance) were inserted in the chromosome, close to a transposon or an insertion sequence (e.g. tnpV was linked to tetO in some isolates). Only 2/8 isolates harboured plasmid-borne AMR genes.

Discussion and Conclusion

Preliminary data confirmed the plasticity of the *Campylobacter* spp. genome (4). Resistance determinants towards macrolides, aminoglycosides or tetracyclines were present in almost all the isolates, and can be plasmid- or chromosome-borne. Although these results still need to be strengthened by increasing the number of isolates sequenced, taken together they represent a novelty in the study of the spread of AMR in major zoonotic *Campylobacter* spp.

References

1. ECDC 2024 *Campylobacter*iosis-Annual Epidemiological Report for 2020
2. EFSA;ECDC. doi:10.2903/j.efsa.2024.8583
3. Sheppard et al. 2015 doi:10.1101/cshperspect.a018119

P084

Antimicrobial resistance / susceptibility testing

EMERGING RESISTANCE TO FLORFENICOL IN *ACTINOBACILLUS PLEUROPNEUMONIAE* ISOLATES FROM ITALIAN PIG HERDS

E. Albini[4], F.R. Massacci[4], L. Cucco[4], G. Binucci[4], M.E. Nigro[4], M. Ustulin[3], P. Papa[4], M. Paniccia'[4], D. Vio[3], M. Cinthi[1], S.N. Coccitto[5], E. Giovanetti[1], A. Brenciani[5], C.F. Magistrali[2]

[1]*Department Of Life And Environmental Sciences, Polytechnic University of Marche, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'emilia Romagna, Italy*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*
[4]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche 'Togo Rosati', Italy*
[5]*Unit of Microbiology, Department of Biomedical Sciences And Public Health, Polytechnic University of Marche Medical School, Italy*

Introduction

Actinobacillus pleuropneumoniae is the etiological agent of porcine pleuropneumonia, a highly contagious lung infection [1]. The control of this respiratory disease is still based on antibiotics, such as phenicols [2]. We describe three isolates of *A. pleuropneumoniae* resistant to florfenicol due to the presence of the *floR* gene, originated from two pig farms in Italy.

Materials and Methods

Three isolates of *A. pleuropneumoniae* (B2278, B2176 and B2177) were collected from lungs of pigs affected by contagious pleuropneumonia. Antibiotic susceptibility was assessed using a MIC commercial test and the MIC values were interpreted using the breakpoints recommended by CLSI [3]. Genomic DNAs were extracted and the whole genome sequenced using the Illumina MiSeq platform. Bioinformatic analysis were also carried out.

Results

The isolates were susceptible to all antibiotics tested, except for the florfenicol, for which B2176 had an intermediate susceptibility profile, while B2177 and B2278 were resistant. The three isolates belonged to the serovar 6 and were found positive for the presence of the *floR* gene. Phylogenetic analysis displayed that the isolates were closely related (SNPs min 8 - max 19). The *floR* gene was locat- ed on a new 5,588 bp plasmid, here named pAp-*floR*, which had a high nucleotide identity (99%) and coverage (60%) with the pMVSCS1 plasmid of *Mannheimia varigena* of porcine origin (Figure 1).

Discussion and Conclusion

Here, we first describe the emergence of florfenicol-resistant *A. pleuropneumoniae* isolates carrying *floR* gene in Italy, in which the *floR* gene was carried by a plasmid never reported in the literature, thus confirming the wide genetic variability of plasmids. The bacterial pathogen probably spread from the sow herd to the finishing farm, where persisted for at least two years under the selective pressure generated by the use of florfenicol. Our findings raise concerns about a possible spread of florfenicol-resistant *A. pleuropneumoniae* clones in the pig population in Italy.

References

1. Zimmerman J. et al. 2019. Disease of Swine
2. Guo X. et al. 2024. Environ. Res
3. CLSI, 2024

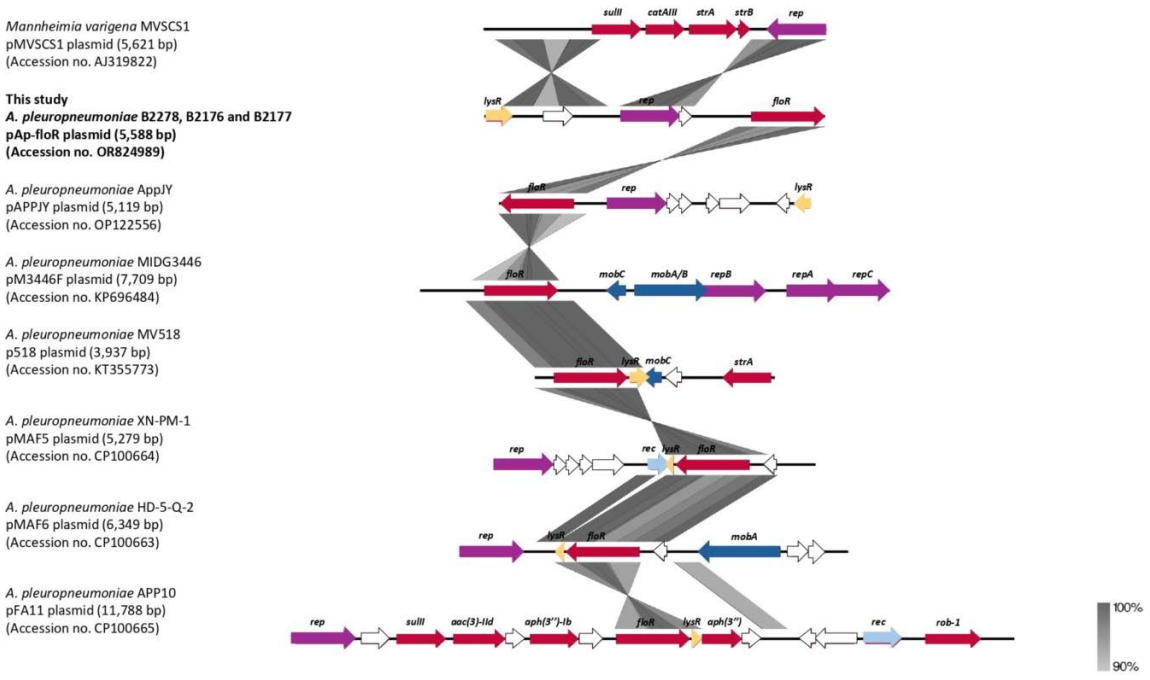


Figure 1. Linear map of the pAp-*floR* plasmid of *A. pleuropneumoniae* strains B2176 and B2177 compared, using Ea- syfig (https:// mjsull. github. io/ Easyfig/), both with the pMVSCS1 plasmid of *M. varigena* and with three plasmids *floR* of A. pleuropneumoniae: (i) pMAF5 of *A. pleuropneumoniae* XN-PM-1; (ii) pMAF6 from *A. pleuropneumoniae* HD-5-Q-2; (iii) pFA11 of *A. pleuropneumoniae* APP10. The positions and transcriptional direction of the ORFs are rep-resented by colored arrows. All antibiotic resistance determinants and relevant plasmid genes are also shown.

P085

Antimicrobial resistance / susceptibility testing

ANTIMICROBIAL RESISTANCE TRENDS IN EQUINE POPULATION OF CENTRAL ITALY FOR *STREPTOCOCCUS EQUI* SUBSP. ZOOEPIDEMICUS

A. Alessiani[1], M. Rulli[1], C. Pompilii[1], F. Cito[1], A. Petrini[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Italy*

Introduction

Streptococcus equi subsp. zooepidemicus (SEZ) is a major concern in equine veterinary medicine. Usually a commensal in horses, SEZ can cause severe diseases like respiratory infections, septicemia, and reproductive tract infections under certain conditions. Recently, evidence indicates SEZ may also cause severe diseases in other animals and humans (1,2). Studies show SEZ has developed resistance to several common antibiotics used in horses (1). This study examines SEZ antimicrobial resistance from equidae nasal swabs in Abruzzo and Molise regions in 2023.

Materials and Methods

A total of 63 SEZ strains were isolated from equidae nasal swabs, grown in a CO2-enriched atmosphere on Edwards medium. Antimicrobial resistance was evaluated using MIC with GPALL1F plates and the Sensititre SWIN Software System (ThermoFisher Scientific, Italy). Results were assessed using EUCAST references version 13.1, except for Penicillin, which was evaluated according to CLSI supplement M100 ed. 31.

Results

Sensitive strains to all antimicrobial were 24 (38%), 11 strains (17%) were 6 MDR, 4 (6%) were 3 and 4 MDR, one strain (2%) were MDR 5, as shown in Figure 1. Sixteen (25%) strains were resistant to Penicillin, as shown in Figure 2.

Discussion and Conclusion

SEZ tends to be more resistant than other pyogenic streptococci, as demonstrated by EFSA (1). In this study, resistance to sulfonamide-trimethoprim exceeds the EFSA intermediate value, whereas other antimicrobial classes exhibit greater susceptibility. Comparing data is challenging due to different methods (MIC vs. Kirby Bauer). Notably, Penicillin resistance was only 25%, much lower than EFSA's find-

ings (1). The high incidence of multi-drug resistant strains, with resistance to up to six classes, is concerning, especially given the zoonotic risk. Future genomic evaluations are recommended to investigate the correlation between pathogenicity and resistance.

References

1. EFSA AHAW Panel, Nielsen SS et al. 2021. Scientific Opinion on the assessment of animal diseases caused by bacteria resistant to antimicrobials: Horses. EFSA Journal 2021;19(12):7112, 43 pp. https://doi.org/10.2903/j.efsa.2021
2. Bosica S et al. "Severe *Streptococcus equi* Subspecies zooepidemicus Outbreak from Unpasteurized Dairy Product Consumption, Italy". Emerg Infect Dis. 2023;29(5):1020-1024. https://doi.org/10.3201/eid2905.221338

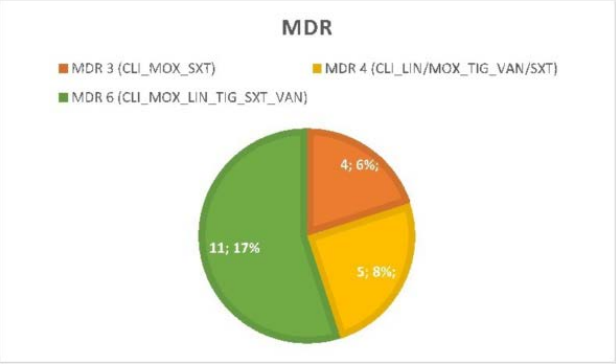


Figure 1. Number and % of MDR strains. Sensitive strains to all antimicrobial were 24 (38%), 11 strains (17%) were 6 MDR, 4 (6%) were 3 and 4 MDR, one strain (2%) were MDR 5.

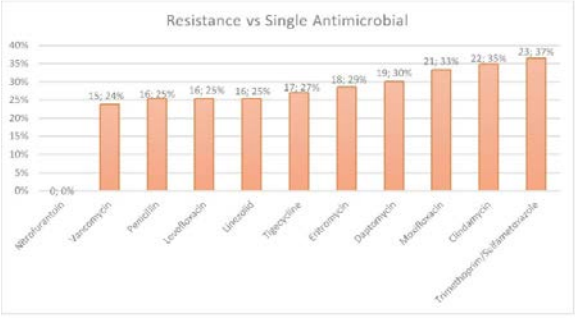


Figure 2. Number and % of resistance vs single antimicrobial. Notably 16 (25%) strains were resistant to Penicillin.

P086

Antimicrobial resistance / susceptibility testing

ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *P. MULTOCIDA* IN DIFFERENT APPROACHES

L. Barta[1], T. Sattler[2], F. Schmoll[1]

[1]*Institute for Veterinary Investigations Mödling, Austrian Agency for Health and Food Safety, Mödling, Austria*
[2]*University of Leipzig, Clinic for Ruminants and Swine, Leipzig, Germany*

Introduction

Pasteurella multocida is one of the most important pathogens when it comes to respiratory infections in farm animals (Wilson & Ho, 2013). To ensure a proper treatment the correct choice of antibiotics is crucial (Richter et al., 2020). For this purpose, we examined *P. multocida* from cattle and pigs for their antimicrobial susceptibility. Due to the heterogenic growth of *P. multocida* we decided to test every strain in 3 different methods to get insights how the incubation method influences the phenotypical resistance.

Materials and Methods

95 isolates and 70 bovine strains were tested for their minimal inhibitory using a commercial 96 well microdilution plate. For the statistical analysis Fisher`s exact test and the the Chi-square test was used with a significance value of $p<0,05$.

Results
For swine the broad majority of CLSI listed antibiotics was considered as effective. The most strains were resistant against Tetracycline (15%) followed by Tilmicosin (6%) and Ampicillin (3%). 59% of the tested bovine strains were resistant to Ampicillin, 21% showed a phenotypically resistance against Tetracycline. For the remaining antibiotics low resistance rates were detected Tulathromycin 7%, Gamithromycin 6%, Tildipirosin 4%, Enrofloxacin 4%, Florfenicol 3% and Penicillin with 1%. The supplemented methods resulted in a significant better growth rate. Especially increased CO^2 atmosphere seems to influence macrolide resistance.

Discussion and Conclusion
Overall, the resistance rate for Austrian *P. multocida* isolates is rather low. Especially in swine the were no alerting results obtained from this study. Cattle showed high resistance rates for Ampicillin and more multiresistant isolates. Derivation in incubation methods led to significant discrepancies in their resistance patterns.

References
Pasteurella multocida: From Zoonosis to cellular microbiology Wilson B, Ho M,Clinical Microbiology Reviews (2013) 26(3)
Reasons for antimicrobial treatment failures and predictive value of in-vitro susceptibility testing in veterinary practice: An overview Richter A, Feßler A, […] Schwarz S Veterinary Microbiology (2020) 245 108694

P087

Antimicrobial resistance / susceptibility testing

OCCURRENCE OF ANTIBIOTIC RESISTANCE GENES IN WILD BOARS

R. Paradiso, A. Rinaldi, M.C. Cuomo, R. Scognamiglio, N. D'Alessio, M. Paone, G. De Luca, F. Del Piano, O. Valvini, G. Borriello

Animal Health Department, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy

Introduction
Antimicrobial resistance (AMR) is an emerging public health problem worldwide. Currently, knowledge of AMR bacteria circulating in wildlife is still limited. In Europe the populations of wild boars (*Sus scrofa*) have increased enormously. Wild boars are ubiquitous, can live close to urban centres and livestock farms and are omnivorous. Their meat is also used for human consumption. It is therefore an important model species for the study of occurrence and persistence of AMR genes in the human-livestock-wildlife interface. The aim of this study was to assess the presence of AMR genes in wild boars by AmpliSeq analysis of faeces and WGS characterization of enterobacteria from their gut.

Materials and Methods
We analyzed 38 faeces samples from as many wild boar carcasses collected in the Campania Region. Samples were processed by cultural method for the isolation of *E. coli* and *Salmonella* strains. Positive samples were also processed for the characterization of the presence of AMR genes by AmpliSeq technology. All the isolated strains were characterized for antibiotic resistance and by WGS analysis.

Results
We isolated 19 *E. coli* strains exhibiting different antibiotic susceptibilities. WGS and AmpliSeq analysis indicated a wide presence of AMR genes, confirmed in the harbouring strains by disk diffusion assay and MIC determination, among isolated strains and wild boar faeces samples (Table 1).

Discussion and Conclusion
These data highlight the presence of AMR genes and bacterial strains among wild boars. The wide presence of AMR genes in a wildlife species, likely not submitted to direct antibiotic treatments, suggests their wide dissemination in the environment, and their ability to be accumulated in the gut of wildlife animals. These results indicate that AMR is a widespread phenomenon in wild boar and can therefore contribute to better understand the role of this animal species in the dissemination and transmission of AMR genes.

References
Arnold, K.E., et al., 2016. Disperse abroad in the land": The role of wildlife in the dissemination of antimicrobial resistance. Biol. Lett. 12(8):20160137
Dhama et al., 2013. Adv. Anim. Vet. Sci 1(1):5- 13
Fredriksson-Ahomaa, M., 2019. FoodbornePathog. Dis. 16, 153–165.

E. coli strains	matrix	MLS	MLD	QU	VN	LC	AZ	CH	DP	T/S	AMX
100459	Strain						x				x
	Faeces			x			x	x			x
100462	Strain						x				
	Faeces		x	x		x	x			x	x
102857	Strain					x	x				
	Faeces	x	x	x	x	x	x	x	x	x	x
102859	Strain						x				
	Faeces	x	x	x	x	x	x		x	x	x
106569	Strain					x	x				
	Faeces	x	x			x	x			x	x
106584	Strain						x				
	Faeces	x					x				x
106587	Strain					x	x				
	Faeces		x			x	x			x	x
109542	Strain						x				x
	Faeces		x	x			x				x
109551	Strain						x				
	Faeces		x	x			x				x
109556	Strain						x				
	Faeces										
111777	Strain					x	x				
	Faeces		x	x	x	x	x	x		x	x
111781	Strain						x				
	Faeces	x	x	x	x		x	x		x	x
111783	Strain						x				
	Faeces						x				
111789	Strain					x	x				
	Faeces		x			x	x			x	x
111791	Strain						x				
	Faeces	x	x	x	x	x	x			x	x
111793	Strain					x	x				
	Faeces					x	x				
121905	Strain						x				
	Faeces	x				x	x			x	x
121909	Strain					x	x				
	Faeces	x	x	x		x	x			x	x
121915	Strain					x	x				
	Faeces			x	x	x	x	x			x
Freq (%)	Strain					42%	100%	26%	10%	58%	10%
	Faeces	42%	63%	58%	32%	58%	100%				84%

MLS=macrolides, lincosamide, streptogramin; MLD=multidrug; QU=quinolones; VN=vancomicin; LC= lincosamide; CH= chloramphenicol; DP=diaminopirimidines; T/S= thrimethoprim/sulphametoxazole; AMX=amoxicillin

Table 1. Antibiotic resistances in the analyzed samples

P088

Antimicrobial resistance / susceptibility testing

OCCURRENCE OF EXTENDED-SPECTRUM β-LACTAMASES AND CARBAPENEMASE-PRODUCING ENTEROBACTERALES FROM SURFACE WATER IN THE APULIA REGION

G. Nobili[3], A. Cocomazzi[3], M.G. Basanisi[3], G. La Bella[3], R. Coppola[3], A.M. Damato[3], A.M. D'Antuono[5], T. Scirocco[2], M. Muscarella[3], S. Lomagro[3], S. Summa[3], I. Franconieri[1], A. Stallone[1], M. Notarangelo[1], R. Petruzzelli[1], C. Pedarra[3], L. Dattoli[3], L. Serrecchia[3], V. Vetritto[3], F. Arena[7], M. Iurescia[4], V. Bortolaia[6], G. La Salandra[3]

[1]*ARPA Puglia, Foggia, Italy*
[2]*Istituto per le Risorse Biologiche e le Biotecnologie Marine del Consiglio Nazionale delle Ricerche (IRBIM CNR), Lesina (FG)*
[3]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy*
[4]*Laboratorio di Riferimento Nazionale (NRL-AR) e Centro di Referenza Nazionale per l'Antibioticoresistenza (CRN-AR). Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy*
[5]*PhD National Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy*
[6]*Statens Serum Institut, Copenhagen, Denmark*
7*Università degli Studi di Foggia, Foggia, Italy*

Introduction

Antibiotic-resistant bacteria (ARB) and antibiotic-resistance genes (ARGs) circulate constantly in various environmental niches, linked to human activities (1-2). Extended-spectrum β-lactamases (ESBLs) and carbapenemases represent the most important mechanism of resistance to β-lactams in Enterobacterales (1) worldwide, including Italy (1-3). The aim of this study was to evaluate the presence of ESBL- and carbapenemase-producing Enterobacterales (CPE) in surface water samples of the Apulia Region.

Materials and Methods

From May 2023 to April 2024, 64 surface water samples were collected from 6 rivers and 2 lakes of the Apulia region (three sampling points for one river and for each lake) (Figure 1). For the isolation of CPE and ESBL, 100-ml water samples were filtered and incubated at 42°C for 24 h in Mossel broth and Buffered Peptone water, respectively. The enrichments were plated on CHROMagar mSuperCarba and CHROMagar ESBL plates and incubated at 42°C for 24 h. Subsequently, the isolated strains with a typical aspect were identified using MALDI-TOF MS and genotypically characterized by Real- Time PCR by Allplex EnteroDR Assay kit.

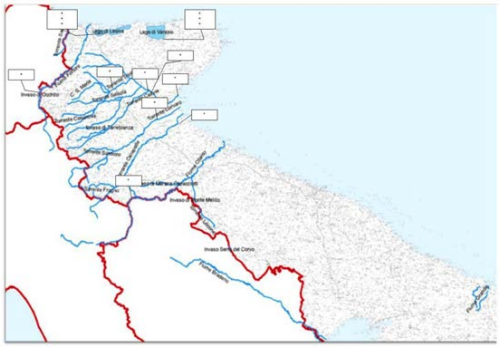


Figure 1. Map of sampling.

Organism	No. of isolates	Resistance phenotypes						
		CTX-M	OXA-48	KPC	VIM	CTX-M, OXA-48	CTX-M, VIM	No genes detected
K. pneumoniae	32	23			3		2	4
E. coli	45	33	3	1	1	2	2	3

Table 1. Resistance phenotypes of *K. Pneumoniae* and *E. coli* isolated from water samples.

Results
From 48 of 64 water samples, 77 non-duplicate Enterobacterales strains were isolated (Table 1). The blaCTX-M was the most prevalent gene identified. Carbapenemase genes were detected in 14 isolates (5 *K. pneumoniae* and 9 *E. coli*).

Discussion and Conclusion
To the best of our knowledge, this represents the first study about the presence of CTX-M-type ESBLs and VIM-, KPC- and OXA-48-type carbapenemases among Enterobacterales collected from the surface water in Southern Italy.
The detection of carbapenemase-producing bacteria in surface water samples is of concern. These could serve as vectors for the spread of carbapenemase genes in the environment that are also associated with additional mechanisms of resistance to other antibiotic classes which, together, result in multidrug resistant bacteria. Further studies are needed to investigate the clonality and possible virulence characters and to evaluate a possible link with clinical isolates of the Apulia Region. Project was funded by Italian Ministry of Health (IZSPB 05/22 RC).

References
1. Piccirilli et al., 2019. doi: 10.1089/mdr.2019.0016
2. Waśko et al., 2022 doi: 10.3390/ijerph192113829
3. Larsson et al., 2022. doi: 10.1038/s41579-021-00649-x

P089

Antimicrobial resistance / susceptibility testing

ESKAPE PATHOGENS: SURVEY IN A CATERING COMPANY

A. Madio[1], A. Salvaggiulo[1], M.M. Dimuccio[1], G. Giustino[2], A. Cavalli[1], M. Corrente[1]

[1]*Department of Veterinary Medicine, University of Bari Aldo Moro, Italy*
[2]*Ladisa srl, Italy*

Introduction
ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and some species of the genus *Enterobacter* spp.) are ubiquitous bacteria. As multidrug resistant bacteria (MDR) are often involved in nosocomial infections. The aim of this survey is to investigate the presence of ESKAPE bacteria in food samples and instruments/ surfaces used for the preparation of hospital meals in a catering company and to assess their antimicrobial resistance pattern.

Materials and Methods
A monitoring plan has been conducted in a private catering company in Apulia (Italy). In a 6-month period (November 2023- April 2024) a randomized systematic sample was performed. Each 2 weeks, several food samples were taken. Also kitchen instruments and surfaces were sampled. All the swabs immersed in transport medium (Nuova APTACA, Brescia, Italy) were subjected to bacteriological analysis, using conventional cultural media. The species identification was confirmed by means of 16S rRNA gene amplification and sequencing. Bacterial isolates were screened for antimicrobial resistance using Thermo Scientific™ Sensititre™ Plates (Thermofisher) for Gram positive and Gram negative bacteria. Breakpoints were determined as recommended by Clinical Laboratory Standards Institute (CLSI).

Results
From a total of 120 food samples (A) and 120 samples from surfaces and instruments used for food preparation (B) , 20 and 30 strains were isolated, respectively. In particular, *P. aeruginosa* (n=5). *S. aureus* (n=5), *Enterococcus* spp. (n=8) were isolated from B. Resistance to more than 3 antimicrobial classes was found in all the bacteria. Interestingly, all *P. aeruginosa* isolates were found to be resistant to imipenem. Other bacteria, not belonging to the ESKAPE complex, were found.

Discussion and Conclusion
As MDR ESKAPE bacteria have been found in some surfaces and instruments, this survey highlights the need of surveillance and environmental sanitation plans.

This research was supported by EU funding within the MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

References
Denissen J., Reyneke B., Waso_Reyneke M. et al. (2022) Int J Hyg Environ Health 244, 114006

P090

Antimicrobial resistance / susceptibility testing

STANDARDIZED PROTOCOL FOR MICROBIOLOGICAL AMR MONITORING IN WASTEWATER

F. Di Timoteo[2], T. Romualdi[2], R. Di Romualdo[2], K. Zilli[2], E. Felicioni[2], L. Di Marcantonio[2], G. Aprea[1], V. Acciari[1], G. Garofolo[2], A. Janowicz[2]

[1]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy*
[2]*National Reference Laboratory for Campylobacter, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy*

Introduction

The spread of antimicrobial resistance (AMR) through antibiotic resistance genes (ARGs) in various environments is a major public health concern. Recent research highlights the impact of AMR in surface and wastewater (WW) on health, following the 'One Health' approach (1). Effective WW monitoring is crucial to combat AMR, but the lack of standardized protocols hinders accurate risk assessment. To address this, we developed a standardized protocol for monitoring ARGs in WW.

Materials and Methods

Five WW samples were spiked with a known mock community (MC) containing reference antibiotic-resistant bacteria (ARB): *E. coli* ESBL, vancomycin-resistant *E. faecium* and *E. faecalis,* carbapenem-resistant (CR) *Acinetobacter* spp, and CR *K. pneumoniae*, along with respective non-resistant strains. Serial dilutions were filtered through 0.45 µm cellulose filter and plated on selected media under defined conditions (Table 1) to determine ARB ratios. The method was then applied to influent WW samples collected from 8 treatment plants between April and June 2024.

Bacteria target	*Culture media agar*	*Incubation*
E. coli	TBX	24 h +37±1 °C
ESBL-E. coli	TBX-cefotaxime	24 h +37±1 °C
CR-E. coli	TBX-meropenem	24 h +37±1 °C
K. pneumoniae	Mc Conkey	24 h +37±1 °C
CR-K. pneumoniae	Mc Conkey-Meropenem	24 h +37±1 °C
Enterococchi	Slanetz Bartley agar	44±4 h 36 ±2 °C
VRE	Slanetz Bartley- vancomycin	44±4 h 36 ±2 °C
A. baumannii	CHROMagar™ Acinetobacter	24 h +37±1 °C
CR-A. baumannii	CHROMagar™ Acinetobacter-Meropenem	24 h +37±1 °C

Table 1. Bacteria and their respective agar used with incubation conditions.

Results

The recovery rate of ESBL and non-ESBL *E. coli* exceeded 80% in all samples, while CR *E.coli* was above 50% only in samples 2 and 3. Sensitive and CR *Acinetobacter* spp. and *K. pneumoniae* had high recovery rates (71.2-100%). *Enterococci* spp. showed high percentages (75-80.4%), unlike VRE, which had low recovery rates (2.16-25.72%). Evaluation of ARB in uncontaminated samples showed varying distributions among bacterial groups. ESBL-producing *E. coli* exhibited higher resistance (1-11%), while VRE also showed high percentages with occasional peaks. CR *K. pneumonia*e and *Acinetobacter* spp. showed lower but stable percentages. Overall, ARB distribution remained stable over time within the same sampling sites (Figure 2).

Discussion and Conclusion

Our data confirms the validity of the developed protocol for analyzing clinically relevant bacterial contamination in WW samples. The stable ARB percentages over three months suggest consistent selective pressure, potentially indicating sources of WW contamination persisting over time. Therefore, continued application of this protocol and ongoing monitoring are crucial for developing effective strategies to control and reduce the spread of antibiotic resistance.

References

1. Nguyen, et al, 2021. Doi: 10.1016/j.scitotenv.2021.146964

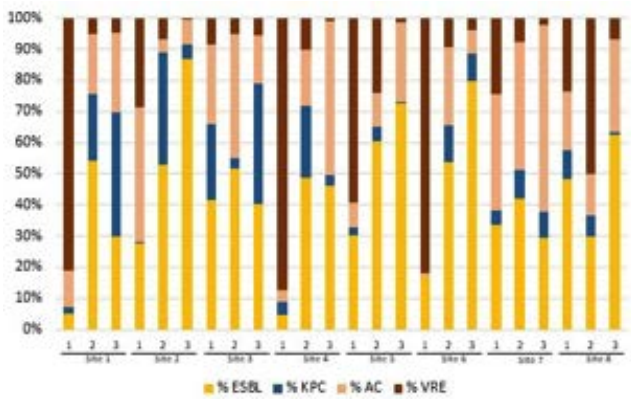


Figure 1. Recovery percentages of the mock community after contaminating 5 wastewater (WW) samples with the Mock Community MC.

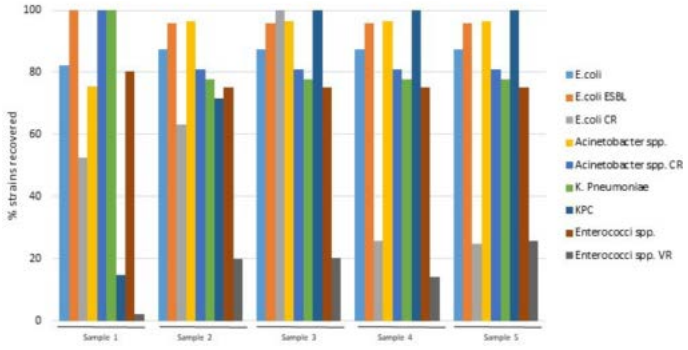


Figure 2. Results of the percentages of antibiotic-resistant bacteria (ARB) for three distinct bacterial groups (indicated by the colors yellow, rose, brown and blue) in wastewater (WW) samples collected from various sampling sites over the course of three months.

P091

Antimicrobial resistance / susceptibility testing

CARBAPENEM AND COLISTIN RESISTANT SHEWANELLA ALGAE ISOLATED FROM LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) IN AN ITALIAN REGION APULIA

L.M. Difato, A. Donatiello, A. Bianco, L. Marino, A. Petrella, S. Castellana, V. Rondinone, L. Del Sambro, V. Manzulli, D. Farina, B. Scalese, D. Galante, A. Parisi, M. Caruso, R. Fraccalvieri

Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy

Introduction
Shewanella algae is an aquaculture and an emerging human pathogen, with a relatively strong association with exposure to seawater during warm seasons (1). There are increasing reports of carbapenem resistance *S. algae* from different hosts. *Caretta caretta* turtles can act as reservoir species for resistant microorganisms in the aquatic environment (2).

Materials and Methods
A cloacal swab, from a turtle found dead on the Mattinata beach in Apulia, an Italian Region, was collected to culture the carbapenem and colistin resistant microorganisms, using CHROMAGAR COL-APSE and mSuperCarba plates. Typical colonies were identified by MALDI-TOF mass spectrometry. Antimicrobial susceptibility of isolate was evaluated by the Minimum Inhibitory Concentration (MIC) method according to the CLSI for Enterobacteriales, using Sensititre™ panels (Trek Diagnostic Systems, USA).

Results
From the cloacal swabs was isolated a colistin and carbapenem resistant *Shewanella algae* (Table 1). Gene *qnrA8*, associated with resistance to quinolones and β-lactamase-encoding blaOXA-SHE, associated to carbapenem-resistant, were identified from *S. algae.*

Discussion and Conclusion

Colistin and carbapenems are often used as the last-resort treatment for many bacterial infections. The aquatic environment is a reservoir for antibiotics-resistant bacteria (3). The antimicrobial resistance of bacteria from wild animals, like turtles, that have never been subjected to antibiotic therapy, represents an interesting finding.

References

1. Torri A., Ambri V. Shewanella algae infection in Italy: report of 3 years' evaluation along the coast of the northern Adriatic Sea. New Microbes New Infect. 2018 Mar 12;23:39-43
2. Trotta, A., Corrente, M. Antimicrobial Resistance in Loggerhead Sea Turtles (*Caretta caretta*): A Comparison between Clinical and Commensal Bacterial Isolates. Animals 2021, 11, 2435.
3. Biyela, P.T., Lin, J., Bezuidenhout, C.C., 2004. The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. Water Sci. Technol. 50, 45–50

Antimicrobial class (Subclass)	Agent	Range µg/ml	MIC value	R/I/S
Penicillins	AMP - Ampicillin	1-32	>32	R
β-lactam combination agents	AUG2 - Amoxicillin / Clavulanic acid	1/0,5-32/16	>32/16	R
	A/S2 - Ampicillin / Sulbactam	4/2-16/8	64/32	R
	P/T4 - Piperacillin/Tazobactam	8/4-128/4	≤8/4	S
	TIM2 - Ticarcillin/Clavulanic acid	8/2-64/2	>64\2	R
Macrolides	AZI - Azithromycin	0,25-32	≤0.25	S
Aminoglycosides	AMI - Amikacin	4-32	≤4	S
	GEN - Gentamicin	0,25-16	1	S
	STR - Streptomycin	2-64	32	R
	TOB - Tobramycin	2-8	≤2	S
Phenicols	CHL - Chloramphenicol	2-32	8	S
Quinolones	CIP - Ciprofloxacin	0,015-4	1	R
	LEVO - Levofloxacin	1-8	≤1	S
	NAL - Nalidixic Acid	0,5-32	2	S
Cephems (Cephalosporins I[c])	FAZ - Cefazolin	1-16	≤1	S
Cephems (Cephalosporins II[c])	FOX - Cefoxitin	0,5-64	4	S
Cephems (Cephalosporins III[c])	AXO - Ceftriaxone	0.25-64	≤0.25	S
	FOT - Cefotaxime	0,25-64	0.5	S
	TAZ - Ceftazidime	0,25-128	0.5	S
Cephalosporin/ combination agent	F/C - Cefotaxime / Clavulanic acid	0,06/4-64/4	0.5/4	S
	T/C - Ceftazidime / Clavulanic acid	0,12/4-128/4	0.5/4	S
Cephems (Cephalosporins IV[c])	FEP - Cefepime	0,06-32	0.12	S
Tetracyclines	MIN - Minocycline	1-8	≤1	S
	TET - Tetracycline	4-32	≤4	S
Tetracycline (Glycylcycline)	TGC - Tigecyline	0,5-8	1	S
Sulfonamides	FIS - Sulfisoxazole	16-256	>256	R
	SXT - Trimethoprim / Sulfamethoxazole	0,12/2,38-	0,5/9,5	S
Monobactam	AZT - Aztreonam	1-16	≤2	S
Penems (Carbapenems)	MERO - Meropenem	0,03-16	1	S
	DOR - Doripenem	0,5-8	1	S
	ETP - Ertapenem	0,25-8	2	R
	IMI - Imipenem	0,12-16	8	R
Lipopeptides (Polymyxins)	COL - Colistin	0.0625 - 64	8	R
			TOTAL R ANTIMICROBIAL AGENTS	10
			TOTAL R ANTIMICROBIAL CLASSES	7

Table 1. Antibiotic susceptibility of *Shewanella algae* from turtle. R=resistant, I=Intermediate resistant; S=sensible

P092

Antimicrobial resistance / susceptibility testing

ANTIMICROBIAL RESISTANCE AND BIOFILM-FORMING ABILITY OF *ESCHERICHIA COLI* AND *KLEBSIELLA* SPP. ISOLATED FROM URINE SAMPLES IN DOGS

A. Facchin[1], J. Filipe[1], F. Tagliasacchi[1], G. Grilli[1], T. Vitiello[1], L. Musa[1], S. Lauzi[1]

[1]*Department of Veterinary Medicine and Animal Sciences, Università degli Studi di Milano, Milan, Italy*

Introduction

The emergence of multidrug-resistant (MDR) bacteria causing urinary tract infections (UTI) in dogs represents both a therapeutic challenge and a public health concern. This study aimed to evaluate the presence of antibiotic resistant and biofilm-forming *E. coli* and *Klebsiella* spp. in dog urine samples.

Materials and Methods
Urine samples were collected from dogs admitted to the Veterinary Teaching Hospital of Lodi, University of Milan, from April to October 2023. Following microbiology, *E. coli* and *Klebsiella* spp. strains were screened for ESBL-/ AmpC-production on MacConkey agar supplemented with 1 mg/L cefotaxime. Positive colonies were phenotypically characterized for ESBL, AmpC, and carbapenemase using commercially available tests, and tested by PCR to detect antibiotic resistance-associated genes (1). Minimum inhibitory concentration was determined by the broth microdilution method, and biofilm formation was evaluated through the microtiter plate assay (2).

Results
UTI was detected in 39 out of 94 (41.9%) samples, with 25 *E. coli* (64.1%) and 4 *Klebsiella* spp. (10.3%) positive dogs. Two ESBL-producing *K. pneumonia*e strains (5.1%) were identified, supported by the presence of *blaCTX-M, blaTEM,* and *blaSHV* genes. MDR was observed in 9 (31%) strains. The frequency of MDR *E. coli* isolates (24%) was lower compared to *Klebsiella* spp. strains (75%). Biofilm production was observed in 24 (82.8%) strains, including weak producers in 20 (69.9%) *E. coli* and both weak and moderate producers in 4 (100%) *Klebsiella* spp.

Discussion and Conclusion
The identification of *E. coli* as the most prevalent bacterium aligns with previous reports on UTI in dogs. The presence of MDR in *E. coli* was higher compared to previous reports in dogs with UTI (3), whereas high level of MDR in *Klebsiella* isolates confirms previous findings (4). MDR and biofilm producer bacteria, along with ESBL *K. pneumonia* are of concern and highlight the need for continuous monitoring on AMR bacteria in dogs.

References
1. Monstein et al. 2007. AMPIS, 115:1400-1408, 2007.
2. Stepanović et al., 2007. APMIS, 115(8), 891–899.
3. Temmerman et al., 2024. Antibiotics, 13(6), 500.
4. Marques et al., 2019. Journal of Antimicrobial Chemotherapy, 74 (3), 594–602.

P093

Antimicrobial resistance / susceptibility testing

SURVEY ON ANTIBIOTIC RESISTANCE OF MASTITIS BACTERIA ISOLATED FROM DAIRY SHEEP IN SARDINIA (ITALY), 2023-2024

I. Fadda[1], D. Brundu[3], L. Pinna[2], F. Cauli[5], M.P. Firinu[5], S. Ghisu[4], B. Zulato[2], S. Dore[1]

[1]*Centro di Referenza Nazionale per le Mastopatie degli Ovini e dei Caprini, Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Sardegna, Cagliari, Italy*
[3]*Istituto Zooprofilattico Sperimentale della Sardegna, Nuoro, Italy*
[4]*Istituto Zooprofilattico Sperimentale della Sardegna, Nuoro, Italy*
[5]*Istituto Zooprofilattico Sperimentale della Sardegna, Oristano, Italy*

Introduction
Mastitis is the most prevalent disease in dairy sheep associated with economic losses due to reduced yield and quality of milk, animal culling and health-care costs. It is mainly determined by bacterial intra-mammary infection, which is the main cause of antibiotic treatment, increasing the risk of selection and spread of antimicrobial resistance.

Materials and Methods
From 1st July 2023 to 31st May 2024, a total of 404 half-udder/individual sheep milk samples were cultured for mastitis diagnosis. After the isolation on blood agar plates, the microbial identification was performed by mass spectrometry using MALDI-TOF MS and tested for the antibiotic sensitivity by Kirby-Bauer's disk diffusion method. Antimicrobial susceptibility was performed testing prototype molecules, representative of antimicrobial classes with a similar chemical structure and mechanism of action.

Results
The main microorganisms isolated were non-aureus staphylococci (NAS) (39%), followed by *Staphylococcus aureus* (24%), streptococci/enterococci (20%), enterobacteriaceae (11%), and non-fermenting gram-negative bacteria (i.e., *Mannheimia haemolityca*, *Pasteurella* sp.) (5%). NAS showed resistance for penicillin (33%), tetracycline (21%) and gentamicin (3%). Among *Staphylococcus* aureus isolates resistance was observed against tetracycline (13%), penicillin (10%) and gentamicin (8%). Streptococci were resistant for tetracycline (37%), penicillin (24%) and enrofloxacin (20%). Resistance against ampicillin (63%), tetracycline (37%) and gentamicin (9%) were found for enterobacteriaceae. More than 50% of non-fermenting gram negative was resistant to macrolides (i.e., tilmicosin).

Discussion and Conclusion
Environmental microorganisms showed the highest prevalence of resistance against penicillins and tetracycline, the antibiotic prescribed

more frequently to treat bacterial disease in sheep. Antimicrobial resistance is one of the most relevant global public health issues and dealing with its development is always challenging; therefore, data on antibiotic sensitivity must be systematically collected to survey the evolution of the resistances and periodical reports should be released to promote the antimicrobial stewardship in veterinary medicine.

References

Cannas EA, Dore S, Lollai S, Liciardi M., Addis MF. Mastitis in small ruminants. IZS Sardegna

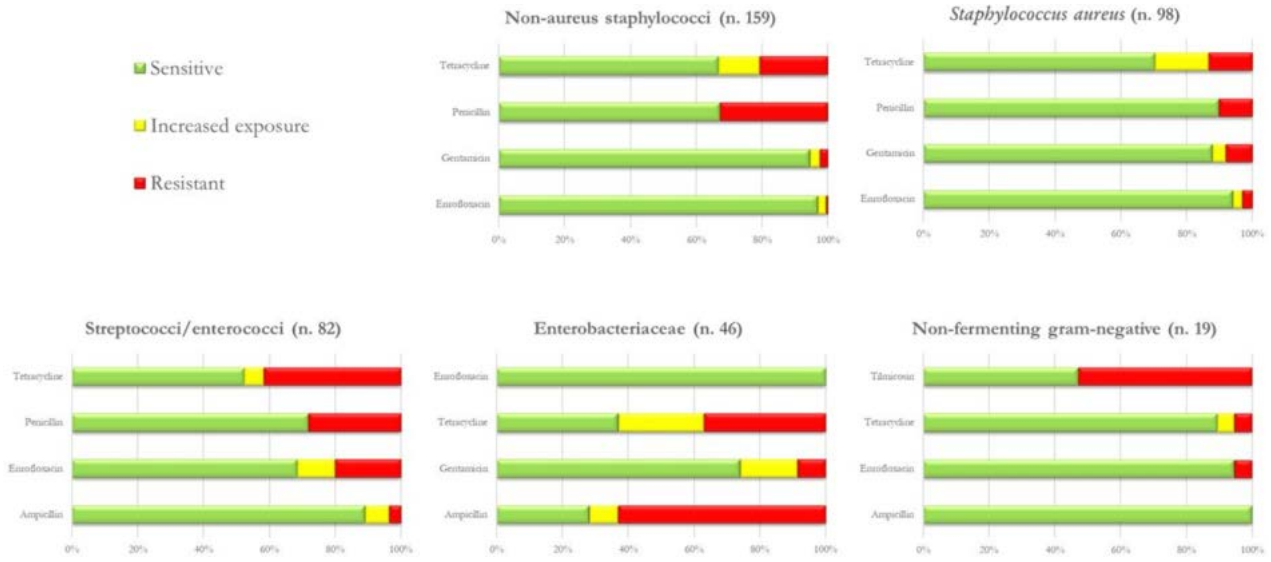


Figure 1. Prevalence of antibiotic resistance in mastitis bacteria isolated from dairy sheep milk (2023-2024).

P094

Antimicrobial resistance / susceptibility testing

LOW PREVALENCE OF COLISTIN RESISTANCE IN *ESCHERICHIA COLI* FROM SWISS LIVESTOCK

C. Fleury[1], J.E. Fernandez[2], V. Perreten[2], O. Gudrun[1]

[1]*Center for Zoonoses, Animal Bacterial Diseases and Antimicrobial Resistance, Institute of Veterinary Bacteriology, University of Bern*
[2]*Division of Molecular Bacterial Epidemiology and Infectious Diseases, Institute of Veterinary Bacteriology, University of Bern*

Introduction

The increase of infections with multi-drug resistant pathogens intensifies the need for alternative treatment options. Therefore, the antibiotic colistin has become one of the last resort options in human medicine. In veterinary medicine, colistin has long been extensively used in livestock. The publication of a novel plasmid-mediated colistin resistance gene (mcr-1) in early 2016 marked a turning point for veterinary medicine, as made it clear that colistin resistance genes can be transferred by mobile elements [1]. In the wake of the renewed use of colistin in human medicine, the European medical agency (EMA) restricted the use of colistin in veterinary medicine.

Materials and Methods

Colistin-resistant *Escherichia (E.) coli* in caecal samples from Swiss broilers, fattening pigs and calves at slaughter were isolated by a two-step method including a selective enrichment and plating. Suspicious colonies were confirmed for colistin resistance by broth microdilution and sequenced by Illumina technology for in silico detection of mcr genes.

Results

Five samples from broilers were positive with colistin-resistant *E. coli* (prevalence of 0.8%), eight from calves (2.7%) and ten from fattening pigs (3.3%). From these 23 colistin-resistant *E. coli*, mcr-genes were detected only in one strain from broilers (mcr-2.1), one strain from calves (mcr-1.26), and in two strains from pigs originating from different farms (mcr-2.1).

Discussion and Conclusion

Our results are in line with findings of the European harmonised monitoring program on antimicrobial resistance. Resistance to colistin

was uncommon among indicator *E. coli* isolates recovered from food-producing animals in 2021 and 2022 [2]. Our results indicate that the long-term use of colistin in Swiss livestock has not led yet to an increased prevalence of colistin-resistant *E. coli*. Therefore, Swiss livestock have so far been negligible as a reservoir for mcr-mediated colistin resistance.

References

1. Liu, Yi-Yun, et al. "Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study." The Lancet infectious diseases 16.2 (2016): 161-168
2. EFSA & ECDC (2024). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2021–2022. EFSA Journal, 22

P095

Antimicrobial resistance / susceptibility testing

ENTEROCOCCUS FAECIUM VRE AND *STAPHYLOCOCCUS AUREUS* MRSA FROM MAGPIE (*PICA PICA*) FROM ITALIAN REGION APULIA

R. Fraccalvieri[2], L.M. Difato[2], A. Donatiello[2], B. Scalese[2], G. Caldarola[2], L. Capozzi[2], S. Castellana[2], V. Rondinone[2], V. Manzulli[2], L. Pace[2], D. Galante[2], M. Tempesta[1], A. Parisi[2], M. Caruso[2]

[1]*Department of Veterinary Medicine, University Aldo Moro of Bari, Valenzano (Ba), Italy*
[2]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy*

Introduction

Magpie, *Pica pica*, is a resident bird common in suburban areas. Birds were considered to be a natural reservoir of *Staphylococcus aureus* (MRSA) (1). Studies have shown that enterococci resistant to antimicrobials occur also in wild mammals and birds that are not directly affected by antibiotic treatment (2).

Materials and Methods

A cloacal swab and a throat swab were examined from a magpie found dead in an urban area, using CHROMAGAR VRE (CHROMagar) and MRSA Select plates to isolate *Enterococci* Vancomycin resistant and *S. aureus* MRSA (3), respectively. Typical colonies were identified and characterized by MALDI-TOF, PCR and PCR Real Time (3; 4). Antimicrobial susceptibility of isolates was evaluated by the Minimum Inhibitory Concentration (MIC) method according to the CLSI, using a Sensititre™ panel GPALL1F®.

Results

Enterococcus faecium VRE was isolated from cloacal swab and *Staphylococcus aureus* MRSA from throat swab. The phenotypic resistances are shown in Table 1. The *mecA* gene and SCC-mec typing IVa were detected in *S. aureus*.

Antimicrobial class (Subclass)	Agent	Range µg/ml	E. faecium		S. aureus	
			MIC µg/ml	R/I/S or N.I	MIC µg/ml	R/I/S or N.I
Penicillins	AMP - Ampicillin	0,12-8	>8	R	>8	R
	OXA - Oxacillin	0,25 -4	>4	N.I.	**>4**	**R**
	PEN - Penicillin	0,06-8	>8	R	>8	R
Macrolides	ERY - Erythromycin	0,25-4	4	I	>4	R
Aminoglycosides	GEN - Gentamicin	2-16	8	N.I.	<2	S
	STR - Streptomycin	1000	>1000	N.I.	<1000	N.I.
Phenicols	CHL - Chloramphenicol	2-16	8	S	8	S
Quinolones (Fluoroquinolones	CIP - Ciprofloxacin	1-2	>2	R	≤1	S
	LEVO - Levofloxacin	0,25-4	4	I	≤0,25	S
Quinolones	MXF - Moxifloxacin	0,25-4	2	N.I.	≤0,25	S
Cephems (Cephalosporins II[c])	**FOX - Cefoxitin**	6	<6	N.I.	**>6**	**R**
Tetracyclines	TET - Tetracycline	2-16	>16	R	>16	R
Tetracycline (Glycylcycline)	TGC - Tigecyline	0,03-0,5	0,25	N.I.	0,25	N.I.
Sulfonamides	SXT - Trimethoprim/Sulfamethoxazole	0,5/9,5-4/76	>4	N.I.	≤0,5/9,5	S
Lincosamides	CLI- Clindamycin	0.5 - 2	>2	N.I.	≤0,5	S
Liptopeptides	DAP - Daptomycin	0,5-4	1	S	≤0,5	S
Oxazolidionones	LZD - Linezolid	1-8	2	S	2	S
Ansamycins	RIF - Rifampin	0,5-4	≤0,5	S	≤0,5	S
Glycopeptides	**VAN - Vancomycin**	0,25-32	**>32**	**R**	1	S
Streptogramins	SYN - Quinupristi/Dalfopristin	0,5-4	2	I	≤0,5	S
Nitroheterocyclics	NIT - Nitrofurantoin	32-64	64	I	≤32	S

Table 1. Antimicrobial susceptibility of isolates *Enterococcus faecium* VRE and *Staphylococcus aureus* MRSA. R=Resistant; I= intermediate resistant; S= Sensible; N.I.= Not Interpretable.

Discussion and Conclusion
The results obtained in the current survey confirm that wild birds can act as reservoirs and spreaders of various pathogens, including antimicrobial-resistant bacteria and so they represent a potential risk for human health.

References
1. Porrero MC, Mentaberre G, Sánchez S, (2013): Methicillin resistant *Staphylococcus* aureus (MRSA) carriage in different free-living wild animal species in Spain. Vet J, 198(1), 127 – 30
2 Cagnoli, G.; Bertelloni, F.; Interrante, P.; Ceccherelli, R.; Marzoni, M.; Ebani, V.V. Antimicrobial-Resistant *Enterococcus* spp. in Wild Avifauna from Central Italy. Antibiotics 2022, 11, 852. https://doi.org/10.3390/antibiotics11070852
3 Caruso M., Latorre L., Santagada G., Fraccalvieri R., Miccolupo A., Sottili R., Palazzo L., Parisi A. - "Methicillin - resistant *Staphylococcus* aureus (MRSA) in sheep and goat bulk tank milk from Southern Italy". Small Ruminant Res. Doi: 10.1016/j.smallrumres.2015.12.023; 2015
4 Cheng S, McCleskey FK, Gress MJ, Petroziello JM, Liu R, Namdari H, Beninga K, Salmen A, DelVecchio VG. 1997. A PCR assay for identification of *Enterococcus* faecium. J Clin Microbiol 35: https://doi.org/10.1128/jcm.35.5.1248-1250.1997

P096

Antimicrobial resistance / susceptibility testing

PREVALENCE OF METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS* IN SLAUGHTERHOUSES AND MEAT SHOPS OF NORTHERN AREA OF PAKISTAN

S. Khan[2], H.S. Siddique[1]

[1]*Veterinary Resarch and Disease Investigation Center Balogram Swat Pakistan*
[2]*Veterinary Research Institute Peshawar Pakistan*

Introduction
Livestock Associated Methicillin Resistant *Staphylococcus aureus* (LA-MRSA) is a zoonotic pathogen of public health importance. The aim of this study was to investigate the prevalence of MRSA in meat shops and slaughterhouses in Malakand Division of Pakistan.

Materials and Methods
Overall 300 sample- 30 each from working area, knives, hooks, meat mincers, worker hands, beef, mutton, chicken meat, and nasal and rectal swabs from animals/chickens were collected. All the samples were cultured for bacterial growth. MRSA isolate were identified among *S. aureus* positive isolates by performing cefoxitin disk diffusion assay. Molecular confirmation of MRSA was further carried out through PCR by targeting mecA gene.

Results
164 isolates of the total samples were confirmed to be *S. aureus* by phenotypic identification through gram staining and biochemical tests. Out of 164 *S. aureus* isolates 99 (60.36%) were resistance to cefoxitin. These isolates were also resistance to methicillin, ciprofloxacin and tetracycline. All the 99 isolates were further confirmed to be MRSA through PCR by targeting *mecA* gene. The prevalence of MRSA was highest in Beef 63.3% (19 /30) followed by meat mincer 53.3% (16/30), chicken meat 50% (15/30), working area 43.3% (13/30), knives 40% (12/30), Nasal swabs and hooks 23.3% (07/30), worker hands 16.6% (5/30), mutton 10% (3/30), and rectal swabs 6.66% (2/30).

Discussion and Conclusion
The study therefore revealed that MRSA is prevalent in meat as well as environment samples from northern area of Pakistan. This demand for implementation of strict hygienic measure in the meat production, processing and supply chain in order to prevent the contamination of environment and ultimate spread of the bacteria to the human population.

P097

Antimicrobial resistance / susceptibility testing

PATHOGEN AND ANTIBIOTIC RESISTANCE SURVEILLANCE IN SWINE FARM SLURRY BY NANOPORE METAGENOMICS

J. Marti-Carreras[3], G. Melgarejo[3], M. Carrasco[3], M. Noguera-Julián[3], X. Roura[2], E. Mateu[1], O. Francino[4]

[1]Departament de Sanitat i d'Anatomia Animals, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain [2]Hospital Clínic Veterinari, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain
[3]Nano[1]Health (N[1]H), Edifici EUREKA, PRUAB, Bellaterra, Barcelona, Spain
[4]Servei Veterinari de Genètica Molecular, Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain

Introduction

Wastewater surveillance is a potent method for monitoring pathogens in aquatic environments, particularly within agricultural settings to trace zoonotic diseases in farm slurry. We aim to apply metagenomic surveillance on an experimental swine farm, using nanopore sequencing to identify pathogens and assess antibiotic resistance (AMR) genes, while evaluating different sampling techniques.

Materials and Methods

The research was conducted at the experimental swine farm at Facultat de Veterinària, Universitat Autònoma de Barcelona. Two pens of 13 piglets were monitored for 2 weeks, sampling their feces (rectal swabs) and the pen's slurry via two methods: passive (Moore swab) and active (sterile immersion bottle) sampling. Samples were run in duplicate. DNA was extracted using a microbiome-specific DNA extraction kit (ZymoBIOMIs DNA Miniprep). DNA samples were pooled, a nanopore sequencing barcoded library was prepared by ligation (SQK-LSK114.24) and sequenced in a R10.4.1 flowcell for 70 hours. Taxonomy classification and AMR prediction were performed using the N1H-Metagenomics v1.1 and N1H-AMR v1.0 pipelines, available at N1H Cloud. Diversity indices were analyzed using the Vegan R package.

Results

Over 932 bacterial taxa were identified. No significant differences were observed in microbial richness or Shannon index among the sampling techniques, indicating comparable microbial diversity across methods (rectal swab, Moore swab, or immersion bottle). However, Jaccard and Bray-Curtis dissimilarity analyses showed that microbial community structures differed significantly between rectal swabs and both Moore swabs or immersion bottle sampling. These results suggest passive sampling is suitable for identifying species of interest, such as pathogens responsible for farm breakouts, but not preferred for quantitative microbiome studies.

Discussion and Conclusion

Taxonomic analysis revealed *Prevotella* spp. as the predominant genus in rectal swabs and *Arcobacter* spp. in Moore swab and immersion bottle samples. The prevalence of AMR genes was consistent across all sampling methods, with tetracycline resistance genes as the most prevalent. These findings advocate integrating genomic surveillance into routine farm management to enhance disease tracking and management.

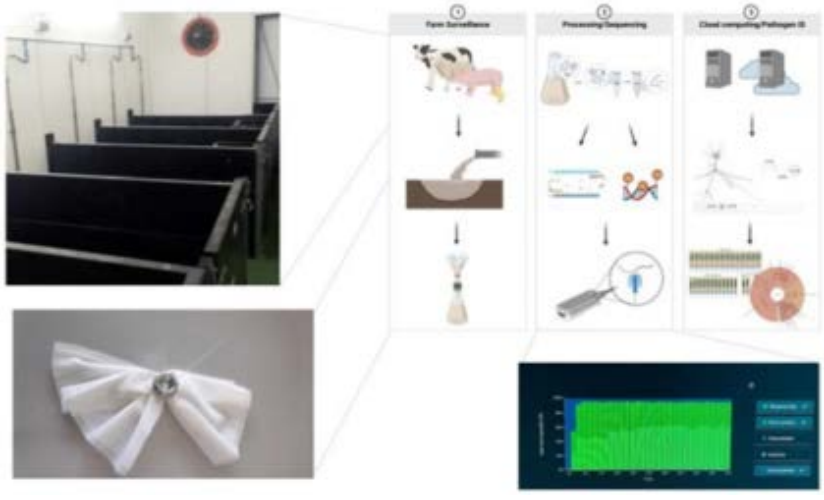


Figure 1. Graphical abstract of the study.

P098

Antimicrobial resistance / susceptibility testing

ENTEROCOCCUS SPECIES AS AN ANTIMICROBIAL RESISTANCE BAROMETER IN WILDLIFE: PRELIMINARY RESULTS

E. Massella[4], S. Russo[4], S. Restori[4], C. Siclari[2], L. Cirasella[2], S. Perulli[2], R. Taddei[1], A. Luppi[3]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Bologna, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Forlì, Italy*
[3]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Parma, Italy*
[4]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Piacenza, Italy*

Introduction

Wildlife is playing an increasing role in antimicrobial resistance (AMR) dynamics. It represents a gauge of AMR pollution and an AMR reservoir, mostly investigated with Gram negative bacteria. The present study investigates AMR indicator *Enterococci* in wild mammals (WMs) and birds (WBs), focusing on Critically Important antimicrobials (CIAs) resistance, especially to vancomycin.

Materials and Methods

In the period between January and June 2024, 116 faeces were collected from 84 WMs and 32 WBs, analysed for the infectious disease surveillance program of the Emilia Romagna region. Microbiological analysis was implemented to isolate *Enterococcus* indicators, identified by MALDI-TOF. AMR profiles were assessed determining Minimal Inhibitory Concentration, interpreted according to CLSI breakpoints. Strains phenotypically resistant to vancomycin were screened for van gene presence with a multiplex PCR (a).

Results

We isolated 106 *Enterococcus* strains belonging to 8 different species. The most common were E. faecalis (48) and *E. faecium* (28). Multidrug resistance (MDR) was observed in 69/106 (65.09%) strains (Table 1). The most common resistance frequencies were to quinupristin/dalfopristin (92/106, 86.79%), tigecycline (51/106, 48.11%), ciprofloxacin (49/106, 46.23%) and erythromycin (47/106, 44.34%), followed by chloramphenicol (31/106, 29.25%), tetracycline and daptomycin (27/106, 25.47% both), gentamycin high dosage (7/106, 6.60%) and ampicillin (2/106, 1.89%). Resistance to teicoplanin was not observed (Figure 1). Vancomycin resistance was observed in 1 *E. gallinarum,* carrying *vanC1* gene. MDR was mostly associated with WMs (54/81, 66.67%) compared to WBs (15/25, 60%). However, WBs showed the highest number of isolates resistant to more than 5 antimicrobials (10/25, 40%; WMs: 20/81, 24.69%).

Discussion and Conclusion

Notably, resistance to antimicrobials with sanitary implications was detected in a high percentage of enterococci isolated from wildlife, which could represent a reservoir of CIAs and a useful tool to evaluate the impact of anthropic pressure.

References

1. Dutka-Malen S. et al., 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J. Clin. Microbiol. 33, 1:24-27.

Species	Isolates		MDR isolates		Isolates with ≥5 R	
	N	%	N	%	N	%
Enterococcus casseliflavus	3	2.83	2	66.67	1	33.33
Enterococcus durans	1	0.94	0	0.00	0	0.00
Enterococcus faecalis	47	44.34	27	57.45	10	21.28
Enterococcus faecium	28	26.42	27	96.43	14	50.00
Enterococcus gallinarum	1	0.94	1	100.00	1	100.00
Enterococcus hirae	17	16.04	7	41.18	3	17.65
Enterococcus mundtii	8	7.55	5	62.50	1	12.50
Enterococcus thailandicus	1	0.94	0	0.00	0	0.00

Table 1. Number and percentage of isolates, MDR isolates and isolates resistant to at least 5 different antimicrobials considering different *Enterococcus* species.

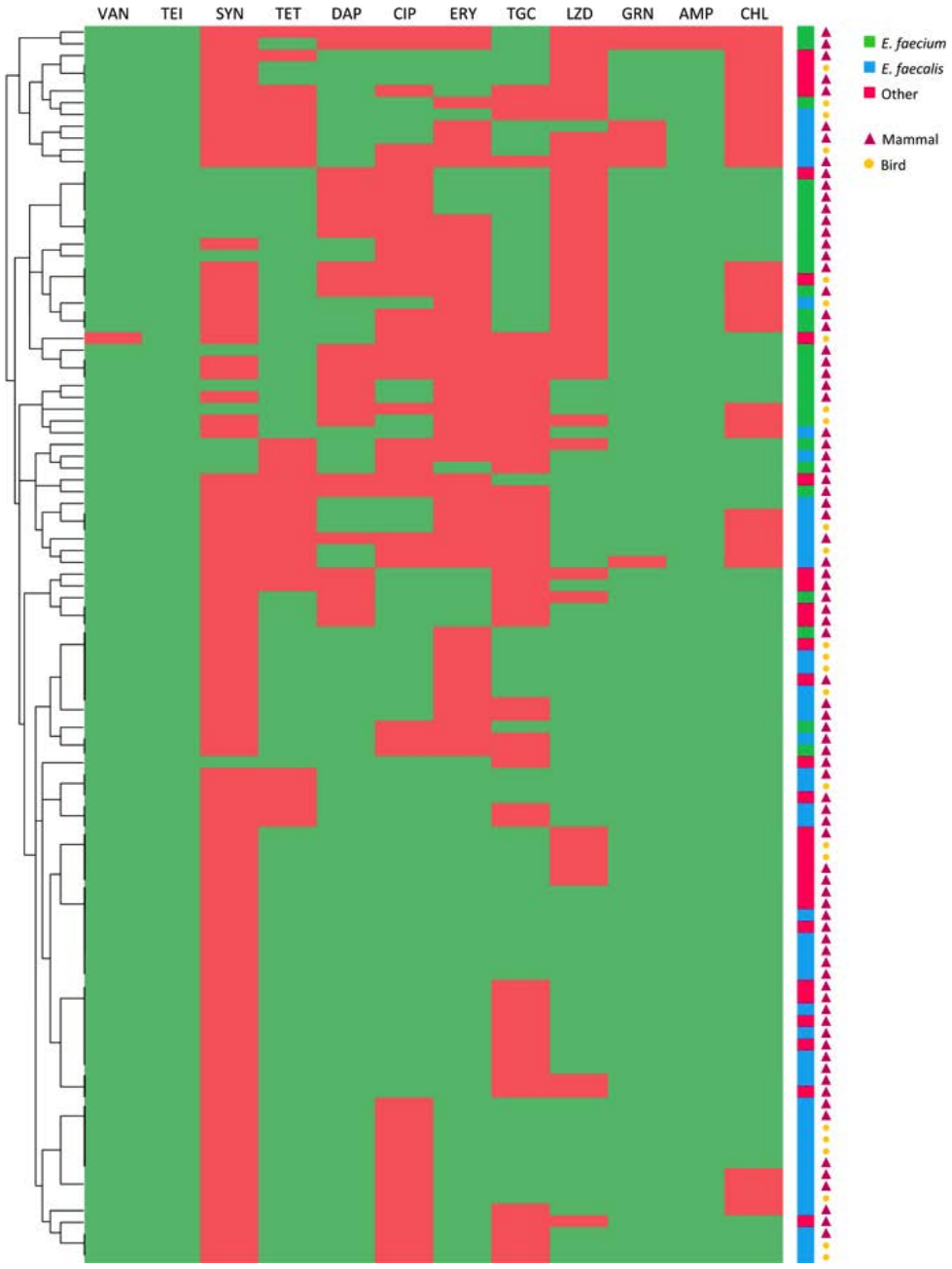


Figure 1. Heat map depicting phenotypic AMR profiles of the strain collection. Resistance to antimicrobials is shown in red, while sensitivity in green. The dendogram on the left represents clustering of *Enterococcus* isolates according to their phenotypic AMR profile. Additional strain information (*Enterococcus* species and source) is provided in the columns on the right of the heatmap. VAN= vancomycin; TEI: teicoplanin; SYN= quinupristin/dalfopristin.

P099

Antimicrobial resistance / susceptibility testing

WILDLIFE AS A RESERVOIR OF MULTIDRUG RESISTANT ESCHERICHIA COLI AND KLEBSIELLA SPP. STRAINS: AN OVER 2-YEAR MONITORING IN THE EMILIA ROMAGNA REGION (ITALY).

E. Massella[3], M. Sampieri[2], S. Bonardi[1], M. Conter[1], G. Galletti[2], L. Bardasi[2]

[1]*Department of Veterinary Science, University of Parma, Italy*
[2]*Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna, Bologna, Italy*
[3]*Experimental Zooprophylactic Institute of Lombardy and Emilia Romagna, Piacenza, Italy*

Introduction

Antimicrobial resistance (AMR) is a health challenge worldwide. Wildlife is considered an indicator of AMR pollution, spreading from human related environments to the natural ones. Here we present an AMR monitoring in wildlife, investigating foxes (F), corvids (C) and aquatic birds (AB) as AMR sentinels and focusing on multidrug resistant (MDR) Enterobacteriaceae.

Figure 1. Animal sources (F= foxes, C= corvids, AB= aquatic birds) and antimicrobial resistance profile of the 98 MDR *Escherichia coli* isolates. Phenotypic resistance to antimicrobials is shown in red, absence in green. AMP= ampicillin, CTX= cefotaxime, CAZ= ceftazidime, MEM= meropenem, NA= nalidixic acid, CIP: ciprofloxacin, TET= tetracycline, COL= colistin, CN= gentamicin, T= trimethoprim, S= sulfa2m50ethoxazole, C= chloramphenicol, AZ= azithromycin, TIG= tigecycline, AK= amikacin.

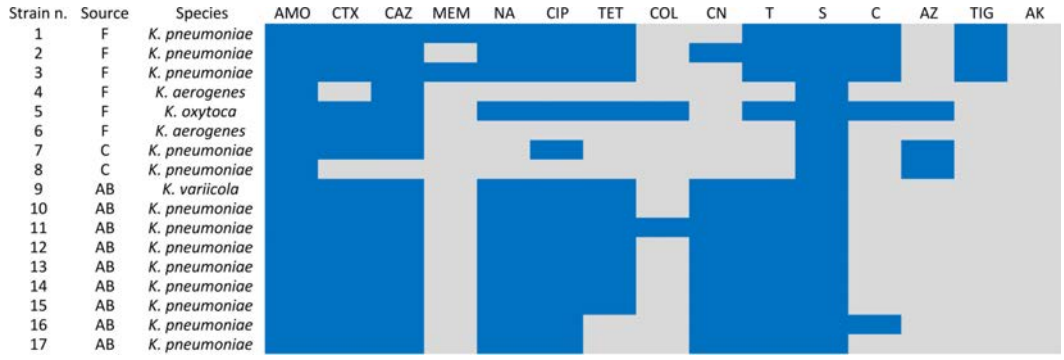


Figure 2. Animal sources (F= foxes, C= corvids, AB= aquatic birds), *Klebsiella* species and antimicrobial resistance profile of the 17 MDR *Klebsiella* spp. isolates. Phenotypic resistance to antimicrobials is shown in blue, absence in grey. AMP= ampicillin, CTX= cefotaxime, CAZ= ceftazidime, MEM= meropenem, NA= nalidixic acid, CIP: ciprofloxacin, TET= tetracycline, COL= colistin, CN= gentamicin, T= trimethoprim, S= sulfamethoxazole, C= chloramphenicol, AZ= azithromycin, TIG= tigecycline, AK= amikacin.

	n. of antimicrobial classes							
	3	4	5	6	7	8	9	10
Foxes	6	14	11	6	4	3	3	4
E. coli	4	14	11	6	4	3	3	-
K. aerogenes	2	-	-	-	-	-	-	-
K. oxytoca	-	-	-	-	-	-	-	1
K. pneumoniae	-	-	-	-	-	-	-	3
Corvids	8	4	7	3	1	1	-	-
E. coli	7	4	6	3	1	1	-	-
K. pneumoniae	1	-	1	-	-	-	-	-
Aquatic birds	1	4	2	11	5	14	3	-
E. coli	1	4	2	11	4	7	2	-
K. pneumoniae	-	-	-	-	1	6	1	-
K. variicola	-	-	-	-	-	1	-	-
Total	15	22	20	20	10	18	6	4

Table 1. Number of MDR isolates according to animal sources, bacterial species and number of antimicrobial classes the isolates were resistant to.

Materials and Methods

Between August 2020 and February 2023, in the context of the project funded by the Italian Ministry of Health (PRC2019002), 495 faecal samples were collected from 184 foxes (F), 210 corvids (C) and 101 aquatic birds (AB) as part of the infectious disease surveillance program of the Emilia Romagna region (Italy). The samples were also tested for MDR Enterobacteriaceae, identified by MALDI-TOF. The AMR profiles were evaluated determining the Minimal Inhibitory Concentration, interpreted according to the EUCAST breakpoints (2020). MDR was assessed according to Magiorakos et al. (2012) (1).

Results

Of main interest was the isolation of 467 *Escherichia coli* and 36 *Klebsiella* spp. strains. 98/467 (21%) *E. coli* and 17/36 (47%) *Klebsiella* spp. isolates were resistant from 3 to 10 antimicrobial classes. Overall, the most common resistance rates in the MDR isolates (115) were to sulfamethoxazole (102; 89%), ampicillin (99; 86%), ciprofloxacin (78; 68%) and tetracycline (75; 65%), followed by cefotaxime (67; 58%), ceftazidime (62; 54%), trimethoprim (64; 56%), nalidixic acid (50; 43%), chloramphenicol (29; 25%%), azithromycin (24; 21%), colistin (23; 20%), gentamicin (20; 17%), tigecycline (15; 13%) and amikacin (2; 2%) (Figure 1, Figure 2). MDR was mostly related to F (n=51; 44%), followed by AB (n=40; 35%) and C (n=24%; 29%) (Table 1). Notably, 17/28 strains (61%), isolated from AB, were found resistant to $\geq$ 8 antimicrobial classes.

Discussion and Conclusion

Our study underlines the important role of wildlife in monitoring the spread of AMR from anthropogenic drivers to the natural environment. Aquatic birds, foxes and corvids were all effective AMR indicators, which can be used for future AMR surveillance programs.

References

1. Magiorakos, A. P. et al., 2012. Clin Microbiol Infect, 18(3), 268-281.

P100

Antimicrobial resistance / susceptibility testing

EXTENDED-SPECTRUM β-LACTAMASE (ESBL) AND METALLO-β-LACTAMASE (MBL) PRODUCING *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED FROM CANINE OTITIS EXTERNA.

F.P. Nocera[1], A. Chiaromonte[1], R. Schena[1], F. Pizzano[1], S. Arslan[1], E. Ipek[1], L. De Martino[1]

[1]*Department of Veterinary Medicine and Animal Production, University of Naples "Federico II", Italy*

Introduction
Pseudomonas aeruginosa has become a challenging and worrisome pathogen in veterinary medicine, since it is often associated with-chronic otitis externa in dogs, poorly responding to antimicrobial treatments [1, 2]. This study aimed to evaluate the antimicrobial resistance profiles and to detect the extended-spectrum β-lactamase (ESBL) and metallo-β- lactamase (MBL) genes in canine *Pseudomonas aeruginosa* strains.

Materials and Methods
Seventeen *Pseudomonas aeruginosa* strains, recovered from auricular specimens of dogs affected by otitis externa attending the University Veterinary Teaching Hospital of Naples in 2023, were identified by MALDI-TOF MS. Antimicrobial susceptibility testing was carried out against twelve clinically relevant antimicrobials using the Kirby Bauer disk diffusion method. PCR assay was performed to detect ESBL blaCTX-M, blaTEM, blaSHV, blaPER, and MBL blaIMP, blaOXA-48, blaVIM, blaNDM, blaGES genes.

Results
The results showed *Pseudomonas aeruginosa* isolates had a phenotypic resistance value of 100% to ceftazidime, imipenem and meropenem, followed by piperacillin-tazobactam and sulfamethoxazole-trimethoprim (94%), and aztreonam (88%). The ESBL genotypic resistance was driven by blaPER (100%; 17/17), followed by blaSHV (29.4%; 5/17), blaTEM (23.5%; 4/17), and lastly by blaCTX-M (17.6%; 3/17). Referring to MBL-genotypic resistance, blaVIM was detected in all 17 *Pseudomonas aeruginosa* isolates (100%), followed by blaGES (76.5%; 13/17), blaOXA-48 (29.4%; 5/17), blaNDM (23.5%; 4/17) and blaIMP (17.6%; 3/17).

Discussion and Conclusion
The findings of this study revealed worrying antimicrobial resistance profiles of *Pseudomonas aeruginosa*- associated canine otitis externa. Furthermore, to the best our knowledge this is the first investigation carried out to detect extended-spectrum β-lactamase and metallo-β-lactamase genes in *Pseudomonas aeruginosa* of animal origin in Italy.

References
1. Nocera F.P. et al., On Gram-positive- and Gram-negative-bacteria-associated canine and feline skin infections: a 4-year retrospective study of the University Veterinary Microbiology Diagnostic Laboratory of Naples, Italy. Animals (Basel) 2021, 11(6):1603.
2. Elfadadny A. et al., Antimicrobial resistance and genotyping of *Pseudomonas aeruginosa* isolated from the ear canals of dogs in Japan. Front Vet Sci. 2023, 10:1074127.

P101

Antimicrobial resistance / susceptibility testing

OCCURRENCE OF *ENTEROCOCCUS* SPP. RESISTANT TO VANCOMYCIN AND LINEZOLID IN CLAMS FROM THE CENTRAL ADRIATIC SEA

L. Petrucci[1], A. Borgognoni[2], S. Pieralisi[2], E. Calandri[2], E. Albini[2], F.R. Massacci[2], F. Barchiesi[2], F. Leoni[2]

[1]*Dipartimento di Sanità pubblica, Medicina Sperimentale e Forense Università degli Studi di Pavia;*
[2]*Istituto Zooprofilattico Sperimentale dell'Umbria e Delle Marche 'Togo Rosati', Perugia, Italy*

Introduction
Bivalves are filter-feeding animals able to concentrate bacteria from surrounding waters, including those resistant to antibiotics [1], often used as "sentinels" to biomonitor the environments in which they live. The aim of the study was to investigate the presence of *Enterococcus* spp. resistant to certain classes of antimicrobials, in bivalve molluscs collected from clam harvesting areas.

Materials and Methods
A total of 152 samples from 9 sampling points of clam harvesting areas of coastal areas of the Marche region were monthly monitored between August 2022 and December 2023 for the occurrence of *Enterococcus* spp. resistant to highly important antimicrobial classes (e.g. glycopeptides and oxazolidinones). Strain isolation was performed by the inoculating enriched broths of the initial shellfish suspension over the surface of Slanetz-Bartley medium supplemented with vancomycin or florfenicol. Isolates were identified by MALDI-TOF mass spectrometry. Susceptibility to different antimicrobials was determined by the minimum inhibitory concentration (MIC) test according to EUCAST/ CLSI breakpoints.

Results
Enterococcus spp. were isolated in selective mediums for the 9.2% (14 of 152) of the samples. Of these, 5 (35.7%) and 9 (64.3%) were identified as *E. faecalis* and *E. faecium*, respectively. The highest prevalence of *Enterococcus* spp. was found in spring (14.8%).In 5.3% (8 out of 152) and 7.9% (12 out of 152) of the samples were identified *Enterococcus* spp. resistant to vancomycin and linezolid, respectively. Multidrug resistance was found in 13 of the isolates.

Discussion and Conclusion
Enterococcus spp. isolates resistant to last-resort antimicrobials antibiotics have been recovered from bivalves. In addition, the majority of isolates was also multidrug resistant. Therefore, bivalves could be used as environmental sentinel for antimicrobial resistance in the marine environment, also providing epidemiological information on the circulation of antimicrobial resistant bacteria in a One-health approach. Further, those isolates will be screened for the presence of resistance genes and subjected to Whole Genome Sequencing.
Research partially supported by Project PNRR no. PE00000007, INF-ACT.

References
1. Albini E. et al., 2022. A systematic review and meta-analysis on antimicrobial resistance in marine bivalves. Front. Microbiol. 13.

P102

Antimicrobial resistance / susceptibility testing

USING OF SURROGATE ENTEROVIRUS 71 FOR THE STUDY OF HONEYBEE VIRUSES INACTIVATION

J. Prodělalová[1], R. Moutelíková[1], E. Čukanová[1]

[1]*Department of Infectious Diseases and Preventive Medicine, Veterinary Research Institute, Brno, Czech Republic*

Introduction
Testing of the effectiveness of chemical and physical disinfection of honeybee viruses is limited by the unavailability of a suitable experimental model based on the honeybee virus and a permanent cell line. Therefore, it is necessary to choose a surrogate. The surrogate should meet the following criteria: (1) taxonomic relatedness; (2) resistance of the surrogate under the test conditions; (3) the ability to grow on a cell line to a sufficiently high titre; (4) safety for laboratory workers.
The aim of the study was to specify the long-term survival ability of the surrogate EVA71 in sugar syrup at cold storage temperature (+11 °C) and to predict the probability of viral contamination in carbohydrate stocks after cold storage.

Materials and Methods
Human enterovirus A 71 (EVA71), strain MY104-9-SAR-97 (CAPM V- 677) was chosen for testing. It is structurally similar to the black queen cell virus and, like most of honey bee viruses, it is a small, non-enveloped virus with a single-stranded RNA genome; classified into the *Picornavirales* order. EVA71 was propagated on VERO cells. Virus inactivation was tested in sugar syrup imitating honey (i.e. glucose and fructose in a 1:1 weight ratio and final concentration 80%); moreover buffered saline solution (DPBS) with the same virus concentration was used as control. Mixture of sugars and EVA71 was stored at 11°C for 140 days and quantified by end-point titration in weekly intervals.

Results
The initial titer of EVA71 in the sugar syrup was 107.2 and in the control DPBS was 107.2 TCID50/ml. At the end of the experiment, the titre of EVA71 in the mixture of carbohydrates simulating honey reserves was 105.6 and in the control DPBS was 105.9 TCID50/ml. The titre of the virus in the carbohydrate test solution, i.e. between the beginning and the end of the experiment, dropped by a maximum of 99%.

Discussion and Conclusion
EVA71 showed the ability to long-term survival in sugar syrup at 11°C; results are similar to our previous study which used honey stored at 4°C instead of sugar syrup.

This study was supported by Ministry of Agriculture of the Czech Republic (RO0523) and Technology Agency of the Czech Republic (TN02000017).

P103

Antimicrobial resistance / susceptibility testing

PREVALENCE OF EXTENDED-SPECTRUM β-LACTAMASES, AMPC-β-LACTAMASES, FLUOROQUINOLONES AND CARBAPENEMS-RESISTANCE IN BACTERIA ISOLATED FROM WILD EUROPEAN HEDGEHOGS (*ERINACEUS EUROPAEUS*) FAECAL SAMPLES IN NORTHERN ITALY: PRELIMINARY RESULTS

M.C. Rapi, L. Musa, M. Penati, F. Santandrea, G.B.M. Bianchi, L. Filippone Pavesi, M.F. Addis, G. Grilli

Department of Veterinary Medicine and Animal Sciences, University of Milan, Lodi, Italy

Introduction

The rise of antimicrobial-resistant bacteria (ARB) in animals is a significant concern for modern medicine. Monitoring antimicrobial resistance (AMR) in wildlife is crucial to understanding ARB dynamics, with omnivorous and anthropophilic species serving as key sentinels, such as the European hedgehog (*Erinaceus europaeus*) [1].

Materials and Methods

This study evaluated the presence and prevalence of bacteria producing extended-spectrum β-lactamases (ESBLs) and AmpC-type β-lactamases (AmpC), and showing resistance to carbapenems and fluoroquinolones, in wild European hedgehogs in Lombardy. Fecal samples from untreated, deceased animals at the Vanzago Wildlife Rescue Center were cultured, identified by MALDI-TOF MS [2], and subjected to ESBL screening and phenotype confirmation tests. The AmpC profile and resistance to carbapenems and fluoroquinolones were assessed phenotypically.

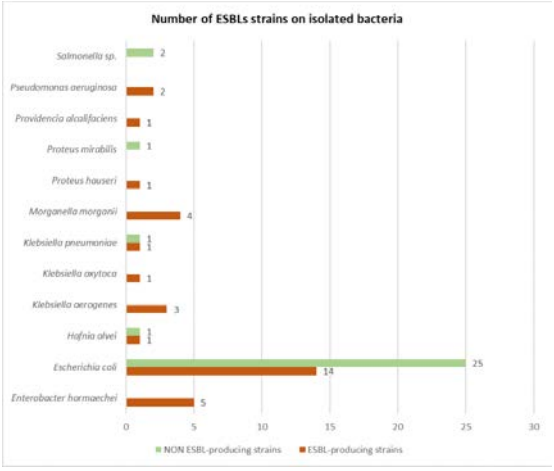


Figure 1. Number of ESBL-producer strains considering all the isolated bacteria. Phenotypically, 33 out of 63 strains isolated from faecal samples exhibited an ESBL-producer profile.

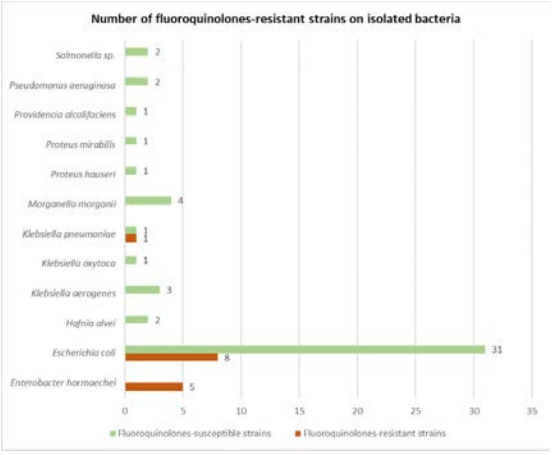


Figure 2. Number of fluoroquinolones-resistant strains considering all the isolated bacteria. Phenotypically, 14 out of 63 strains isolated from faecal samples were recognized as fluoroquinolones-resistant.

Results

From 45 animals, 61 Enterobacterales and 2 Pseudomonadales isolates were identified, with *Escherichia coli* (n=39) as the most predominant. Other identified species included *Klebsiella* spp. (n=6), *Enterobacter hormaechei* (n=5), *Morganella morganii* (n=4), *Proteus* spp. (n=2), *Hafnia alvei* (n=2), *Salmonella* spp. (n=2), *Providencia alcalifaciens* (n=1) and *Pseudomonas aeruginosa* (n=2). Phenotypically, 74% (47/63) of the strains exhibited an ESBL-producer and fluoroquinolones-resistance profile, with 52% (33/63) recognized as ESBL producers and 22% (14/63) as fluoroquinolones-resistant. Notably, all *E. hormaechei* strains (5/5) were fluoroquinolone-resistant. All strains were susceptible to carbapenems and no positivities for acquired AmpC-resistance were detected.

Discussion and Conclusion

Our findings suggest that European hedgehogs may act as carriers of ARB with various AMR-phenotypes. Monitoring this species, that often lives in an urbanized environment, could serve as a warning system for health threats to humans, animals, and the environment.

References

1. Dolejska. Antibiotic-resistant bacteria in wildlife. In Antibiotic resistance in the environment: A worldwide overview. Springer International Publishing, 2020, 19-70.
2. Rosa et al. Species identification by MALDI-TOF MS and gap PCR-RFLP of non-aureus *Staphylococcus*, Mammaliicoccus, and *Streptococcus* spp. associated with sheep and goat mastitis. Vet. Res., 2022, doi:10.1186/ s13567-022-01102-4.

P104

Antimicrobial resistance / susceptibility testing

ANTIMICROBIAL SUSCEPTIBILITY PROFILING OF *STREPTOCOCCUS UBERIS* ISOLATED FROM A BOVINE MASTITIS IN DAIRY FARMS IN APULIA REGION

V. Rondinone[1], L. Addante[2], L. Capozzi[2], L. Serrecchia[1], V. Manzulli[1], D. Farina[1], P. Di Taranto[1], L. Marino[1], D. Cipolletta[1], L. Del Sambro[2], D. Galante[1], L. Pace[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Putignano (BA), Italy*

Introduction

Bovine mastitis is a common disease that causes inflammation of the mammary gland and udder tissues, resulting in a significant reduction in the quantity and quality of milk produced and can consequently lead to significant losses in the dairy industry. In particular, *Streptococcus uberis* is capable of causing cases that are difficult to treat with possible relapses, causing both clinical and subclinical mastitis in dairy cattle. The aim of the work is first of all to investigate the prevalence of *S.uberis* in the milk and mammary secretions of dairy cows affected by mastitis, and to carry out the evaluation of their susceptibility to antibiotics.

Materials and Methods

Antimicrobial resistance patterns were tested on 40 isolates of *S.uberis*, by minimum inhibitory concentration (MIC) using the broth microdilution method on commercially prepared 96-well microtiter plates from Sensititre System (Thermo Fisher), that have a panel of 10 commonly used antimicrobials. Results were interpreted using available CLSI resistance breakpoints according to VET01S 5th edition guidelines.

Antimicrobials	Range (µg/mL)	Breakpoints (µg/mL) and Susceptibility				MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
		S[1]	[%]	R[2]	[%]		
AMP[3]	0.12-8	≤ 0.25	90	≥1	10	0.25	0.25
XNL[4]	0.5-8	≤ 0.5	72.5	≥8	27.5	0.12	4
CEP[5]	2-16	≤ 2	97.5	≥8	2.5	2	2
ERY[6]	0.25-4	≤ 0.25	65	≥1	35	0.25	1
OXA+[7]	2-4	≤ 2	90	≥4	10	2	2
PEN[8]	0.12-8	≤ 0.25	90	≥2	10	0.25	1
P/N[9]	1/2-8/16	≤ 1/2	100	≥4/8	0	1/2	1/2
PIRL[10]	0.5-4	≤ 0.5	40	≥4	60	4	4
SDM[11]	32-256					>256	>256
TET[12]	1-8	≤ 2	32.5	≥8	67.5	8	8

[1]Susceptible, [2]Resistant, [3]Ampicillin, [4]Ceftifur, [5]Cephalothin, [6]Erythromycin, [7]Oxacillin+2%NaCl, [8]Penicillin, [9]Penicillin/Novobicin, [10]Pirlimycin, [11]Sulphadimethoxine, [12]Tetracycline

Table 1. Antimicrobials tested, dilution range, breakpoints values, percentage of susceptible and resistant *S.uberis* isolates, MIC inhibiting the growth of at least 50% (MIC50) and 90%(MIC90) of the 40 isolates analyzed.

Results

Table 1 reports the distribution of the MIC inhibiting the growth of 50% and 90% of the isolates for all the antimicrobials tested, and shows the highest inhibiting concentrations for erythromycin, pirlimycin and tetracycline, with MIC90 values even higher than the resistance breakpoints. Furthermore, resistance to β-lactam antibiotics was present in four isolates resistant to penicillin, ampicillin and oxacillin, and eleven isolates resistant to third-generation cephalosporins (ceftiofur).

Discussion and Conclusion

In the present study, in line with recent literature, we found a high resistance phenotype for lincosamides and tetracycline. On the contrary, compared with other European reports, we observed an increased resistance to erythromycin, demonstrating the importance also for an increasing control of bacteria causing environmental mastitis. In conclusion, these preliminary surveillance data can be meaningful for practical management and helpful for the identification of the most appropriate antibiotic agents against *S.uberis* mastitis.

References

Monistero V. et al. Genotyping and Antimicrobial Susceptibility Profiling of *Streptococcus* uberis Isolated from a Clinical Bovine Mastitis Outbreak in a Dairy Farm. Antibiotics vol. 10,6 644. 28 May. 2021

P105

Antimicrobial resistance / susceptibility testing

ANTIMICROBIAL RESISTANCE OF *SALMONELLA ENTERICA* SEROVAR CHOLERAESUIS ISOLATED FROM PIGS DURING OUTBREAKS OF SEPTICEMIC FORM IN HERDS OF NORTHERN ITALY

C. Rosignoli[1], G. Santucci[1], S. Faccini[1], L. Gintoli[1], G. Ventura[1], M. Gradassi[1], L. Marocchi[1], M. D'Incau[1], M. Boldini[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy*

Introduction

Salmonella enterica serovar Choleraesuis (*Salmonella Choleraesuis*) is a host-adapted swine pathogen. It can also infect humans, typically through direct or indirect contact with infected animals or consumption of contaminated animal products. In pigs, *Salmonella* Choleraesuis causes a severe septicemic disease characterized by fever, lethargy, cianosis and rapid death (1). In Northern Italy, area of intensive pig farming, severe clinical outbreaks of this infection have increased significantly in recent years. In this cases, early intervention with appropriate antibiotics can be effective, but resistance is a concern. This retrospective study aimed to assess the antibiotic resistance pattern in *Salmonella Choleraesuis* isolates from pigs during severe outbreaks of septicemic form.

Materials and Methods

Thirty-four *Salmonella Choleraesuis* isolates collected between 2022 and 2024 in pigs from different herds located mainly in the Province of Mantua (Lombardy Region– Northern Italy) were examined by broth micro-dilution tests (Sensititre TM, Thermo Scientific) to assess the minimum inhibitory concentration (MIC). Antimicrobial tested included amoxicillin/clavulanic acid, ampicillin, cefotaxime, enrofloxacin, florfenicol, sulfisoxazol, tetracycline and trimethoprim/sulfamethoxazole.

Table 1. Distribution of minimum inhibitory concentrations (MICs) for Salmonella Choleraesuis isolates (n = 34) and the percentage of isolates classified as resistant against different antimicrobial agents.

Antimicrobial agent	Distribution of isolates (count) across MIC values (µg/ml)[1]																% of resistant isolates
	0.0156	0.0312	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	
Amoxicillin/clavulanic acid[2]							5	2	1	23	3						0.0
Ampicillin						1	2	3			1		27				79.4
Cefotaxime						33	1										0.0
Enrofloxacin			3	13		2	1	7	6		2						91,2
Florfenicol									8	2				24			70,6
Sulfisoxazole														5		29	85.3
Tetracycline								9	2			23					67.6
Trimethoprim/sulfamethoxazole[3]			6	1	5	5						17					50.0

[1]The dilution ranges tested are those contained in the white area. Values above this range indicate MIC values higher than the highest concentration within the range. Values corresponding to the lowest concentration tested indicated MIC values lower or equal to the lowest concentration within the range. Resistance breakpoints are indicated by vertical red lines, as specified by CLSI M100 for amoxicillin/clavulanic acid, ampicillin, cefotaxime, and sulfisoxazole; by CLSI VET01S for florfenicol, tetracycline, and trimethoprim/sulfamethoxazole; and by EUCAST v11 for enrofloxacin. [2] Amoxicillin is tested at a concentration ratio of 2:1 (amoxicillin/clavulanic acid). [3]Trimethoprim is tested at a concentration ratio of 1:19 (trimethoprim/sulfamethoxazole).

Results

The highest frequency of resistance was observed with enrofloxacin (91.2%), followed by sulfisoxazol (85.3%), ampicillin (79.4%), florfenicol (70.6%), tetracycline (67.6%) and trimethoprim/sulfamethoxazole (50.0%). There was no isolate resistant to amoxicillin/clavulanic acid and cefotaxime (Table 1). Multidrug resistance was common, with 85% (29/34) of isolates showing resistance to three or more antimicrobial classes.

Discussion and Conclusion

Salmonella Choleraesuis strains isolated from clinical septicemic cases between 2022 and 2024 in Italian pig herds showed a high percentage of multidrug resistance. These data suggest that implementation of effective biosecurity measures and use of autogenous vaccines should be considered in alternative to antimicrobial medication to contrast this type of salmonellosis. The study underlines the importance of promoting responsible antimicrobial use.

References

1. Diseases of swine, 11th Edition, Wiley-Blackwell, 2019.

P106

Antimicrobial resistance / susceptibility testing

MONITORING OF VANCOMYCIN RESISTANT ENTEROCOCCI (VRE) IN WILD ANIMALS IN PIACENZA PROVINCE, ITALY.

S. Russo[4], S. Restori[4], A. Di Cesare[5], T. Sbaffi[5], G. Borgomaneiro[5], L. Cirasella[2], C.A. Garbarino[4], R. Taddei[1], A. Luppi[3], E. Massella[4]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Bologna, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Forlì, Italy*
[3]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Parma, Italy*
[4]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Piacenza, Italy*
[5]*National Research Council of Italy (CNR) – Water Research Institute (IRSA), Verbania, Italy*

Introduction

Vancomycin resistant *Enterococci* (VRE) are an important cause of nosocomial infections, increasingly reported in all Europe. Monitoring of antimicrobial resistance (AMR) spread has often been implemented using indicators (including wildlife), representing sentinels of AMR pollution. Here we present a VRE monitoring in wild animals in the Piacenza province and, to the best of our knowledge, the first record of a VR *E. gallinuarum* (Eg) in a wild owl in Italy.

Materials and Methods

In the period between January 2023 and February 2024, faeces were collected from 285 wild animals, analysed for the infectious disease surveillance program of the Emilia Romagna region. Samples were enriched in a selective broth for enterococci isolation, added with 10 µg/ml vancomycin, and incubated overnight. DNA was extracted with a commercial kit. van genes presence was screened with a multiplex PCR (1). Samples tested positive were streaked on Kanamycin Aesculin agar (37°C, 24h). The suspected colonies were typed using MALDI-TOF. Antimicrobial resistance profile was determined with broth microdilution method. Minimum Inhibitory Concentration (MIC) results were interpreted according to CLSI or, when not present, EUCAST breakpoints. Whole genome sequencing (WGS) was carried out to identify sequence types (STs), antibiotic resistant (ARGs) and virulence genes (VRGs).

Antimicrobials	MIC (µg/ml)	Interpretation
Vancomycin	8	R
Teicoplanin	2	S
Quinupristin/Dalfopristin	4	R
Tetracycline	2	S
Daptomycin	4	R
Ciprofloxacin	2	R
Erythromycin	<1	S
Tigecycline	0,5	R
Linezolid	4	R
Gentamicin	<8	S
Ampicillin	2	S
Chloramphenicol	16	R

Table 1. Phenotypic antimicrobial resistance profile of the vanC *Enterococcus gallinarum*, isolated from a wild owl.

	Genes
Antimicrobial resistance	*efrB*, *vanY* gene in *vanM*, *vanC*, *vanXY*, *vanT*, *vanR*, *vanS* gene in *vanC* cluster
Virulence	*ebpA, ebpB, ebpC, srtC, efaA, pavA, slrA, cpsA/uppS, cpsB/cdsA, cpsI, cpsJ, bopD, cylR2, cesC, fliP, fliQ, htrA/degP, cheY, bscN, lgt*

Table 2. Antimicrobial resistance and virulence genes carried by the vanC *Enterococcus gallinarum*, isolated from a wild owl.

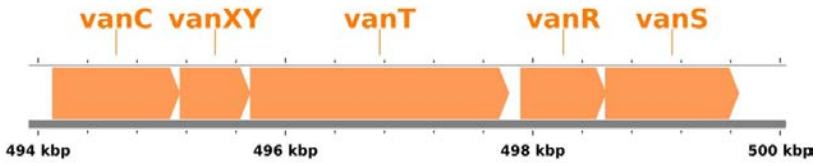


Figure 1. Organization of enterococcal vancomycin-resistance gene cluster in the vanC *Enterococcus* gallinarum, isolated from a wild owl.

Results
Out of 285 samples, only one was associated with a vanC Eg, isolated from a wild owl. The strain was phenotypically multidrug resistant (MDR), including to vancomycin (MIC=8 µg/ml) (Table1). ARGs and VAGs are reported in Table 2. vanC gene and associated genomic elements are shown in Figure 1. No ST was identified.

Discussion and Conclusion
Here we present the first report of a MDR vanC Eg in a wild owl in Italy. Interestingly, wild animals were not associated with clinically important van genes (i.e. vanA, vanB), despite vanC ones have been already reported in human outbreaks. The study underline the importance of wildlife as AMR sentinel, to monitor and predict diffusion of critical resistances.

References
1. Dutka-Malen S. et al., 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J. Clin. Microbiol. 33, 1:24-27.

P107

Antimicrobial resistance / susceptibility testing

ANTIMICROBIAL SUSCEPTIBILITY PROFILES OF STRAINS OF *CLOSTRIDIUM* SPP. ISOLATED FROM DIFFERENT DOMESTIC AND WILD ANIMAL SPECIES

G. Schiro'[1], A. Carrozzo[1], F. Mira[1], M. Vitale[1], M. La Giglia[1], D. Vicari[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia "A. Mirri", Palermo, Italy*

Introduction
Clostridium spp. are anaerobic and ubiquitous bacteria, producing a high number of toxins, and some of them are involved in severe diseases of humans and animals (1). Therapy towards *Clostridium* spp. infection commonly includes antimicrobial treatment (metronidazole, vancomycin, fidaxomicin), but resistance to several antibiotics has been reported. However, the role of animals in the spread of antimicrobial resistance is still unknown, particularly for domestic and wild species (2). The aim of this study was the evaluation of antimicrobial susceptibility profiles of strains of *Clostridium* spp. isolated from tissue samples of different animals (bovine, ovine, equine, dog, cat, hare, stork, blackbuck).

Materials and Methods
Samples were plated on Blood agar and anaerobically incubated at 37°C for 24-48h. Strains identification was carried out with the biochemical API® and VITEK® systems, confirmed by MALDI-TOF MS. Antimicrobial susceptibility was evaluated by the disk diffusion method, and isolates were considered resistant, intermediate, or susceptible according to the CLSI ranges; twelve antibiotic molecules were tested. The presence of toxin genes for *C. perfringens* strains was also evaluated by a Multiplex PCR assay, for the detection of genes encoding for α, β, ε, ι, β2 toxins and for enterotoxins.

Results
Strains of different *Clostridium* species (*C. perfringens, C. sordelli, C. baratii, C. septicum, C. histolyticum, C. glycolicum*) were isolated, showing different antimicrobial resistance profiles and in particular, almost all strains towards metronidazole, tilmicosin, erythromycin, vancomycin, lincomycin. Some *C. perfringens* strains tested positive for toxin genes (cpa, a).

Discussion and Conclusion
Antimicrobial resistance is one of the main threats to human and animal health and it is crucial to investigate this phenomenon with a One Health perspective. This study highlights the importance of monitoring antimicrobial profiles of pathogenic and zoonotic *Clostridia* species both in domestic and wild animal species to evaluate related risks.

References
1. Czepiel J., Dróżdż M., Hanna Pituch H., et al. European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:1211–122.
2. O'Grady, K., Knight D. R., Thomas, Riley T. V. European Journal of Clinical Microbiology & Infectious Diseases (2021) 40:2459–2478.

P108

Antimicrobial resistance / susceptibility testing

ANTIMICROBIAL RESISTANCE AND AMR GENES OCCURRENCE IN *STREPTOCOCCUS SUIS*

M. Zouharova[1], K. Nedbalcova[1], J. Matiasovic[1], K. Matiaskova[1], N. Kralova[2]

[1]*Veterinary Research Institute, Czech Republic*
[2]*Veterinary Research Institute, Department of Experimental Biology Faculty of Science Masaryk University, Czech Republic*

Introduction

Diseases caused by *Streptococcus suis* are a significant economic concern for pig farms globally and have also zoonotic potential (1). Antimicrobials are commonly used to treat *S. suis* infections, and it is therefore essential to monitor antimicrobial resistance (AMR).

Materials and Methods

A total of 525 *S. suis* isolates were collected from diseased pigs on Czech farms between 2018 and 2022. Antimicrobial susceptibility testing (AST) was performed by determining the minimum inhibitory concentrations (MICs) using the microdilution broth method. In selected 173 isolates, genes encoding AMR were detected by searching the whole genome sequences using the ResFinder database.

Results

None of the isolates tested were resistant to ceftiofur, a very high level of susceptibility of *S. suis* isolates was also found to amoxicillin with clavulanic acid, sulfamethoxazole potentiated with trimethoprim, enrofloxacin and florfenicol. On the contrary, high levels of resistance were found to tetracycline, clindamycin, tilmicosin, tulathromycin and tiamulin (Figure 1, Table 1). AMR genes were predominantly present for macrolides and lincosamides (erm(B)), tetracyclines (tet(O)) and aminoglycosides (ant(6)-Ia) (Table 2).

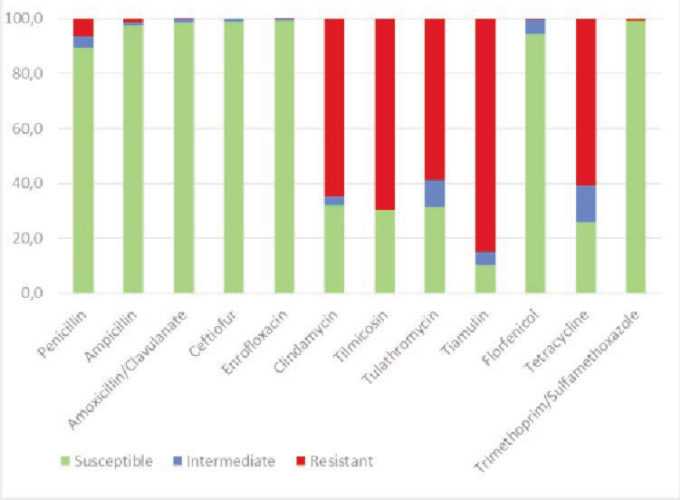


Figure 1. Percentage representation of susceptible, intermediate susceptible and resistant strains of *S. suis*. (n=525).

	MIC (mg/L)														S (%)	I (%)	R (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)
	0,03	0,06	0,125	0,25	0,5	1	2	4	8	16	32	64	128	256					
PEN	212	199	32	26	23	17	12	4							89,3	4,4	6,3	0,06	0,25
AMP	257	185	46	19	6	5	4	2	1						97,7	1,0	1,3	0,06	0,125
AMC				511	7	6	1								98,7	1,1	0,2	≤0,25	≤0,25
EFT			312	109	34	36	28	6							98,9	1,1	0,0	≤0,125	1
ENR		13	102	286	121	2	1								99,4	0,4	0,2	0,25	0,5
CLI			136	31	18	5	4	44	20	12	255				31,8	3,4	64,8	0,25	0,5
TIL						1	4	11	20	123	114	8	15	229	30,3	0,0	69,7	0,25	0,5
TUL						12	15	32	47	58	53	50	11	247	31,2	10,1	58,7	0,25	0,5
TIA				13	40	27	50	111	49	41	48	146			10,1	5,1	84,8	0,25	0,5
FFC					4	123	369	27		2					94,5	5,1	0,4	2	2
TET				99	36	70	24	5	17	48	194	32			25,7	13,3	61,0	2	32
SXT		398	78	34	8	3	1	3							99,2	0,2	0,6	≤0,06	0,125

Table 1. MICs distribution for antimicrobials; percentages of susceptible, intermediately resistant, and resistant isolates, and MIC50 and MIC90 values in *S. suis* isolates

Antibiotic group	Percentage of the strains carrying AMR gene	Resistance genes (ResFinder database)	Percentage of strains carrying the gene
Macrolides	54.9	*mef(A)*	3.5
		msr(D)	2.9
		erm(B)	53.8
Lincosamides	57.2	*erm(B)*	53.8
		lnu(B)	6.4
		lnuC	0.6
		lsa(E)	5.8
Tetracyclines	66.5	*tet(W)*	4.6
		tet(L)	0.6
		tet(44)	2.3
		tet(40)	3.5
		tet(O)	49.1
		tet(M)	12.7
Aminoglycosides	22.5	*ant(6)-Ia*	15.6
		ant(6)-Ib	4.0
		aph(3)-III	5.2
		aac(6)-aph(2')	1.7
Amphenicols	1.7	*optrA*	1.7

Table 2. Antimicrobial resistance genes in *S. suis* isolates (n=173).

Discussion and Conclusion

Resistance to certain antimicrobials may be related to their high consumption in pig farms for the treatment of infections (2). The prevalence of AMR genes in our study is consistent with the results of phenotypic AST. However, the presence of AMR genes in the isolates did not always correspond to the AST results. This discrepancy is likely due to the presence of silent genes or, conversely, the presence of genes not identified by the available database. Any use of antimicrobials must be well justified to increase the likelihood of treatment success and prevent the spread of resistence. This work was supported by grants RO0523 and TN02000017.

References

1. Goyette-Desjardins et al. (2014) Emerg Microb Infect 3, e45.
2. Vela (2005) Vet Microbiol 105, 143–147.

P109

Biosecurity in animal health

OVERVIEW OF BIOSECURITY MEASURES IN SALMONID FARMS IN THE AUTONOMOUS PROVINCE OF TRENTO (NORTHEASTERN ITALY)

E. Franzago[5], M. Mazzucato[5], T. Dorotea[3], P. Mulatti[5], G. Marchetti[4], C. Casarotto[5], A. Fabris[1], N. Ferrè[5], A. Toffan[5], A. Marsella[5], A. Callegaro[5], D. Dellamaria[5], L. Bortolotti[2], G. Manca[5], M. Dalla Pozza[5]

[1]*Associazione Piscicoltori Italiani (API), Italy*
[2]*Azienda Provinciale per i Servizi Sanitari di Trento (APSS), Italy*
[3]*Azienda ULSS [6] - Euganea, Italy*
[4]*Freelance, Italy*
[5]*Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Italy*

Introduction

The Autonomous Province of Trento (APT), in the North-Est of Italy, has the bigger number of trout farms of the country. In order to have a high production it is necessary that establishments don't suffer losses due to diseases and therefore a policy of prevention must be followed. The scope of the work has been to identify the biosecurity measures (BM) and assess how much each of them can affect the level of biosecurity in a farm and classifying the establishments accordingly.

Materials and Methods

The study included 62 farms of salmonids of the APT. Data regarding BM of each farms (from 2020 to 2022) were collected by means of a national checklist that applied the 2006/88/CE Directive, still in use by competent authority. The biosecurity's questions of the checklist were submitted to a group of 12 experts. Using the expert elicitation method, they evaluated which BM are mostly linked to the risk of introduction and spread of diseases.

Results

The following five were identified as the most critical measures: 1) Trucks' cleaning and disinfection; 2) Regularity in remove dead fishes; 3) Protection against ichtiophagous birds; 4) Use of dedicated equipment for the different sectors of the farm; 5) Presence of separated

areas dedicated to the loading of dead fish. From combining the checklists answers with the importance of the BM, a score for each farm was obtained. Farms were ranked in five classes as follow: "low" (0%), "medium-low" (1.6%), "medium" (24.2%), "medium-high" (55.8%), and "high" (19.4%).

Discussion and Conclusion

In general, the BM considered are basically always, or in most cases, present in the APT's establishments. The differences are due to the variety of the farm's structures and management, but all the APT's establishments can be considered fairly protected by diseases.

References

Oidtmann, B.C.; Crane, C.N.; Thrush, M.A.; Hill, B.J.; Peeler, E.J. Ranking freshwater fish farms for the risk of pathogen introduction and spread. Prev. Vet. Med. 2011, 102, 329–340.

Oidtmann, B.C.; Peeler, E.J.; Thrush, M.A.; Cameron, A.R.; Reese, R.A.; Pearce, F.M.; Dunn, P.; Lyngstad, T.M.; Tavornpanich, S.; Brun, E.; et al. Expert consultation on risk factors for introduction of infectious pathogens into fish farms. Prev. Vet. Med. 2014,115, 238–254.

P110

Emerging and re-emerging diseases

GLAESSERELLA [HAEMOPHILUS] *PARASUIS* REAL-TIME PCR AS A DIAGNOSTIC METHOD FOR GLäSSER'S DISEASE IN PIGS.

L. Arais[1], S. Williamson[2], M. Horney[1], D. Loy[3], M. Kirchner[1]

[1]*Animal and Plant Health Agency, Centre of Excellence for Bacteriology, Addlestone, Surrey, United Kingdom*
[2]*Animal and Plant Health Agency, Veterinary Investigation Centre, Bury St Edmunds, Suffolk, United Kingdom*
[3]*Current address: Astrobiology OU Faculty of Science, Technology, Engineering and Mathematics, The Open University, Milton Keynes, Buckinghamshire, United Kingdom*

Introduction

Glaesserella parasuis is the causative agent of Glässers' disease. It is endemic globally in pig-producing countries and can be associated with high morbidity and mortality, and economic losses. It is detected by bacteriological culture, but this can be insensitive due to the fastidious nature of the bacterium. Therefore, a real-time PCR was developed for detection of *G. parasuis* in clinical samples.

Materials and Methods

Two PCRs based on published methods were trialled. In silico analysis was used to design real-time PCR primers. Primers for rRNA 16S were included as an internal control. Specificity was determined using a large panel of bacteria including *G. australis*. A panel of 33 *G. parasuis* isolates representing 13 known serotypes, was used for validation. Swabs (n=156) collected from 40 cases of serositis were used to assess the test. Swabs from culture/PCR negative submissions were pooled and used in a spiking study with known *G. parasuis* cell concentrations to assess test sensitivity and its performance in detecting *G. parasuis* combined with the microbiota and potential inhibitors. Test sensitivity was also assessed using dilutions of *G. parasuis* DNA.

Results

The HSP gene was selected as the target for the PCR (Howell et al. 2015) as it had good specificity. There was no amplification in the panel of non-*G. parasuis* tested and the PCR could detect all *G. parasuis* serotypes analysed. This PCR detected *G. parasuis* at 7.3 x10-2 cfu/µL (Cq=31.07, SD=0.13) and 826.7 fg/µL (Cq=30.81, SD=0.43) of DNA. In four submissions where *G. parasuis* was isolated, nine of 18 swabs were positive for *G. parasuis* by PCR. In culture-negative submissions, 136 swabs from 36 submissions were negative for *G. parasuis* by PCR, while two swabs from a single submission tested positive.

Discussion and Conclusion

The PCR developed detected *G. parasuis* DNA in swabs collected from 50% of the submissions where *G. parasuis* was isolated in culture, and in one additional culture-negative submission. Usually, a swab from a submission sent for culture wasn't the same received for the PCR validation. The PCR was highly specific for *G. parasuis*. It had good sensitivity with spiked DNA, and when *G. parasuis* cells were spiked into negative clinical samples. This test could be applied in a diagnostic setting for the rapid detection of *G. parasuis*.

P111

Emerging and re-emerging diseases

APPLICATION OF THE DROPLET DIGITAL PCR (DDPCR) TECHNOLOGY FOR THE DETECTION OF SARS-COV-2 IN BIVALVE MOLLUSCS AND ENVIRONMENTAL SAMPLES

M.G. Basanisi[4], G. La Bella[4], G. Nobili[4], R. Coppola[4], A.M. Damato[4], A.M. D'Antuono[5], T. Scirocco[2], G. Occhiochiuso[4], S. Faleo[4], P. Selicato[4], M.C. Nardella[4], S. Tola[6], L. Cilenti[1], E. Suffredini[3], G. La Salandra[4]

[1]*Istituto di Scienze delle Produzioni Alimentari del Consiglio Nazionale delle Ricerche (ISPA CNR), Foggia, Italy*
[2]*Istituto per le Risorse Biologiche e le Biotecnologie Marine del Consiglio Nazionale delle Ricerche (IRBIM CNR), Lesina (FG), Italy*
[3]*Istituto Superiore di Sanità, Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria-Laboratorio Nazionale di Riferimento per i Virus di Origine Alimentare, Roma, Italy*
[4]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy*
[5]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy; PhD National Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, Univer*
[6]*Istituto Zooprofilattico Sperimentale della Sardegna "G. Pegreffi", Sassari, Italy*

Introduction
SARS-CoV-2 is the causative agent of an acute respiratory disease responsible for the COVID-19 pandemic emergency in the period 2020-2023. SARS-CoV-2 RNA has been detected in treated sewage, rivers and seawater, and even in bivalve shellfish. The aim of this study was to investigate the presence of SARS-CoV-2 RNA in bivalve molluscs, waters and sediments from harvesting areas of the Apulia region (South-East Italy) in order to evaluate the possible use of shellfish for monitoring the spread of SARS-CoV-2 in coastal environment.

Materials and Methods
The study was carried out on 222 samples of bivalve molluscs and 190 water and sediment samples collected between January 2022 and June 2024. Bivalve molluscs were collected from harvesting areas or fish shops. Water and sediment samples were collected at the Lesina and Varano lagoons (North Apulia region), where there are molluscs farms. After viral concentration and subsequently RNA extraction, the samples were tested by Reverse Transcription droplet digital PCR (RT-ddPCR) targeting E, N and RdRp genes of Sars-CoV-2.

Results
During the period considered, none of the bivalve molluscs and water and sediment samples tested positive for the presence of SARS-CoV-2 RNA by RT-ddPCR method. The limit of detection (LOD) of each target gene was found to be 1 copy/µl.

Discussion and Conclusion
The results of this study suggest that SARS-CoV-2 did not reach coastal environment of Apulia region and Lesina and Varano lagoons at significant levels to allow a bioaccumulation in molluscs during the period considered. The viral concentration in bivalve molluscs is significantly influenced by the viral load in the farming waters, which depends both on the virus input through wastewater and the virus dispersion in marine waters. Furthermore, the efficiency of the wastewater treatment plants and the distance between the discharges and the place of production must also be considered. The RT-ddPCR assays applied in this study confirmed the high sensitivity of the method to detect low levels of nucleic acids and could be used in routine practice to provide reliable data for risk analysis or in case of epidemic events. Project funded by the Italian Ministry of Health (IZSPB 04/21 RC).

References
Corman et al., 2020. Euro Surveill. 25(3):2000045

P112

Emerging and re-emerging diseases

HISTORICAL MALARIA VECTORS IN SOUTHERN ITALY (APULIA AND BASILICATA REGIONS): FIVE YEARS OF ENTOMOLOGICAL SURVEILLANCE ON RESIDUAL ANOPHELISM, FROM 2017 TO 2023

M.A. Cafiero[6], D.A. Raele[6], F. Severini[5], L. Toma[5], M. Menegon[5], D. Boccolini[5], E. Franco[4], P. Miccolis[4], V. Nola[3], P. Salerno[3], G. Francavilla[1], M.G. Cariglia[6], G. Tortorella[2], M. Di Luca[5]

[1]*Azienda Sanitaria Locale SIAV-A, Foggia, Italy*
[2]*Azienda Sanitaria Locale SIAV-A, Lecce , Italy*
[3]*Azienda Sanitaria Locale SIAV-A, Matera, Italy*
[4]*Azienda Sanitaria Locale SIAV-C, Taranto, Italy*
[5]*Istituto Superiore di Sanità, Dipartimento Malattie Infettive, Reparto Malattie Trasmesse da Vettori, Roma, Italy*
[6]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*

Introduction

Malaria remains a potential public health issue in Italy due to the presence of former Anopheles vectors and the increasing number of yearly imported cases. In presence of both gametocyte carriers and competent vectors, the reemergence of local transmission could be possible. In the Apulia region, in October 2017 the occurrence of 4 suspected indigenous malaria cases prompted an entomological Surveillance Plan, funded by the Ministry of Health, carried out by ISS, IZSPB, and Local Health Authorities, aimed to investigate on the presence/distribution of malaria vectors.

Materials and Methods

Entomological surveys were carried out in 2017 in the areas where the 4 cases occurred (Taranto province), in 2019-2023 in other areas in Apulia and Basilicata regions, historically prone to the malaria. Resting mosquitoes, larvae and pupae were collected in rural and natural sites. Mosquitoes were morphologically identified, specimens of the maculipennis complex molecularly confirmed at the species level.

Results

A total of 2,320 mosquitoes of 20 species belonging to 6 genera were collected; 1,217 (52,45%) of them were anophelines, identified as shown in the table. In the investigated areas, *An. labranchiae* was the most abundant species, except for Lecce province where only An. sacharovi was found (2022-2023); *An. algeriensis* was predominant in the Gargano sites; *An. superpictus* was recorded (2017) in Matera province, *An. plumbeus* was found in a wooded area in the same province.

Discussion and Conclusion

Our findings show the significant receptivity of the studied areas, evidenced by the presence of the three historical malaria vectors, *An. labranchiae, An. sacharovi* and *An. superpictus*. These species occur at different densities related to environmental, climatic and anthropic factors. The notable rediscovery of *An. sacharovi* after over 50 years allow us to reintegrate it into the Italian Culicidae fauna and strongly suggests for a continuous surveillance of residual anophelism in vulnerable areas.

References

Boccolini D. et al., 2020 doi: 10.1186/s12889-020-08748-9 Di Luca M. et al., 2024, Atti XXXIII Congresso SOIPA Menegon M. et al., 2022 doi: 10.3390/pathogens11090990 Raele DA et al., 2021 doi: 10.3390/pathogens10111521
Raele DA et al., 2024 https://doi.org/10.1186/s13071-024-06252-2 Severini F et al., 2022 Rapporti ISTISAN 22/3

Species	N. specimens
Anopheles algeriensis	494
Anopheles labranchiae	401
Anopheles maculipennis sl.	244
Anopheles superpictus	69
Anopheles sacharovi	8
Anopheles plumbeus	1
Total	1,217

Table 1. Number of mosquito specimens collected during the entomological surveys carried out between 2017 and 2023.

P113

Emerging and re-emerging diseases

IN-DEPTH STUDY OF MAMMALIAN ORTHOREOVIRUS (MRV) IN OWNED DOGS AND CATS IN THE VENETO REGION: PRELIMINARY RESULTS

M. Campalto[5], E. Mazzotta[5], L. Ceglie[5], L. Lucchese[5], P. Danesi[5], M. Berlanda[4], G. Barina[3], E. Baroni[1], G. Barbieri[2], A. Natale[5]

[1]*AniCura - Clinica Veterinaria Baroni, Rovigo, Italy*
[2]*Centro Veterinario Montecchio, Vicenza, Italy*
[3]*Clinica Veterinaria Santa Rita, Padova, Italy*
[4]*Department of Animal Medicine, Production and Health (MAPS), University of Padova, Padova, Italy*
[5]*Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Padova, Italy*

Introduction

Recently, reports of human Mammalian orthoreovirus (MRV) infections have increased, identifying human MRVs as reassortant strains resulting from spillover from animal reservoirs. Conversely, data on MRV infections in companion animals remain limited, although three serotypes have been isolated from dogs and cats. In 2021, a preliminary investigation on 257 dogs and 389 cats detected 12 positive rectal swab samples (1 dog, 11 cats). Early detection of potential interspecies transmission and potential viral reassortment is pivotal in a One Health vision. This study, based on voluntary participation, aims to assess MRV circulation in owned dogs and cats in the Veneto region and to perform an epidemiological and genetic analysis to understand its zoonotic potential.

Materials and Methods

In collaboration with various veterinary facilities in the Veneto region, faecal samples from owned dogs and cats, both asymptomatic and with gastrointestinal symptoms, have been tested for MRV using an ad hoc real-time RT-PCR (rRT- PCR). The analytical panel for differential diagnoses includes Rotavirus (rRT-PCR), Parvovirus (rPCR), Coronavirus (rRT- PCR), and a qualitative coprological examination. Epidemiological data were collected by veterinarians during sampling. All MRV-positive samples will be subjected to viral isolation in cell cultures and subsequent characterization through NGS sequencing.

Sample ID	Province	rRT-PCR results MRV	rRT-PCR results RVA	rPCR results CPV-2	rRT-PCR results CCoV	QLCOPR	Symptoms
1D	Vicenza	Negative	Negative	Negative	Positive	Negative	Diarrhea, Fever
2D	Vicenza	Negative	Negative	Negative	Negative	Positive - Coccids	Diarrhea
3D	Vicenza	Negative	Negative	Negative	Positive	Negative	None
4D	Vicenza	Negative	Negative	Negative	Negative	Positive - Coccids	Diarrhea
5D	Vicenza	Negative	Negative	Negative	Negative	Positive - Coccids	None
6D	Padova	Negative	Negative	Negative	Positive	Positive - Coccids	Diarrhea
7D	Vicenza	Negative	Negative	Negative	Negative	Positive - Coccids	Diarrhea
8D	Vicenza	Negative	Negative	Negative	Positive	Negative	Diarrhea
9D	Vicenza	Negative	Negative	Negative	Negative	Positive - Trichuris	Diarrhea
10D	Vicenza	Negative	Negative	Negative	Negative	Positive - Ascarids	Diarrhea
11D	Verona	Negative	Negative	Negative	Negative	Positive - Coccids	Diarrhea
12D	Padova	Negative	Negative	Positive	Negative	Negative	None
13D	Padova	Negative	Negative	Negative	Positive	Negative	None

Legend: **MRV**: Mammalian orthoreovirus, **RVA**: Rotavirus A, **CPV-2**: Canine parvovirus type 2, **CCoV**: Canine coronavirus, **QLCOPR**: Qualitative coprological examination.

Table 1. Positive results from real-time PCR and qualitative coprological examinations of analyzed dog faecal samples. For each sample (Sample ID), the province of origin and the symptoms of the sampled animal are listed.

Sample ID	Province	rRT-PCR results MRV	rRT-PCR results RVA	rPCR results FPV	rRT-PCR results FCoV	QLCOPR	Symptoms
1C	Vicenza	Negative	Negative	Negative	Positive	Negative	Diarrhea
2C	Vicenza	Negative	Negative	Negative	Negative	Positive - COCCIDS, ASCARIDS	Diarrhea
3C	Ferrara	Negative	Negative	Positive	Positive	Negative	Vomiting
4C	Vicenza	Negative	Negative	Negative	Positive	Negative	Diarrhea
5C	Rovigo	Negative	Negative	Negative	Positive	Negative	Diarrhea
6C	Rovigo	Negative	Negative	Negative	Positive	Negative	Vomiting, Diarrhea
7C	Padova	Negative	Negative	Negative	Positive	Negative	None
8C	Padova	Negative	Negative	Negative	Positive	Negative	None
9C	Padova	Negative	Negative	Negative	Positive	Negative	None
10C	Padova	Negative	Negative	Negative	Positive	Negative	None
11C	Padova	Negative	Negative	Negative	Positive	Positive - ASCARIDS	None
12C	Padova	Negative	Negative	Negative	Positive	Negative	None
13C	Padova	Negative	Negative	Negative	Positive	Negative	None
14C	Padova	Negative	Negative	Negative	Positive	Negative	Polyphagia, Weight loss
15C	Rovigo	Negative	Negative	Negative	Positive	Negative	None
16C	Padova	Negative	Negative	Negative	Positive	Negative	None
17C	Padova	Negative	Negative	Negative	Positive	Negative	None
18C	Padova	Negative	Negative	Negative	Neg	Positive - ASCARIDS	Diarrhea
19C	Padova	Negative	Negative	Negative	Positive	Negative	None
20C	Vicenza	Negative	Negative	Negative	Positive	Negative	None
21C	Padova	Negative	Negative	Negative	Positive	Negative	None
22C	Padova	Negative	Negative	Negative	Positive	Negative	None
23C	Venezia	Negative	Negative	Negative	Positive	Negative	None

Legend: **MRV**: Mammalian orthoreovirus, **RVA**: Rotavirus A, **FPV**: Feline parvovirus, **FCoV**: Feline coronavirus, **QLCOPR**: Qualitative coprological examination.

Table 2. Positive results from real-time PCR and qualitative coprological examinations of analyzed cat faecal samples. For each sample (Sample ID), the province of origin and the symptoms of the sampled animal are listed.

Results

To date, 104 dogs and 85 cats have been tested, and none of them resulted positive for MRV. Table 1 and 2 show the positive results obtained in differential analyses for dogs and cats, respectively.

Discussion and Conclusion

Sampling is ongoing and we expect to add some new MRV strains to our previous collection. The strains will be characterized and compared with human MRVs to assess the zoonotic risk. Given the increasing presence of pets in households and the interest in Animal-Assisted Interventions for children and vulnerable people, surveillance for new potential zoonoses is recommended.

FUNDING

Ministero della Salute, Ricerca Finalizzata 2021 (SG-2021-12374649).

References

Decaro N et al., 2005. DOI: 10.1016/j.vetmic.2005.05.014
Rosa U A et al., 2019. DOI: 10.1038/s41598-019-55216-5
Mikuletic T et al., 2019. DOI: 10.1016/j.meegid.2019.06.002
Yamamoto S P et al., 2020. DOI: 10.1038/s41598-020-58003-9

P114

Emerging and re-emerging diseases

NON TOXIGENIC *CORYNEBACTERIUM ULCERANS* IDENTIFIED IN A DOG

M. Cocchi[2], G. De Zan[2], N. Genero[2], M. Monaco[1], G. Errico[1], S. Giancristofaro[1], M.C. Riva[3], F. Agnoletti[2]

[1]*Istituto Superiore di Sanita, Italy*
[2]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*
[3]*Veterinary Clinic Best is Udine, Italy*

Introduction

In dogs, *Corynebacterium* (C.) *ulcerans* is a commensal of the mucous membranes but is able to cause upper respiratory and skin infections. Phylogenetically, it is related to *C. diphtheriae*, the etiological agent of human diphteria, a rare but potentially fatal disease. Recently, diphtheria caused by toxigenic *C. ulcerans* is increasingly reported across Europe, and it is often ascribed to a zoonotic trasmission, mainly involving pets. In this study, the identification of *C. ulcerans* from an ulcerative lesion in a dog is presented.

Materials and Methods
A sample, obtained from an ulcerative nasal lesion, was submitted for routine culture. The isolate was identified by MALDI-TOF (MALDI Biotyper® Sirius System, Bruker) and tested for the susceptibility to antimicrobials, assessing the minimum inhibitory concentration (MIC). To confirm species identification and the presence of the diphtheria toxin gene, Real-time PCR was performed at the National Reference Laboratory for Diphtheria (Istituto Superiore di Sanità). API-Coryne® test system was also used to identify phenotipically the species.

Results
After 24 hours incubation, a growth suggestive of coryneform bacteria associated with rare colonies resembling *Streptococci* were identified as *C. ulcerans* and *S. canis*, respectevely. *C. ulcerans* was susceptible to all the tested molecules, except penicillin and clindamycin, showing an intermediate MIC value. The isolate was confirmed as non toxigenic *C. ulcerans*.

Discussion and Conclusion
This report describes the identification of a non toxigenic *C. ulcerans* strain in a dog, affected by an ulcerative lesion in a nostril. This bacterium is considered an emerging zoonotic pathogen, however, both the data about the role of pet in the transmission and the diffusion of this pathogen are scarce. In Italy, Monaco et al. (2017) reported a respiratory diphtheria case transmitted by a companion dog, suffering from mouth ulcers. Carfora et al. (2018) described the identification of non toxigenic strains from dogs with skin lesions; one of the isolates was resistant to clindamycin. The characterization of *C. ulcerans* allows the evaluation of its potential risk of transmission to human, providing also useful information for an accurate managment of this pathogen.

P115

Emerging and re-emerging diseases

A NEW PEN-SIDE TEST TO CONFIRM RVF CLINICAL SUSPICIONS IN THE FIELD.

L. Comtet[2], A. Limozin[2], F. Donnet[2], A. Pedarrieu[1], C. Cetre-Sossah[1], P. Pourquier[2]

[1]*CIRAD, INRAE, UMR ASTRE, University of Montpellier, France*
[2]*Innovative Diagnostics, France*

Introduction
Rift Valley fever (RVF) is a zoonotic illness causing an acute infection in domestic ruminants, with a high mortality rate among young animals. This mosquito-borne disease also affects wild animals and humans. As a consequence, a correct identification of infected animals has three purposes: reduce the impacts of the disease on the livestock market, identify areas where the risk of human infection is the highest as well as decrease the number of human cases. In the One health perspective, fast and reliable tools are needed to rapidly diagnose suspected clinical cases.
Diagnostic performances of a new pen-side test to detect RVFV in less than 15 minutes: the ID Rapid® Rift Valley fever is presented here.

Materials and Methods
Diagnostic specificity was assessed on 580 negative sera (292 cattle, 96 small ruminants and 192 camelids) and 184 heparinized whole blood (cattle) from RVF-free areas. Analytical sensitivity was evaluated using a titrated RVFV strain (Smithburn) spiked in negative whole blood or serum (bovine and caprine). Diagnostic sensitivity on serum of a previous similar test with the same limit of detection (LOD) was assessed with:
- 25 isolated strains from different geographical origins mimicking clinical specimens
- 10 RT-qPCR-positive clinical samples from the 2019 Mayotte outbreak (Bird et al., 2007

Results
Measured specificity was:
- Cattle sera: 99.7% [98.1-99.9]
- Small ruminant sera: 99.0% [94.3-99.1]
- Camelids sera: 100% [98.0-100]
- Bovine whole blood: 98.9% [96.1-99.7]

Diagnostic sensitivity was 100% [90,1-100]. Inclusivity, tested on different RVFV viral strains, is 100%. Exclusivity documented with flaviviruses and alphaviruses rules out cross-reactivity with clinically similar viruses. LOD on serum/ whole blood was respectively 3.5x103 pfu and 7.5x104 pfu.

Discussion and Conclusion
The ID Rapid® Rift Valley fever antigen demonstrates very high levels of specificity and inclusivity, and a sensitivity level adapted to a specific, accurate and rapid detection of RVFV in serum or whole blood in ruminants. It is a reliable tool able to confirm a RVF clinical suspicion and enhance virus detection during an outbreak in less than 15 minutes directly on the field, without any specific lab equipment.

References
Rift Valley fever, pen-side test, antigen detection

P116

Emerging and re-emerging diseases

NEWLY UPDATED RT-QPCR FOR AN EFFICIENT BLUETONGUE VIRUS TYPE 8 AND 4 DETECTION, INCLUDING BTV-8 STRAINS REPORTED IN FRANCE AND ITALY IN 2023

L. Comtet[1], L. Despois[1], A. Limozin[1], P. Pourquier[1]

[1]*Innovative Diagnostics, France*

Introduction

Bluetongue virus (BTV), is responsible for bluetongue (BT), one of the WOAH-listed major diseases of ruminants. Since 1998, serotype 1, 2, 3, 4, 6, 8, 9, 11, and 16 have been reported in Europe. During summer 2023, several clinical cases of bluetongue serotype 8 (BTV-8) occurred in cattle and sheep in France and Italy. More clinical signs were observed during that BTV-8 emergence, and the French NRL confirmed the presence of new BTV-8 variants, which were either giving bad PCR signals or not detected by the ID Gene™ Bluetongue genotypes 8 and 4 Triplex. Therefore, Innovative Diagnostics updated its diagnostic tool by modifying the BTV-8 target of the latter kit to allow proper detection of the newly identified BTV-8. This study summarizes validation data of this updated ID GeneTM 2.0 kit.

Materials and Methods

Diagnostic specificity was assessed on 115 negative whole blood samples from cattle, from France, confirmed negative with the ID Gene™ Bluetongue Duplex RT-qPCR. Diagnostic sensitivity was tested on 45 BTV-8 and 15 BTV-4 positive samples. Inclusivity was tested on a panel of BTV-4 and BTV-8 isolates, including the new BTV-8 strains detected in 2023 provided by the French NRL for BTV&EHDV. Exclusivity with respect to other BTV serotypes and to EHDV serotypes was assessed on 38 BTV isolates provided by the French NRL and the FLI (Germany) and on 20 EHDV isolates from the French NRL and The Pirbright Institute (TPI, UK). The WOAH Reference Laboratory for BTV of Teramo (Italy) also conducted a trial of the kit.

Results

The ID GeneTM 2.0 measured specificity was 100 %, [96.8-100], n=115. Measured diagnostic sensitivity with respect to BTV-8 and BTV-4 was respectively 100%, [92-100], n=45 and 100%, [80-100], n=15. All BTV-8 strains tested, including the strains detected in France and Italy in 2023, were efficiently detected by the updated ID GeneTM kit, giving a perfect inclusivity. All other BTV serotypes as well as all EHDV strains included in the panels tested were not detected, showing the ID GeneTM kit's excellent excluvisity.

Discussion and Conclusion

ID GeneTM Bluetongue genotype 8 and 4 Triplex 2.0 offers a specific and exclusive detection of bluetongue type 8 and 4, including the BTV-8 strains reported in France and Italy in 2023.

P117

Emerging and re-emerging diseases

A NEW RT-QPCR KIT ENABLING A SPECIFIC DETECTION OF BLUETONGUE VIRUS SEROTYPE 3

L. Comtet[1], L. Despois[1], A. Polat[1], A. Limozin[1], P. Pourquier[1]

[1]*Innovative Diagnostics, France*

Introduction

Bluetongue (BT), caused by the bluetongue virus (BTV), is one of the WOAH-listed major diseases of ruminants. In september 2023, new BTV-3 outbreaks were reported in sheep farms in The Netherlands. These events were rapidly followed by diffusion to Belgium, Germany and United Kingdom. Consequently, Innovative Diagnostics has developed a new molecular diagnostic tool, the ID GeneTM Bluetongue genotype 3 Duplex. It allows specific and exclusive detection of BTV serotype 3, including the recent BTV-3 strain detected in the aformentionned countries (BTV-3/NET-2023), in blood, spleen and organs from aborted animals (spleen, heart and liver). This study present the validation and performance of this new kit.

Materials and Methods

Diagnostic specificity was assessed on 115 negative whole blood samples from cattle, from France, confirmed negative with ID Gene™ Bluetongue Duplex all serotypes RT-qPCR. Diagnostic sensititivy was tested on 8 BTV-3 positive samples : 5 samples which were tested positive by the French NRL, and 3 RNAs extracted from sheep whole blood samples collected in Belgium. Samples were kindly provided by the respective NRLs. Inclusivity was tested on a panel of BTV-3, including the BTV-3/NET-2023 strains. Exclusivity with respect to other BTV serotypes and to EHDV serotypes was assessed on 3 panels : 38 BTV isolates (French NRL for BTV&EHDV ; FLI, Germany) and on 20 EHDV isolates (French NRL for BTV&EHDV ; The Pirbright Institute, UK)

Results
The ID GeneTM kit measured specificity was 100%, [96.8-100], n=115. Measured diagnostic sensitivity was 100%, [67.6-100], n=8. All BTV-3 strains tested, including BTV-3/NET-2023, were efficiently detected. All other BTV serotypes and all EHDV strains tested were not detected, showing the ID GeneTM kit's excellent exclusivity.

Discussion and Conclusion
The ID GeneTM Bluetongue genotype 3 Duplex kit offers a specific detection of BTV-3, including the BTV-3 strains detected in western Europe in 2023. Results can be obtained in 50 min (rapid amplification program), compatible with all IDGeneTM kits, enabling to test on the same run for different Orbiviruses RT-qPCRs, therefore offering maximum flexibility & testing capacity by optimizing lab equipments ressources.

P118

Emerging and re-emerging diseases

PRELIMINARY SEROLOGICAL AND MOLECULAR EVIDENCES OF INFLUENZA VIRUS IN WILD BOARS IN SOUTHERN ITALY

A.M. D'Antuono[4], N. Cavaliere[3], L. Palazzo[3], D. Galante[3], C. Ortello[5], F. Ferringo[3], A.C. Romano[3], M.T. Toce[3], G. Cascini[2], F. Stefania[1], D.A. Raele[3]

[1]*Azienda Sanitaria Locale di Foggia, Italy*
[2]*Azienda Sanitaria Locale di Potenza, Italy*
[3]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*
[4]*PhD National Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy*
[5]*Università degli Studi di Pavia, Italy*

Introduction
Influenza A viruses (IAV) are of particular concern to human and animal public health. Pigs have both human and avian IAV receptors; therefore, swine could be infected by multiple-origin IAV strains and play a role in the generation of potential new pandemic IAV strains[1]. Due to the role of suids as IAV reassortment species, it is essential develop surveillance also on wild animals such as wild boars to monitor the variability of this virus and the possibility of spill-over events[1].

Materials and Methods
A total 191 wild boars were investigated. Swabs, lungs and muscles were collected from each animal during the hunting period (February to May 2024) in the Apulia, Basilicata and Campania regions. Viral RNA amplification was performed using a Reverse Transcriptase Real Time PCR based on the segment 7 of the AIV genome, coding for the M matrix protein. The M gene is a common gene to all types of influenza A. Lungs were homogenized and swabs diluted in 1ml of PBS and then extracted by a commercial kit. For serological screening the meat juice of each wild boar was extracted from muscles and subsequently tested by an indirect ELISA kit for the detection of antibodies to type A influenza.

Results
Out of 191 wild boards, 5 tested positive for the presence of M gene by rt-qPCR (Table 1). A total of 22/191 (11.5%) of meat juices tested positive for influenza A antibodies by ELISA. Out of 22 sera, 8 have been confirmed as H1 subtype by the National Reference Centre for Avian Influenza (Table 2).

Discussion and Conclusion
This study has allowed to collect information on the circulation of AIV in the wild boar population in Southern Italy for the first time. Further molecular analysis will allow to identify the genotypes of the viruses to better understand the variability with IAV circulating. Serological monitoring has been valuable to better identify circulating subtypes and may be useful for the identification of past infections. The monitoring of AIV in wild animals and the collection of information related to the variability of the virus is the first step to better define profiles of zoonotic viruses in the wild fauna context.

References
Chiapponi C. et al. Genetic Variability among Swine Influenza Viruses in Italy: Data Analysis of the Period 2017-2020.Viruses.2021 Dec 28;14(1):47. doi: 10.3390/v14010047

Figure 1. Map of sampling regions. The areas from which the tested samples originated are marked in light blue.

SAMPLING PERIOD	ORIGIN	SEX	WEIGHT (Kg)
18/02/2024	Castelluccio Inferiore (PZ)	F	44
04/04/2024	Vietri (PZ)	M	52
04/04/2024	Castelluccio Inferiore (PZ)	F	52
11/04/2024	Tricarico (MT)	F	60
21/05/2024	Rignano Garganico (FG)	M	90

Table 1. Details of samples tested positive for the presence of M gene by rt-qPCR

SAMPLING PERIOD	ORIGIN	SEX	WEIGHT (Kg)
06/03/2024	Savoia di L. Pz (PZ)	F	76
14/03/2024	Muro Lucano (PZ)	F	69
14/03/2024	Vietri (PZ)	M	86
14/03/2024	Felitto Sa. (SA)	M	71
14/03/2024	Muro Lucano (PZ)	F	57
14/03/2024	Muro Lucano (PZ)	F	41
14/03/2024	Castelluccio Inferiore (PZ)	F	67

Table 2. Particulars of samples tested positive for influenza A antibodies by ELISA

P119

Emerging and re-emerging diseases

A NEW HIGHLY PERFORMANT COMPETITIVE ELISA FOR THE DETECTION OF *BESNOITIA BESNOITI* ANTIBODIES IN CATTLE

A. Greatrex[1], L. Olagnon[1], L. Comtet[1], A. Limozin[1], P. Pourquier[1]

[1]*Innovative Diagnostics, France*

Introduction
Besnoitiosis is a vector-borne disease due to an apicomplexan parasite, *Besnoitia besnoiti* (Bb), causing economic losses and increasing mortality. As identification of seropositive animals is a key to control the disease, serological tools such as ELISA, western blot (WB) or IFAT play a crucial role for Bb diagnosis. Most of the commercially available solutions are indirect ELISAs. To improve Bb serological diagnosis, Innovative Diagnostics has generated several monoclonal antibodies and the most promising one was used to develop a new blocking ELISA (cELISA).

Materials and Methods
Diagnostic specificity was assessed on 500 serum samples from bovine besnoitiosis-free herds with no history of the disease and/or consistently negative serological result. Diagnostic sensitivity was evaluated on 200 French cattle sera samples which positive status was confirmed by WB. To check the exclusivity with respect to other Apicomplexan, 10 and 5 cattle sera (France) having a seropositive status confirmed by the ID Screen® *Neospora caninum* Indirect and the ID Screen® Toxoplasmosis Indirect, respectively, were tested with the ID Screen® Besnoitia Competition. Repeatability, stability, robustness and interlaboratory reproducibility were also evaluated.

Results
Measured specificity was 100.0 %, [99.2-100]. Measured sensitivity was 100.0 % [98.1 -100]. The percentage of correlation with the WB was 100%, indicating a perfect agreement. All *Neospora caninum* or *Toxoplasma gondii* seropositive samples were found negative with the cELISA, indicating good exclusivity. Repeatability, stability and robustness were validated. The inter-laboratory reproducibility was good, with coefficients of variation inferior to 15%.

Discussion and Conclusion
The ID Screen® kit efficiently detects positive animals, demonstrates excellent specificity and excellent correlation with the WB. Furthermore, the kit performances comply with the requirements of the French Expert Laboratory for Besnoitiosis (ANSES). The new ID Screen® kit is a reliable tool for the detection of cattle antibodies directed against *Besnoitia besnoiti.* This new kit will reinforce IDvet's unique expertise in Besnoitiosis diagnostic, offering the most complete range, with kits for bulk tank milk surveillance, milk or serum testing.

P120

Emerging and re-emerging diseases

IDENTIFICATION OF A NOVEL HUMAN PARVOVIRUS B19-LIKE (ERYTHROPARVOVIRUS) IN CATS

G. Lanave[1], F. Pellegrini[1], G. Diakoudi[1], C. Cristiana[1], A. Cavalli[1], P. Capozza[1], G. Elia[1], B. Di Martino[3], E. Zini[2], N. Decaro[1], M. Camero[1], V. Martella[1]

[1]*University of Bari Aldo Moro, Department of Veterinary Medicine, Bari, Italy*
[2]*University of Padua, Department of Animal Medicine, Productions and Health*
[3]*University of Teramo, Department of Veterinary Medicine, Teramo, Italy*

Introduction
Erythroparvoviruses (EPVs) have been identified in humans (parvovirus B19), nonhuman primates, seals, squirrels, and cows 1. In healthy immunocompetent children, parvovirus B19 causes infectious erythema (fifth disease), while in adults it can be associated with acute polyarthropathy. In this study, we report the identification of a novel EPV in the sera of cats.

Materials and Methods
Two feral colony cats were presented for weakness, weight loss, and anorexia and tested positive for feline leukemia virus and domestic cat hepadnavirus. The animals were part of a study aimed at the investigation of hepatotropic pathogens in cats and the inclusion criterium was the presence of altered liver markers. Metagenomic investigations were carried out using sequence-independent single primer amplification enrichment protocols on Oxford Nanopore Technologies sequencing platform. A specific quantitative PCR (qPCR) assay was designed and optimized testing the sensitivity and specificity.

Results

On metaviromic investigation, parvoviral reads were identified in the sera of the two cats. The feline EPV (FeEPV) genome was 5.3 kb long and displayed two main open reading frames (ORF) and inverted terminal repeats. In the ORF1 (nonstructural proteins) and ORF2 (VP1/VP2 precursor) the feline virus had 44.3% and 48.1% nt identity to human parvovirus B19. On phylogenetic analysis, the FeEPV clustered with chipmunk parvovirus (Figure 1). The two FeEPV strains were highly related (>99.9% nt identity) to each other, based on genome comparison. Using the qPCR assay, the virus load in the serum of cat A (4.9x1010 genome equivalent [GE]/ml) and of cat B (1.7x104 GE/ml) was quantified. The virus was also identified in the sera of an additional 10 cats (prevalence 27.6%, 12/43) presenting altered hepatic markers with viral lower loads (1.73x101 - 4.78x102 GE/ml).

Discussion and Conclusion

The findings of our study extend the list of known parvoviruses in the feline host. Since EPVs have been associated with a broad range of clinical signs in human and non-human primates, alone or in conjunction with immuno-suppressive pathogens/conditions 2, it will be important to investigate if FeEPV has a pathogenic role in cats.

References

1. Jager, et al. 2021 doi: 10.1186/s12985-021-01677-y.
2. Landry et al., 2016 doi: 10.1128/microbiolspec.DMIH2-0008-2015.

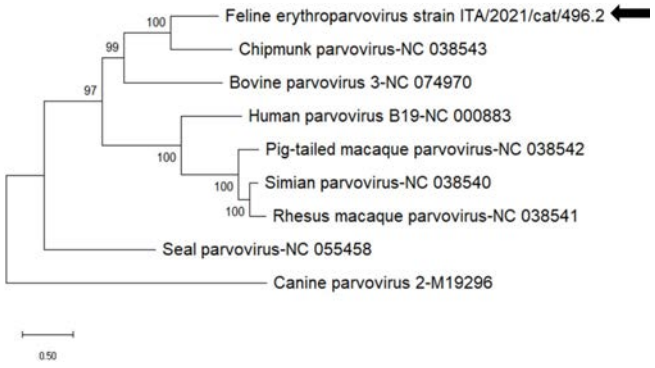


Figure 1. Full-genome-based unrooted phylogenetic tree of Feline Erythroparvovirus identified in this study and reference strains recovered in the GenBank database. The Maximum Likelihood method and LG model with a gamma distribution and invariable sites were used for the phylogeny. A total of 1000 bootstrap replicates were used to estimate the robustness of the individual nodes on the phylogenetic tree. Bootstrap values greater than 75% were indicated. Black arrows indicate strain detected in this study. Numbers of aminoacid substitutions are indicated by the scale bar.

P121

Emerging and re-emerging diseases

A RETROSPECTIVE STUDY OF THE PREVALENCE IN EQUINE POSTMORTEMS OF CRANIAL MESENTERIC ARTERITIS CAUSED BY *STRONGYLUS VULGARIS* IN ALBERTA (2010 TO 2022)

K. Domshy[1], A. Whitehead[1], J. Poissant[1], D. Goldsmith[1], C. Legge[1], C. Knight[1], E. Zachar[1], S. Loch[1], J. Davies[1]

[1]*University of Calgary, Canada*

Introduction

Strongylus vulgaris is one of the most pathogenic nematodes affecting equids. Larval migration through the cranial mesenteric artery (CMA) with attendant arteritis and thromboembolism can result in fatal non-strangulating intestinal infarction. Once considered a historical disease, recent studies have described the reemergence of this pathogen in several European countries; however, little is known of the current prevalence of *S. vulgaris* in the Canadian horse population. The objective was to determine the prevalence of active *S. vulgaris* cranial mesenteric arteritis in horses submitted for postmortem examination to the Diagnostic Services Unit (DSU) at the University of Calgary Faculty of Veterinary Medicine.

Materials and Methods

We conducted a retrospective review of all equine postmortem cases submitted to the DSU between July 1, 2010 and June 30, 2022. Over

the 12 year 510 horses > 2 months of age from Alberta were submitted to the DSU for necropsy. Active cases were defined as those with endarteritis and thrombosis in the CMA or its branches. Those cases with only intimal scarring of the CMA were classified as historical.

Results

The prevalence of all CMA lesions (both historical and active) over the study period was 88/510 (17.3%). Active *S. vulgaris* cranial mesenteric arteritis was documented in 6.1% (31/510) of equine postmortems and the sequelae of verminous arteritis were the cause of euthanasia or death in 1.5% (8/510) of the cases submitted.

Discussion and Conclusion

Even after historically intense efforts to eradicate this parasite, the continued effects of *S. vulgaris* are demonstrated by the results of this study. *Strongylus vulgaris* should not be regarded as a parasite of the past and verminous arteritis remains an important differential diagnosis for horses in western Canada presenting with mild colic or dull demeanor and anorexia of duration > 24 hours. Furthermore, *S. vulgaris* should be taken into careful consideration when implementing antiparasitic control strategies. Practitioners should remain current on prevention, diagnosis and treatment of this potentially reemerging and fatal equine disease.

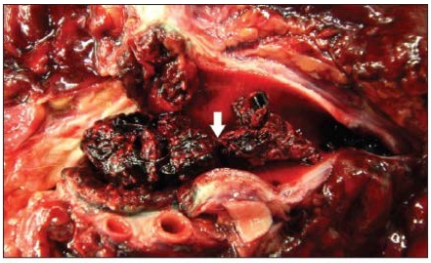


Figure 1. Cranial mesenteric artery from a horse. Visible are endarteritis characterized by thickening of the arterial wall, roughening of the tunica intima, and a large intraluminal thrombus (arrow).

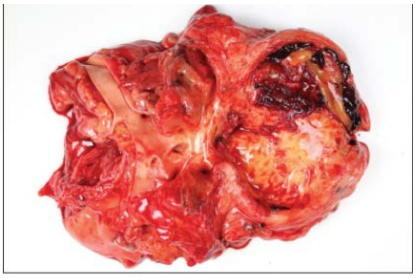


Figure 2. Cranial mesenteric artery from a horse. In addition to endarteritis and thrombosis, there is marked aneurysmal dilation of the cranial mesenteric artery.

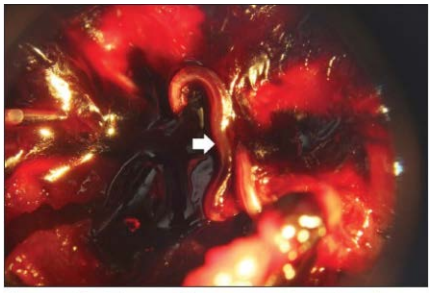


Figure 3. Cranial mesenteric artery from a horse, viewed through a dissecting microscope. Visible are 2 slender, larval nematodes embedded within the thrombus (arrow).

P122

Emerging and re-emerging diseases

GENETIC VARIABILITY OF INFLUENZA A VIRUSES IN SWINE IN NORTHERN ITALY

A. Mescoli[1], A. Prosperi[1], L. Soliani[1], L. Baioni[1], I. Zanni[1], I. Calanchi[1], C. Torreggiani[1], A.M. Moreno Martin[1], L.G. Alborali[1], S. Faccini[1], C. Rosignoli[1], P. Bonilauri[1], G. Pupillo[1], L. Fiorentini[1], G. Tosi[1], A. Luppi[1], C. Chiapponi[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER), Brescia, Italy - WOAH Reference Laboratory for swine influenza, Italy*

Introduction

Influenza A virus in swine (IAV-S) infections have shifted from acute to enzootic/epizootic forms. In Europe, the genetic and antigenic variability of IAV-S (H1N1, H1N2, H3N2, H1N1pdm09) has increased. In Italy, circulating strains have hemagglutinins H1 (1A, pandemic; 1B, human-like; 1C, avian-like) and H3, as well as neuraminidase genes N1 (avian- like or pandemic) and N2 (1).

Materials and Methods

From 2022 to 2024, testing for IAV-S was conducted on diagnostic samples from pig farms in Northern Italy. RT-PCR was used to detect IAV-S, followed by molecular typing for HA and NA lineages and full genome sequencing.

Results

313 samples were fully typed; among them, H1CN2 was the most frequent (42%), followed by H1CN1 (23%), H1AN1 (16%), and H3N2 (6%). Sequencing provided genetic characterization, including HA lineage and internal gene combinations. It revealed high variability, especially within the H1N2 subtype, with 7 HA clades, leading to 18 genotypes. H1N1 had 5 HA clades, resulting in 13 genotypes. H3N2 was the most stable with 2 genotypes. The 1C clade showed great variability with the detection of 4 different sub-clades.

Discussion and Conclusion

The 1C.2.2 sub-clade, prevalent in 2022, was replaced by 1C.2.4 in 2023, continuing into 2024. 1B, mainly 1B.1.2.2, unique to Italy, is now almost absent. 1A.3.3.2 circulated as a new swine-adapted reassortant sub-clade (2). H3N2 was stable genotypically but had no occurrences in early 2024. The diversity of H1 lineages complicates subtype classification, highlighting the importance of genomic sequencing. Given the active circulation of influenza viruses among swine, the persistence of endemic strains, and the introduction of new viruses from regions with high-density of swine farms, systematic epidemiological surveillance within swine populations is crucial. Continuous monitoring and a One Health approach are essential to protect animal and human health against potential pandemics.

This research was supported by Ministry of Health IZSLER RC 2020005 and by PNRR INFACT_NODE_1 project IZSLER-Parma, One Health Basic and Translational Research Actions addressing Unmet Needs on Emerging Infectious Diseases.

References

1. doi:10.1128/mSphere.00275-16
2. doi:10.3390/v14010047

P123

Emerging and re-emerging diseases

EVALUATING DIAGNOSTIC PERFORMANCE: INTER-LABORATORY COMPARISON TESTS (ILCT) FOR AFRICAN SWINE FEVER (ASF) IN THE EUROPEAN UNION AND THIRD COUNTRIES FROM 2014 TO 2024

R. Nieto[1], A. Soler[1], C. Pérez[1], A. Simón[1], N. Casado[1], M. GarcÍa[1], C. Gómez[1], M. Arias[1], C. Gallardo[1]

[1]*European Union Reference Laboratory for ASF (EURL-ASF): Centro de investigación en sanidad animal (CISA-INIA, CSIC), Valdeolmos, Madrid, Spain*

Introduction

ASF is a priority disease for the EU. The EURL-ASF organizes since 2003 an annual ILCT to evaluate the performance of national reference laboratories (NRLs) in the EU Member States (MS) and third bordering countries to identify potential gaps and needs for harmonization in the participating countries. This study evaluates the ILCTs's results since 2014 up to 2024 to ensure the ASF diagnostic confidence of NRLs.

Materials and Methods

Each ILCT panel included an average of 20 samples from pigs infected at the EURL BSL3 facilities with various ASFV genotypes, to resemble different scenarios. From 2014 to 2024, 37 NRLs have participated from the EU MS and non- EU countries. The EURL analyzed the number, types and performance of tests used for antibody and virus detection, and the final diagnostic conclusion for each sample.

Results

Since 2014, all EU MS NRLs have participated in the annual ILCTs. Real-time PCR is the preferred method for virus detection, with WOAH UPL real-time PCR (58%) and commercial kits (42%) being the most widely used. For antibody detection, the ELISA ®INGEZIM PPA COMPAC K3 and the indirect immunoperoxidase technique (IPT) are the primary methods. In 2024 all EU MS NRLs successfully diagnosed ASF in all samples, demonstrating high competence in ASFV diagnosis in the EU.

Discussion and Conclusion

The EU MS NRLs participation in the ILCTs since 2014 underlines their commitment to maintaining high diagnostic standards for ASF. The results demonstrate the robustness of ASF diagnostic methods within the EU, the effectiveness of continuous evaluation through the ILCTs, and the ability of NRLs to implement advanced diagnostic techniques. This high level of diagnostic competence is crucial for the control and management ASF outbreaks, ensuring rapid responses to the ongoing epidemic. This work has been funded by the EURL-ASF (EC contract 2014-2024, DG SANTÉ, EU). With the expertise of the EURL team and the collaboration of the GF-TADs and the FAO Reference Centre for ASF, the EURL established since 2003, an ASF network with NRLs worldwide, working in the harmonisation of ASF diagnosis through the ILCTs.

P124

Emerging and re-emerging diseases

PREVALENCE AND GENETIC DIVERSITY OF KOBUVIRUS IN CATTLE FARMS

F. Pellegrini[1], F. Caringella[1], G. Diakoudi[1], B. Di Martino[2], M. Camero[2], G. Lanave[1], J. Matthijnssens[3], V. Martella[1]

[1]*Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy*
[2]*Faculty of Veterinary Medicine, University of Teramo, Teramo, Italy*
[3]*KU Leuven, Department of Microbiology, Immunology, & Transplantation, Rega Institute, Division of Clinical & Epidemiological Virology, Laboratory of Viral Metagenomics, Leuven, Belgium*

Introduction

The family *Picornaviridae* includes small, single-stranded non-enveloped RNA viruses. Among them, the genus *Kobuvirus* comprises *Aichivirus* (AiV) species A to F [1]. AiVs have been associated with severe enteritis in children and individuals with impaired immune systems [2]. These viruses have a broad host range of several mammalian species. In this study, the presence of KoVs in bovine farms in southern Italy was investigated.

Materials and Methods

The samples were collected between October 2019 and January 2020 from 6 farms in Taranto (n=5, Apulia) and Cosenza (n=1, Calabria) prefectures, Italy. In detail, 38 stool samples were collected from animals with enteric signs (n=17) and healthy calves (n=21). The age of the animals ranged from 20 days to 96 months. A KoV-specific RT-PCR was used for screening [3]. All samples were prepared using the NetoVIR protocol for viral enrichment [4] and sequenced on an Illumina platform (Illumina). The genome of AiV strains was completed using a primer walking strategy and sequenced on a MinION device (ONTTM).

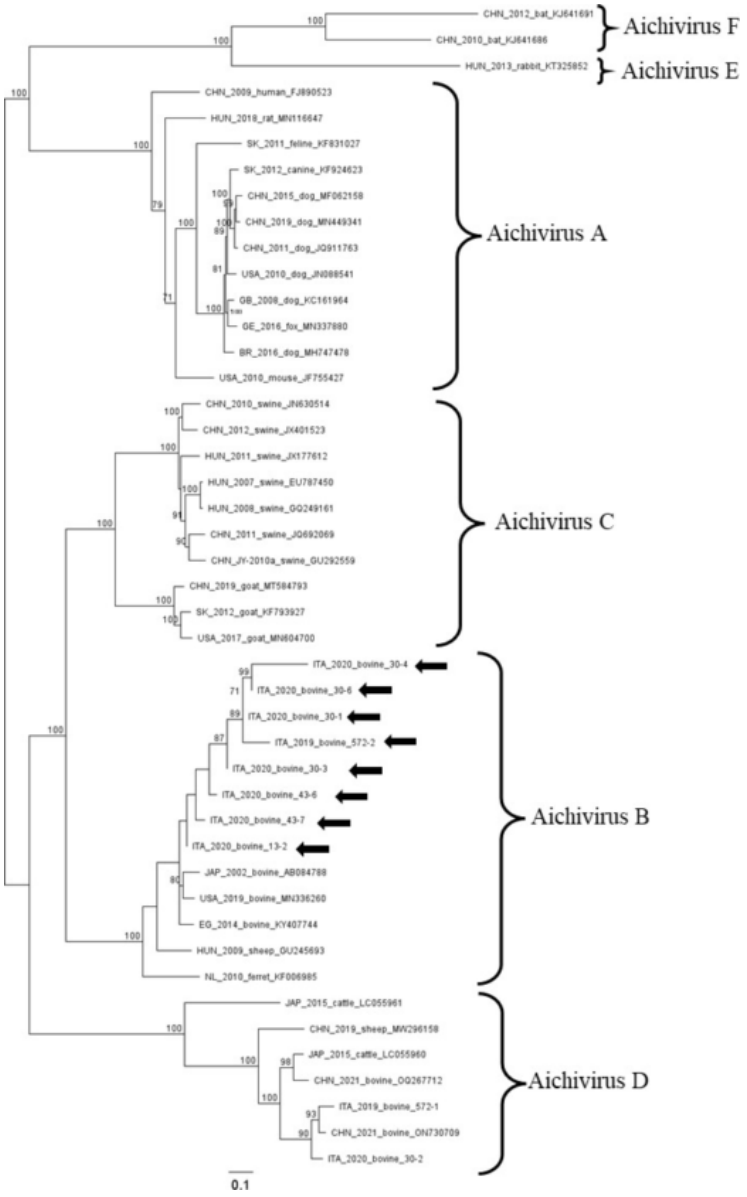


Figure 1. Phylogenetic reconstruction based on partial KoV sequences obtained in this study (arrows) and hit sequences obtained from the Genbank databases. Statistical support was determined using 1000 bootstrap replicates, with gamma distribution and invariant sites.

Results

KoV RNA was detected in 10 samples out of 38 (26.31%). On sequence analysis, eight strains were related to AiV B (94.2-97.7% nt). Two strains showed the highest identity (up to 97.1% nt) to AiV D strains (Figure 1). Six strains were from animals with enteric signs (6/17, 35.3%), including the two AiV D strains, while four were from healthy animals (4/21, 19%). KoV-specific reads (Illumina) were detected in 4 samples (10.5%)(mean=13,716.66; median=1,032.5). On phylogenetic analysis, strains ITA/2019/572-1 and ITA/2020/30-2 clustered with other AiV D from ruminants in Asia. The highest identity (87.7-89.6% nt) was to the Chinese bovine strain CHN/2021/ON730709 (Figure 2).

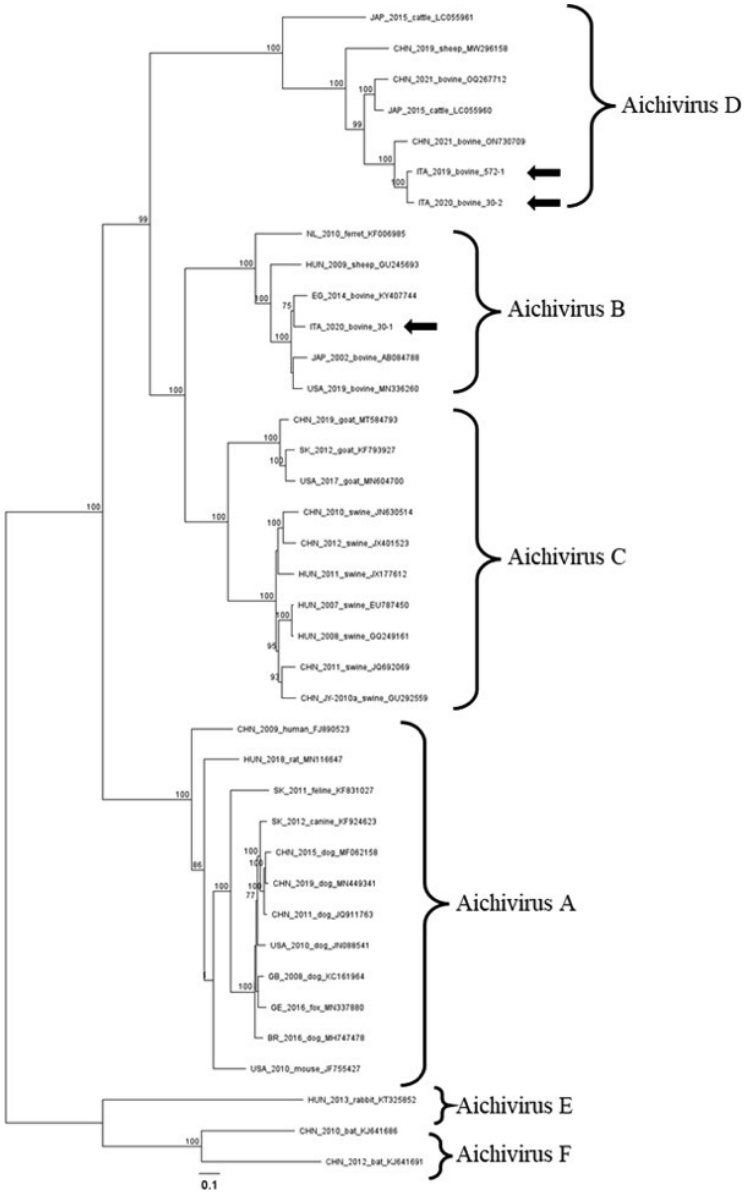


Figure 2. Phylogenetic reconstruction based on complete KoV ge2n9o5mes obtained in this study (arrows) and hit sequences obtained from the Genbank databases. Statistical support was determined using 1000 bootstrap replicates, with gamma distribution and invariant sites.

Discussion and Conclusion

KoVs have been detected in the stool of animals with and without enteric signs, suggesting that they are common components of the bovine enteric virome [5,6]. Understanding the genetic diversity of KoVs in animals will be useful to improve the diagnostics and fill up epidemiological gaps. Also, this will help understand if some peculiar KoV strains, may impact the health of livestock animals.

References

1. doi: 10.1002/rmv.677.
2. doi: 10.3201/eid2601.
3. doi: 10.1007/s00705-008-0288-2.
4. doi: 10.1007/978-1-4939-8682-8_7.
5. doi: 10.1007/s11259-024-10403-2.
6. doi: 10.1128/msystems.00842-23

P125

Emerging and re-emerging diseases

HIGH QUALITY GENETIC SEQUENCING AS A KEY TOOL FOR PATHOGEN SURVEILLANCE. PORCINE RESPIRATORY AND REPRODUCTIVE SYNDROME VIRUS (PRRSV) AS AN EXAMPLE

A. Sánchez Santos[1], L. Valls Vila[1], J. Maldonado García[1]

[1]*HIPRA S.A*

Introduction

Pathogen surveillance in veterinary medicine is crucial for monitoring and to streamline decision-making for better disease control. RNA viruses such as PRRS virus (PRRSV) display high mutation rates demanding fast interventions to control their spread. Modern genomic and bioinformatic tools provide rapid and accurate characterization of pathogens. A divergent PRRSV strain named "Rosalia" emerged in Spain in 2020, hardly impacting the local swine industry1. The aim of this study is to evidence the usefulness of sequencing tools in routine diagnostic laboratories for pathogen surveillance with PRRSV as an example.

Materials and Methods

In January 2020, respiratory tissues from PRRSV-affected animals in Spain were sent to DIAGNOS to confirm infection and characterize the strain involved. Samples were tested and analyzed by PRRSV qPCR and ORF5 sequencing2. A positive sample was subjected to virus isolation and whole genome (WGS) characterization by next generation sequencing (NGS). Subsequently, monitoring of animals with suspicion of Rosalia virus infection was performed (2020-2024) to see its genetic variation.

Results

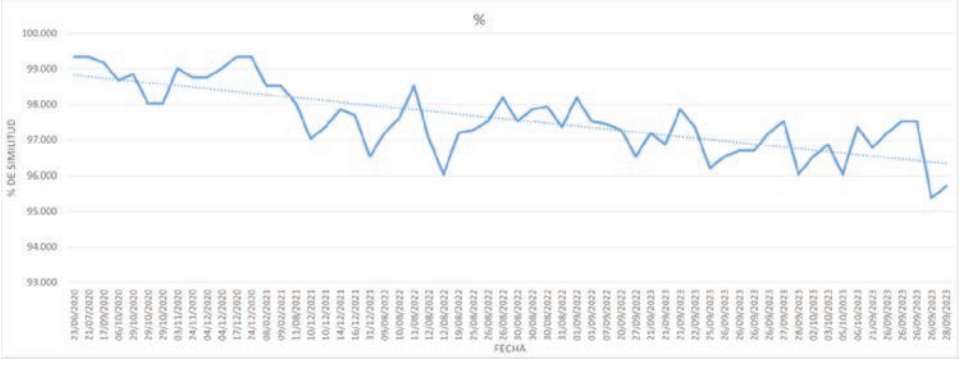
The ORF5 sequence obtained from the first Rosalia strain displayed <95% nucleotide identity against own and publicly available PRRSV sequences. WSG showed recombination events among multiple PRRSV strains from different origins1. A high percentage of the sequences analyzed from clinical samples were classified as Rosalia. Monitoring the evolution and genetic drift comparing with the first Rosalia strain was also assessed (Graphic 1).

Discussion and Conclusion

Effective pathogen surveillance relies on robust infrastructure, interdisciplinary collaboration, and the integration of diverse data sources. The combination of well-equipped genetic laboratories with continuous monitoring is key to understand the spread and the evolution of infectious agents. Aligned to this, HIPRA Diagnos is offering sequencing diagnostics for different pathologies and implementing NGS technologies to reach WGS. The continuous evolution of surveillance methods is essential for prevention.

References

1. Gerard E Martín-Valls et al. Microbiol Resour Announc. 2022.
2. Valls, L et al. Proceedings ESPHM 2019



Graphic 1. Rosalia ORF5 nucleotide identity (%) evolution along time.

P126

Emerging and re-emerging diseases

LONGITUDINAL STUDY MONITORING SWINE INFLUENZA CIRCULATION IN TWO SWINE FARMS IN NORTHERN ITALY AND POST-VACCINATION FOLLOW-UP

L. Soliani[2], A. Mescoli[2], L. Baioni[2], I. Zanni[2], A. De Mattia[2], A. Prosperi[2], C. Torreggiani[2], E. Pileri[1], G. Guadagnini[3], D. Ponzoni[3], A. Luppi[2], C. Chiapponi[2]

[1]*CEVA Animal Health, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER), Brescia, Italy - WOAH Reference Laboratory for swine influenza*
[3]*Swine practitioner, Italy*

Introduction
Swine influenza A viruses (IAV-S) impact the swine industry, leading to decreased productivity and also posing a potential threat to human health, urging to monitor viral spread to implement effective control measures(1,2). Given that, a longitudinal study was performed in 2 swine farms in Northern Italy. During the first sampling, the farms did not vaccinate, while in the follow-up both farms implemented IAV-S vaccination. The study's aim was to provide insights into infections patterns and to determine IAV-S prevalence in different age groups of piglets.

Materials and Methods
In both farms, 2 batches of piglets were selected and sampled during the first round. For each batch, 33 piglets belonging to 11 sows were selected. Sampling began in the farrowing unit at 4 weeks of age, while next samplings took place in the nursery every two weeks. Nasal swabs were collected. In the follow-up sampling, Farm 1 performed vaccination in sows and gilts, and 3 piglet batches were sampled, while Farm 2 vaccinated sows, gilts and 4 out 6 batches of sampled piglets. IAV-S RT-PCR was performed, positive samples were subtyped; viral isolation and WGS were performed(3).

Results
Multiple H1 lineages were detected during the study: in Farm 1, after circulation of H1BN2, H1CN1 (H1C.2.5) and H1AN1 were detected. In Farm 2, after detection of H1CN2 (H1C.2.4) and H1AN1, H1CN1 (H1C.2.1) was also detected. In the follow-up monitoring, one year later, only H1CN2 strains were detected in both farms with multiple introductions during sampling. Moreover, WGS showed that the combination of viral segments was different. Two viral H1CN2 genotypes (HC1.2.4 and H1C.2.2) were detected in Farm 1, while in Farm 2, two genotypes of H1CN2 (H1C2.4 in different combination of internal genes) were detected.

Discussion and Conclusion
The study highlighted a complex situation, with multiple IAV-S lineages circulating in both farms. Despite the vaccination, circulation of different IAV-S genotypes was observed. This could lead to new reassortant strains, hence the need to keep performing surveillance, in order to monitor circulating strains and to track emerging new variants at the human-animal interface. Study funded by ERA-Net ICRAD-PIGIE Grant Agreement No 862605.

References
1. doi.org/10.1186/s40813-021-00196-0
2. doi.org/10.3389/fcimb.2024.1336013
3. doi.org/10.3390/v14010047

P127

Epidemiology / Risk analysis and communication

COMMUNICATING FOOD RISKS: THE EXPERIENCE OF THE ITALIAN GROUP OF NATIONAL EXPERTS ON FOOD RISK COMMUNICATION

B. Tiozzo Pezzoli[3], M.G. Falcone[4], L. Baldi[2], S. Bonati[1]

[1]*Group of National Experts on Food Risk Communication, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*
[4]*Ministry of Health, ex Directorate General of Collegial Bodies for Health Protection (DGOCTS), Office 2 - Food Safety Risk Assessment, Italy*

Introduction
The 2019 Transparency Regulation highlights the importance of coordinated communication at EU and Member State level in maintaining

and strengthening public confidence in the food safety system. In 2022 the Ministry of Health (Moh) -Directorate General of Collegial Bodies for Health Protection, Office 2 Food Safety Risk Assessment – established the Group of National Experts on Food Risk Communication to form a community of professionals, to design and implement risk communication in a coordinated manner in Italy and in Europe with the Communications Experts Network of the EFSA.

Materials and Methods

The Group counts more than 20 experts with specific competence in risk communication from the network of Competent Organisations Art. 36 of EFSA's founding regulation, including Italian Universities, Istituti Zooprofilattici Sperimentali, research centres. Thanks to various professional background, the experts guarantee a multidisciplinary approach to risk communication, ensuring capillarity in the territory.

Results

The Group, coordinated by the MoH, ex DGOCTS - Office 2, is in charge of identifying needs and planning risk communication in collaboration with the MoH - ex Directorate General for Communication and European-International Relations, plans and implements risk communication activities on the national territory, collects and promotes good practices in risk communication carried out by the participating organisations; through the CEN, it participates in and promotes EFSA communication activities on the Italian territory and other requests coming from the Authority.

Discussion and Conclusion

The Group agrees on the need for competent national authority to speak with a single voice, from the national to the territorially competent authorities, also thanks to the preparation and adoption of a National Communication Plan. When fully operational, the Group aims to create an integrated system for food risk communication, consolidating a model and a community capable of responding effectively to the communication requirements of the Transparency Regulation.

References

Regulation (EU) 2019/1381 of the European Parliament and of the Council of 20 June 2019 on the transparency and sustainability of the EU risk assessment in the food chain

P128

Epidemiology / Risk analysis and communication

MACHINE LEARNING MODELS FOR SOURCE ATTRIBUTION OF *SALMONELLA* STRAINS ISOLATED FROM HUMANS

A. Boscolo Anzoletti[1], M. Mancin[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy*

Introduction

In Europe, *Salmonella* is the second most frequently isolated food-borne pathogen and the identification of the main sources of infection is crucial to address risk management strategies. This study compares different machine learning models to attribute domestic human *Salmonella* infections in Italy between 2018 and 2020 to four potential animal sources (laying hens, broilers, fattening turkeys and pigs) based on the serovar information only.

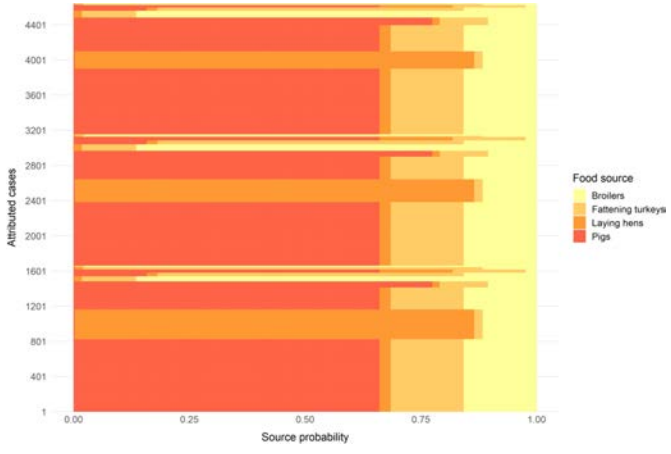


Figure 1. Predictive LB model results.

Materials and Methods
Four machine learning (ML) algorithms - random forest (RF), logit boost (LB), support vector machine radial (SVMR) and linear (SVML) kernel - were compared. Animal data were randomly split into training set (70%) to train the models and testing set (30%) to evaluate their performance using the accuracy (Ac) metric [1]. Cross-validation randomly partitioned the training dataset into 7 subsets. Due to imbalanced data, two additional downsampling training sets (outside and inside the resampling procedure) were also considered. The final model, using the best algorithm, was trained on the entire animal dataset and tested on human dataset to predict the animal source.

Results
The animal dataset included 11755 isolates from broilers, 750 from laying hens, 835 from fattening turkeys and 2644 from pigs. The human dataset had 6446 observations. LB was the best performing model considering the original dataset (Ac=0.91) and the two downsamplings (inside Ac=0.89; outside Ac=0.88). LB predicted 72% (4639) of the domestic human salmonellosis cases attributing 3417 of them to pigs, 799 to laying hens, 250 to broilers, 173 to fattening turkeys (Figure 1).

Discussion and Conclusion
The LB model results are in line with those obtained by the EFSA SAM model [2] that clearly identified pigs as the main animal source of human infections in Italy. ML models, combined with other strains information (MLVA or cgMLST), can potentially improve their performance in attributing the human cases.

References
1. Tanui, Collins K., et al. "A machine learning model for food source attribution of *Listeria monocytogenes*." Pathogens 11.6 (2022):691
2. Cento, Giulia, et al. "Application of the EFSA Source Attribution Model (SAM) to compare the main sources of human salmonellosis in Italy related to the national production over the period 2018–2020." Food Control 160 (2024):110315

P129

Epidemiology / Risk analysis and communication

ISOLATION TIMES OF *BRUCELLA* SPP. FROM SAMPLES COLLECTED FROM LIVESTOCK AND TERRESTRIAL WILDLIFE

M.R. Capone[1], D. Averaimo[1], D. Di Sabatino[1], A. Di Giuseppe[1], A. Alessiani[1], A. Petrini[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Italy*

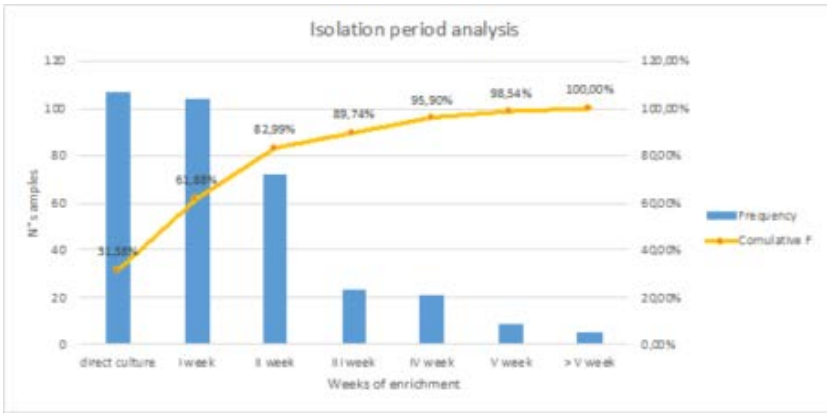
Introduction
An unequivocal diagnosis of *Brucella* infection requires microbiological isolation and, according to the WOAH Manual, it should be performed whenever possible to confirm the disease and to identify the *Brucella* species. As *Brucella* numbers may be lower in some tissue samples than in abortion material, enrichment in broth media with weekly subcultures for up to 6 weeks is advisable. The standard operating procedure adopted at the National Brucellosis Reference Centre (CRNB-IZSAM Teramo, Italy), provided enrichment for 6 weeks to maximise the sensitivity of the method. However, the process is time-consuming, costly, and labor-intensive. The current study aims to determine whether reducing the incubation time can find the right compromise between analysis times and strain isolation capacity.

Materials and Methods
The data analysis was based on 6081 samples collected primarily from cattle, buffalo, goat, sheep, and wild boar, analysed at the CRNB-IZSAM between January 2013 and June 2023. The days required for *Brucella* isolation were calculated for positive samples.

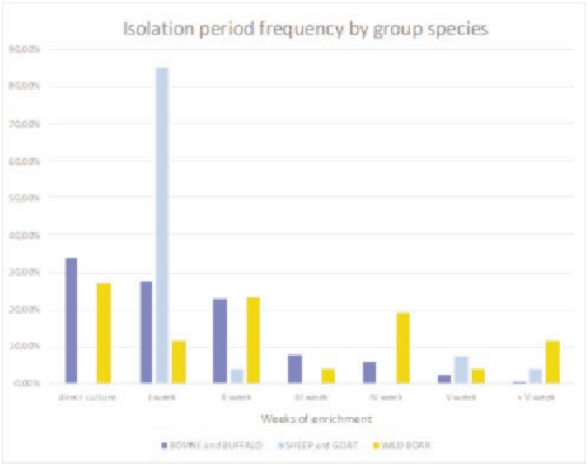
Results
Of the analysed samples, 338 tested positive for *Brucella* spp. (84.3% bovine-buffalo, 8% sheep-goat, 8% wild boar). Of these, 31.4% tested positive in direct culture while 95.9% tested positive within the fourth week of enrichment (Graph 1). Samples tested positive after the fourth week of enrichment, belong to animals in which *Brucella* spp. was isolated from other matrices between the first and second week of enrichment. A focus on the type of matrices is reported in Table 1. Differences in the distribution of the isolation time of *Brucella* are evaluated on different group species and reported in Graph 2. The isolation time for wild boar samples was significantly higher than cattle and buffalo ($p<0.5$) and *Brucella* was isolated within the fourth week of enrichment in the 84.6% samples.

Discussion and Conclusion
Considering the results, it seems to be feasible to reduce the isolation time at the fourth week of culture enrichment. Samples from wildlife may require longer isolation times than domestic animals. Further analyses should be conducted to evaluate the data collected by animal species, sample type, and epidemiological context.

References
WOAH. Brucellosis (infection with *Brucella* abortus, B.melitensis and B.suis). Chapter 3.1.4. Man. Diagn. TestsVaccinesTerr.Anim.2023.



Graph 1. Isolation period analysis.



Graph 2. Distribution and frequency of the isolation time of *Brucella* on different group species

Positive sample type	Direct culture	I week	II week	III week	IV week	V week	> V week	Total n° of samples
Fetus	29%	8%	1%	0%	0%	0%	20%	12%
Milk	6%	12%	9%	0%	5%	11%	0%	9%
Lymph nodes	37%	54%	38%	78%	67%	56%	40%	51%
Udder	2%	3%	4%	0%	0%	0%	0%	3%
Male genitalia	1%	0%	1%	0%	0%	22%	0%	1%
Internal organs	13%	16%	9%	17%	19%	11%	40%	15%
Placenta	8%	2%	1%	0%	10%	0%	0%	4%
Swab (uterine or vaginal)	0%	3%	4%	0%	0%	0%	0%	2%
Uterus	6%	3%	3%	4%	0%	0%	0%	4%
Total n° of samples	104	104	72	23	21	9	5	338

Table 1. Details on positive matrices and isolation times of *Brucella*

P130

Epidemiology / Risk analysis and communication

TESTICULAR CANCER AND ENVIRONMENTAL POLLUTION: A COMPARATIVE ONCO-EPIDEMIOLOGY STUDY IN CAMPANIA REGION

E. Di Napoli[5], D. De Biase[2], B. Degli Uberti[4], M. Dimatteo[4], S. Cavallo[4], L. Baldi[4], G. Rosato[1], G. Piegari[5], S. Papparella[5], S. Rossetti[3], F. Bruzzese[3], A. Budillon[3], O. Paciello[5]

[1]*ASL Napoli [1] Centro - CRIUV Regione Campania, Naples, Italy*
[2]*Department of Farmacy, University of Salerno, Italy*
[3]*Istituto Nazionale Tumori Fondazione G. Pascale-IRCCS, Naples, Italy*
[4]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy*
[5]*Pathology Unit , Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy*

Introduction
Comparative biological analysis between testicular cancer in dogs and humans highlights any similarities in the behaviour of related environmental cancer. The ubiquitous distribution of endocrine disruptors is strongly correlated to the development of testicular cancers. These toxic substances can mimic the action of hormones and interact with their receptors, altering their proper stimulation.

Materials and Methods
For oncological analysis, we extrapolated the data from the Campania Animal Cancer Registry and the management system of the National Cancer Institute "Fondazione G. Pascale-IRCCS", about 2020-2021-2022-2023. We reviewed 221 cases of testicular tumors in dogs and 174 cases of testicular cancer in humans. Furthermore, we analyzed and compared the environmental data from the Ministry of Environment and Energy Security website and the Campania Regional Agency for Environmental Protection (ARPAC).

Results
The areas with the highest incidence of testicular cancer in humans and dogs were the provinces of Napoli, Salerno, and Caserta. In the same locations severe contamination of soil and water by Organostannic Compounds, Dioxin, Ethyl- t-butyl Ether, Phenols, Phytopharmacies, Pesticides, Furans, Hydrocarbons, PAHs, Metals, Metalloids, Methyl-t-butyl Ether, PCBs was recorded.

Discussion and Conclusion
Considering our evidence, we can conclude that the high frequency of testicular cancer in humans and dogs in the reported locations and the high percentages of environmental pollutants correlated to testicular cancer are strongly associated. However, the molecular mechanisms are not yet fully understood. The biological behaviour of testicular cancers in dogs and the possible role of environmental risk factors may provide useful indications for preventing neoplasms affecting humans and animals.

References
1. Faja F., Esteves S., Pallotti F., Cicolani G., Di Chiano S., Delli Paoli E, Lenzi A., Lombardo F., Paoli D. Environmental disruptors and testicular cancer. Endocrine (2022); 78:429-435.
2. Sumner, R.N., Byers, A., Zhang, Z. et al. Environmental chemicals in dog testes reflect their geographical source and may be associated with altered pathology. Sci Rep 11, 7361 (2021).

P131

Epidemiology / Risk analysis and communication

INTEGRATION BETWEEN NATIONAL LIVESTOCK REGISTER AND SIGLA (ISTITUTO ZOOPROFILATTICO DELL'UMBRIA E DELLE MARCHE - IZSUM - LABORATORY INFORMATION MANAGEMENT SYSTEM)

L. Faccenda[2], M. Falcinelli[2], M. Capuccella[2], D. Francia[2], A. Mingolla[2], C. Olivieri[2], A. Paoloni[2], E. Rivosecchi[2], F. Scubla[1], S. Tonazzini[2], G. Scieri[2]

[1]*Invisiblefarm S.r.l., Brescia, Italy*
[2]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy*

Introduction
In public health laboratories samples reception is one of the most important elements of the analytical process. All relevant information must be entered into Laboratory information management system (LIMS) to ensure traceability for reports, data flows and epidemiological investigations. The single identification animal number and the sample collection point are two of the main pieces of information, that must be registered correctly. To avoid risks of errors in the pre-analytical stage, our Institute, with Invisiblefarm srl technical support, has

developed an integrated management system which allows the alignment between BDN-Vetinfo (Italian national livestock register) and our LIMS (SIGLA) [1]. BDN–Vetinfo is the Italian national register where information of interest for veterinary public health could be updated and consulted by various stakeholders. Samples recorded in SIGLA shall be accompanied by identifying data taken from Vetinfo. Although it is currently in use in IZSUM a sharing system of data related to official sampling that allows data transfer in digital way, unofficial samples still require manual data input from paper forms

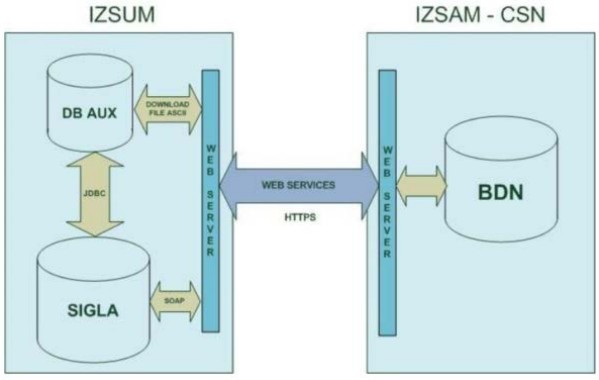


Figure 1. System architecture.

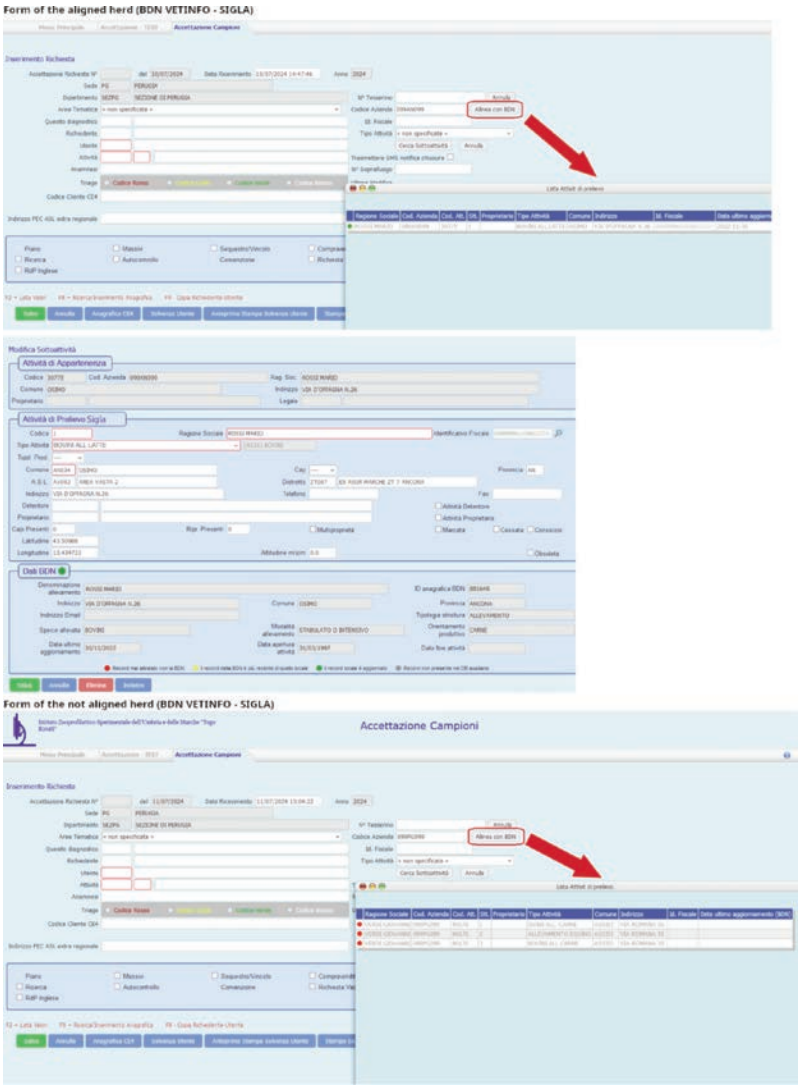


Figure 2. Example of a reception form with an aligned and a not aligned herd (BDN VETINFO - SIGLA)

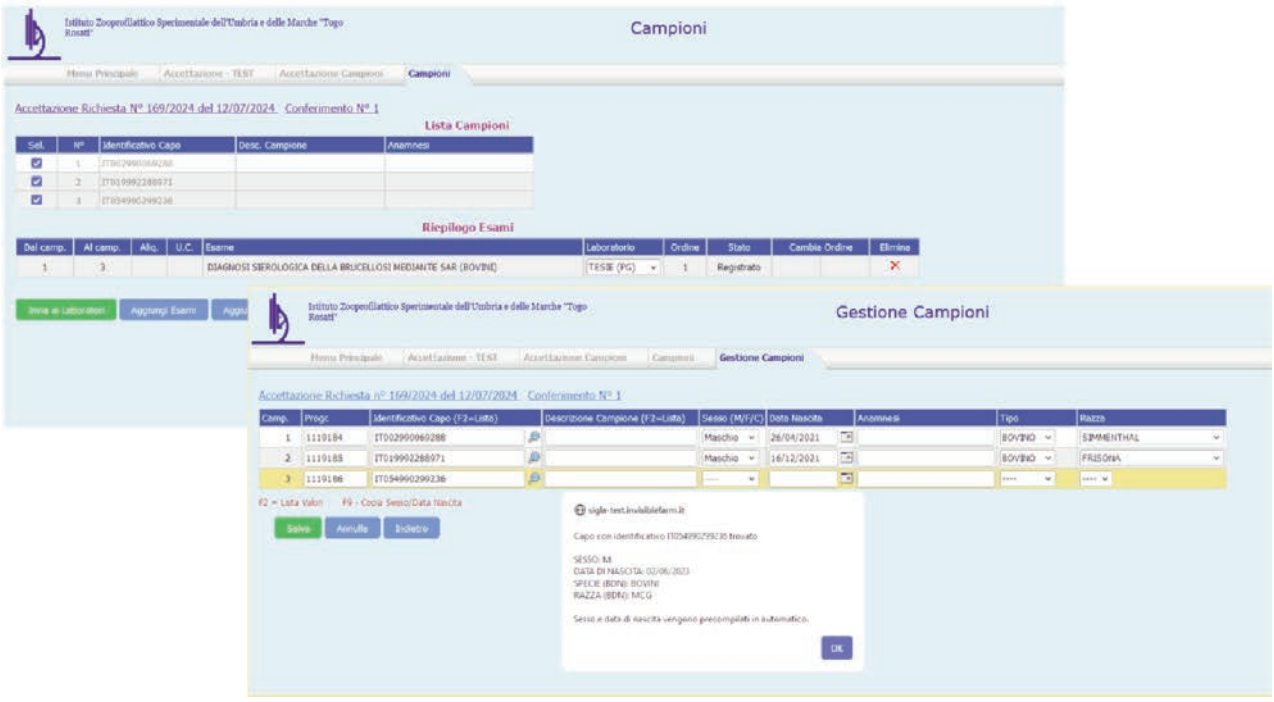


Figure 3. Example of a reception form with aligned single identification animal number (BDN VETINFO – SIGLA)

Materials and Methods

Alignment process involves two components: herd data register and individual animal data register. For herd register the information are retrieved from an auxiliary database created to minimize web service calls and conserve resources. Updated daily, this database, ensures the most current herd data is accurately reflected in the SIGLA system. Animal data alignment is done through web services from the IZS Abruzzo e Molise (IZSAM-CSN), integrating information from the BDN–Vetinfo into SIGLA (Figure1).

Results

The feature is tested and will be installed in a production environment.Figure2-3 represent the screenshots of the main functions implemented in SIGLA

Discussion and Conclusion

This process ensures accurate recording of animal data, enhancing data accuracy and reliability essential for epidemiological investigations and public health reporting.

References

1. Nappo C.,Pizzoni E.,Izzo P.,Desantis E.,Virdis A.,Mingolla A.,Cenni G.,Faccenda L. Utilizzo e evoluzione di un sistema informativo per la gestione laboratori di analisi (SIGLA). Atti XIV Congresso SIDILV. Sorrento 24-26 ott. 2012

P132

Epidemiology / Risk analysis and communication

LOW PREVALENCE OF *PAENIBACILLUS LARVAE* IN *APIS MELLIFERA* IN THE NETHERLANDS

H. Graham[1], Y. Dijkstra[1], R. Ruuls[1], D. Valkenburg[2], H. Goossen[2], M. Counotte[1], D. Panziera[2]

[1]*Wageningen Bioveterinary Research, Wageningen UR, Lelystad, The Netherlands*
[2]*Wageningen Plant Research, Wageningen UR, Wageningen, The Netherlands*

Introduction

Paenibacillus larvae is the causative agent of American Foulbrood (AFB), a disease of the brood in European honey bees (*Apis mellifera*). Historically, the control of AFB in the Netherlands was managed by the Dutch government, until the Dutch government and several beekeeper's associations agreed to delegate this task to the sector in 2005. To be able to assess the effect of these changes in policy on the prevalence of *P. larvae*, a study was conducted in 2008, which served as a baseline measurement. The prevalence of *P. larvae* was found

to be very low: *P. larvae* was detected by culture in 1 out of 170 apiaries (0.6%, 95% confidence interval (CI): 0.03-3.7%) and in six out of 190 honey samples (3%, 95% CI: 0.1-7.1%). In 2023, we conducted a study in order to assess the effect of the changes in policy on the prevalence of *P. larvae* in the long term.

Materials and Methods

A total of 623 brood comb honey samples originating from 224 apiaries across the country were collected and pooled per apiary, resulting in 223 pooled samples. Pooled samples were analysed for the presence of *P. larvae* by real-time PCR. If the result was positive or inconclusive, the PCR was repeated for the individual samples within the pooled sample, to be able to assign the positive result to an individual colony within the apiary.

Results

One pooled sample tested positive for *P. larvae*, resulting in a prevalence of 0.4% (95% CI: 0.02-2.9%) on the level of apiaries. When tested individually, two out of the three samples from the positive pool yielded a positive result, resulting in a prevalence on the level of colonies of 0.3% (95% CI: 0.06- 1.3%).

Discussion and Conclusion

P. larvae was detected by real-time PCR in one out of 223 apiaries (0.4% (95% CI: 0.02-2.9%)), with two out of three colonies in this apiary testing positive. Following communication of the positive results a few months after sampling, the beekeeper informed us that no symptoms of AFB had been observed in these colonies in the meantime. Based on these results, we can conclude that the changes in policy have not led to an increase in the prevalence of *P. larvae* in the Netherlands, which is also in accordance with the very few clinical outbreaks reported annually. The beekeeping practices of Dutch beekeepers might explain the low prevalence of AFB compared to other countries.

P133

Epidemiology / Risk analysis and communication

AN EVIDENCE-BASED FRAMEWORK FOR ANTIBIOTIC RESISTANCE GENE TRANSFER IN DAIRY FARMS

F. Marzoli[2], J. Pinarelli Fazion[2], P. Antonelli[1], A. Dall'Occo[1], S. Belluco[2], C. Losasso[1]

[1]*SCS1 – Lab of microbial ecology and genomics - Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*
[2]*SCS8 – Lab of safety and quality of the food chain - Istituto Zooprofilattico Sperimentale delle Venezie, Vicenza, Italy*

Introduction

The epidemiology of antimicrobial residues and antibiotic resistance genes (ARGs) in microbial communities are highly interconnected (1). The therapeutic use of antibiotics in dairy farms contributes to the selection, persistence and dissemination of mobile antibiotic resistance genes (MARGs) which can be amplified and exchanged among microorganisms, propagate in farm environments and spread into the external environment (2). The study aims to define the risk pathway and spread of MARGs in the dairy farm environment.

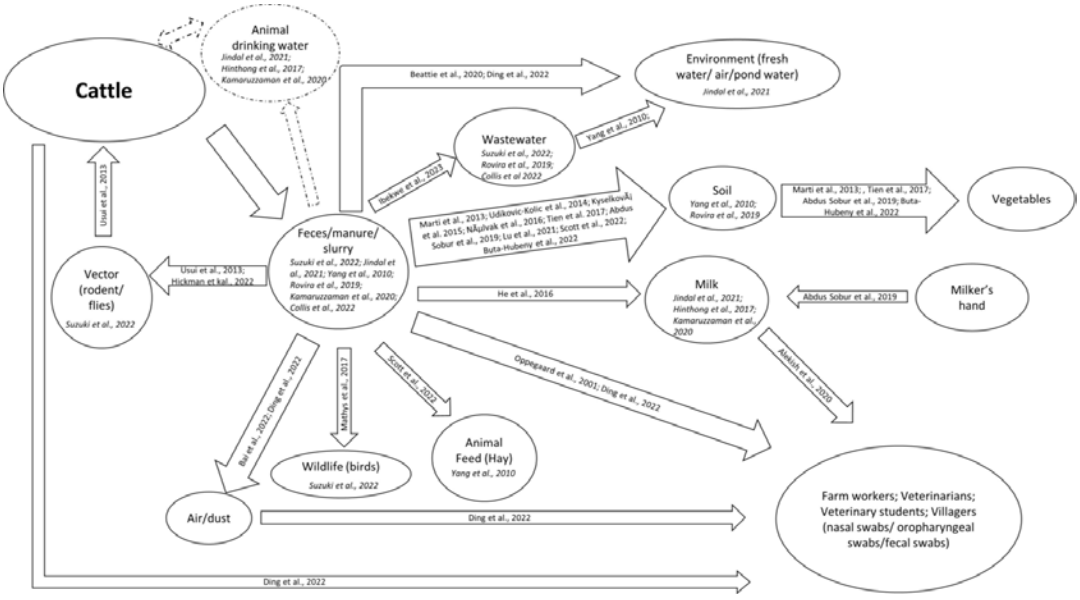


Figure 1. Pathway of mobile antibiotic resistance genes (MARGs) in different matrices characterizing dairy farms.

Materials and Methods
A systematic review was conducted and the review question was: "Which pathway does exist for the bacteria acquisition of MARGs in dairy farms?" We considered studies published in peer-reviewed journals in English, without any time restrictions. We searched PUBMED and EMBASE databases.

Results
A total of 28 studies were included. Of these, 21 identified the pathways by which MARGs are transferred from one specific matrix to another. The remaining 7 reported the presence of MARGs in different matrices without demonstrating the transfer pathway. Faeces were identified as the main source of MARGs dissemination in 17 studies, while raw milk, milker's hand, wastewater, air and rodent faeces were considered the main source in one study each.

Discussion and Conclusion
Faeces play a key role in the dissemination of MARGs in the dairy farm environment. It has been shown that MARGs can spread not only within livestock but also in the surrounding environments, eventually reaching raw food intended for human consumption such as milk and vegetables. However, these studies have notable limitations, as none have defined a transfer rate for MARGs, useful for risk assessment purposes. Future research emphasizes the need to adopt this approach to prevent the increasingly significant rise of antibiotic-resistant bacteria.

References
1. Phillips, I. et al. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. 53, 28–52 (2004).
2. Pérez-Rodríguez F and Mercanoglu Taban B (2019) A State-of-Art Review on Multi-Drug Resistant Pathogens in Foods of Animal Origin: Risk Factors and Mitigation Strategies.Front. Microbiol. 10:2091.doi: 10.3389/fmicb.2019.02091.

P134

Epidemiology / Risk analysis and communication

CHARACTERIZATION OF *STREPTOCOCCUS SUIS* ISOLATES COLLECTED FROM DIFFERENT ANIMAL SPECIES IN THE CZECH REPUBLIC BETWEEN 2018 AND 2022

J. Matiasovic[1], M. Zouharova[1], N. Kralova[2], K. Nedbalcova[1]

[1]*Veterinary Research Institute, Brno, Cechia*
[2]*Veterinary Research Institute, Department of Experimental Biology Faculty of Science Masaryk University, Brno, Cechia*

Introduction
Streptococcus suis is important pathogen primarily associated with pigs. Here we present a description of 622 isolates collected from different animal species between 2018 and 2022.

Materials and Methods
Blood agar plates were used for primoculture (22-48 hours at 37°C) of sectional materials or swabs. Suspected *S. suis* colonies were tested using biochemical test, rapid slide latex agglutination, and MALDI–TOF. Serotyping by multiplex PCR method and PCR-MLST typing were performed on DNA prepared by the boiling method. All isolates untypeable by PCR were tested with the co-agglutination test using antisera prepared via the immunisation of rabbits with reference strains (Zouharová et al. 2022).

Results
The vast majority of isolates were collected from pigs (597 isolates, 95.98%), followed by isolates from cattle (20 isolates, 3.21%), geese (3 isolates, 0.48%), a dog (1 isolate, 0.16%) and a parrot (1 isolate, 0.16%). 22 different serotypes, including the so-called Suis-like serotypes, were identified. The most prevalent were untypeable isolates followed by serotype 7 (Figure 1). Similar to serotypes, a wide spectrum of MLST types was identified, with the most prevalent being unknown sequence types (Figure 2).

Discussion and Conclusion
Similar to other countries in Europe (Rieckmann et al. 2018), a wide diversity of *S. suis* isolates was found in the Czech Republic. Among clinically important serotypes, serotypes 2 and 7 were frequently detected in pathological material. This work was supported by grants RO0523 and TN02000017.

References
Zouharová, M. et al. Characterisation of *Streptococcus suis* Isolates in the Czech Republic Collected from Diseased Pigs in the Years 2018–2022. Pathogens 2023, 12, 5.
Rieckmann, K. et al. *Streptococcus suis* cps7: an emerging virulent sequence type (ST29) shows a distinct, IgM- determined pattern of bacterial survival in blood of piglets during the early adaptive immune response after weaning. Vet Res. 2018;49:48.

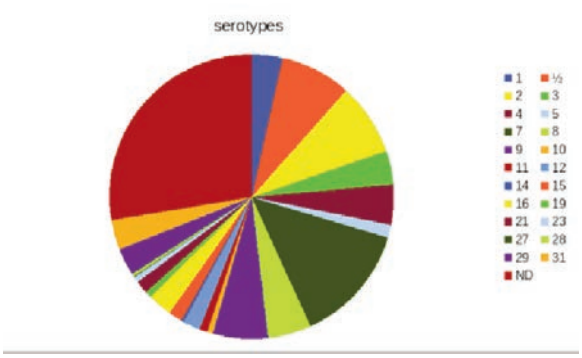


Figure 1. Serotypes of *S. suis* isolates collected in 2018-2022 the Czech republic.

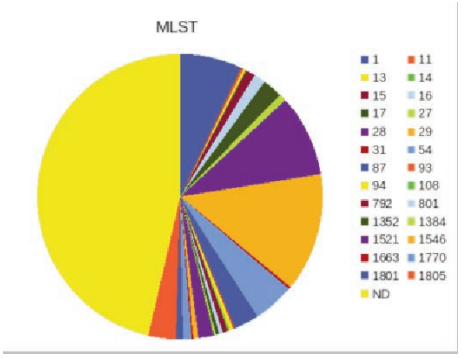


Figure 2. Sequence types of *S. suis* isolates collected in 2018-2022 in in the Czech republic.

P135

Epidemiology / Risk analysis and communication

AUTOMATION OF COMMUNICATIONS IN ANIMAL HEALTH ACCORDING TO LEGISLATIVE DECREE N. 136/2022

A. Mingolla[1], M. Falcinelli[1], D. Francia[1], C. Olivieri[1], A. Paoloni[1], E. Rivosecchi[1], G. Scieri[1], S. Tonazzini[1], A. Valiani[1], L. Faccenda[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy*

Introduction

Recent regulatory changes in EU determined the need for laboratories to communicate to the authorities, in defined times, the detection of some pathogens' positivity. Waiting for a national register to be available to which the information can be transmitted, to avoid the task being carried out manually, a computerized system was developed, that instructed with the positives to report and the authorities to which communicate it, sends an email to the correct recipients with the test report as soon as it is issued. Regulation EU/2016/429 also called Animal Health Law was adopted in order to standardize European policy on animal health management, in particular by identifying specific measures against specific diseases, listed by Regulation EU/2018/1882 and Leg. Decree No. 191/2006, throughout the Union territory. Italy has adapted its national legislation to these amendments with legislative acts, including Leg. Decree No.136/2022 [1], in which it is established that the positivity detected against a list of pathogens by the laboratory must be communicated to the authority. Aim of this work is to describe how our Institute has implemented the LIMS in use (SIGLA) to carry out this task.

Materials and Methods

Automatic sending of e-mails has developed through a new SIGLA external function that, when analytical reports are generated, is called by SIGLA and returns mail subject, text message (Figure 1) and a parameter that represents the category of the positivity. The categories identification is developed by configuration tables populated with analytical test codes and animal species. Combination of the previous elements determines the parameters in Table 1.

Parameters	Category of listed diseases (art.2 Reg.2018/1882 and Legislative Decree No. 191/2006)	Recipients
0	No positivity according to Leg. Dec. 136	None
1	Zoonosis	Local Health Unit
2	C,D,E	Local Health Unit
3	A,B	Local Health Unit, Regions, Ministry of Health

Table 1. Combination of the elements that determines the parameters.

Results
From April to June 2024, when the feature has released, have been send to the Authorities 254 communications, 23 of these were related to A-B diseases, 178 to C-D-E diseases and 53 to zoonoses.

Discussion and Conclusion
Public Administrations have obligation to improve digitalization of their activities, these improvements increase processes efficiency and sustainability. Even in this case, replacing an operation done manually increased the precision of the activity decreasing the workload of the operators

References
1. Leg. Decree No. 136/2022, 5/8/2922, GU Serie Generale 213 12/09/2022

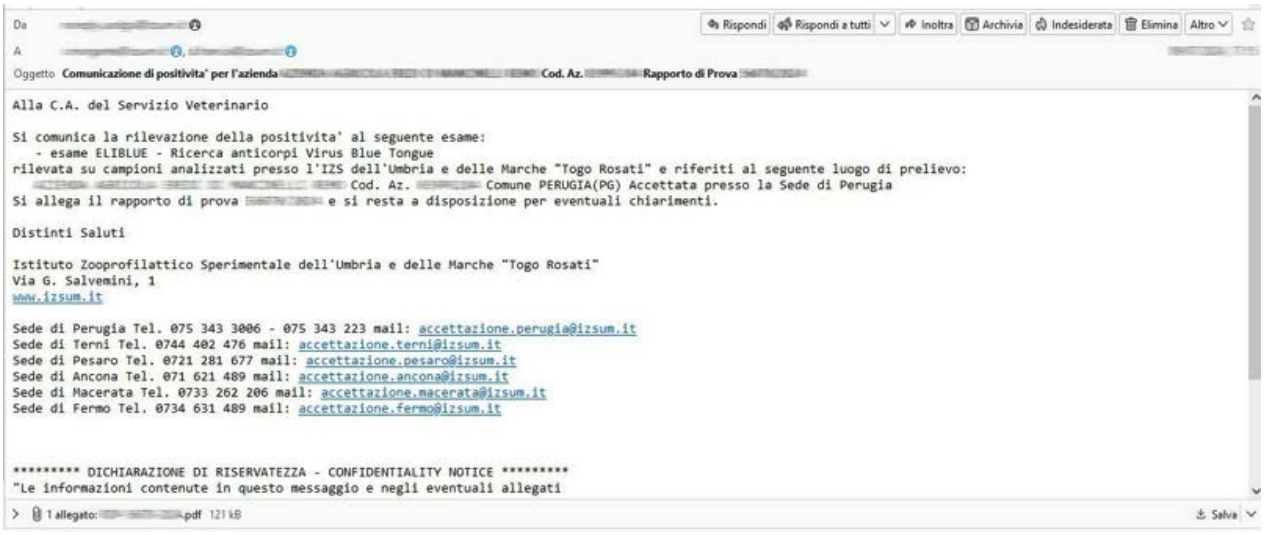

Da
Rispondi | Rispondi a tutti | Inoltra | Archivia | Indesiderata | Elimina | Altro
A
Oggetto Comunicazione di positivita' per l'azienda Cod. Az. Rapporto di Prova
Alla C.A. del Servizio Veterinario
Si comunica la rilevazione della positivita' al seguente esame:
- esame ELIBLUE - Ricerca anticorpi Virus Blue Tongue
rilevata su campioni analizzati presso l'IZS dell'Umbria e delle Marche "Togo Rosati" e riferiti al seguente luogo di prelievo:
Cod. Az. Comune PERUGIA(PG) Accettata presso la Sede di Perugia
Si allega il rapporto di prova e si resta a disposizione per eventuali chiarimenti.
Distinti Saluti
Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati"
Via G. Salvemini, 1
www.izsum.it
Sede di Perugia Tel. 075 343 3006 - 075 343 223 mail: accettazione.perugia@izsum.it
Sede di Terni Tel. 0744 402 476 mail: accettazione.terni@izsum.it
Sede di Pesaro Tel. 0721 281 677 mail: accettazione.pesaro@izsum.it
Sede di Ancona Tel. 071 621 489 mail: accettazione.ancona@izsum.it
Sede di Macerata Tel. 0733 262 206 mail: accettazione.macerata@izsum.it
Sede di Fermo Tel. 0734 631 489 mail: accettazione.fermo@izsum.it
********* DICHIARAZIONE DI RISERVATEZZA - CONFIDENTIALITY NOTICE *********
"Le informazioni contenute in questo messaggio e negli eventuali allegati
1 allegato: pdf 121 kB
Salva

Figure 1. Example of a notification e-mail.

P136

Epidemiology / Risk analysis and communication

ROTAVIRUSES A, B AND C DETECTED ON PIG FARMS IN THE CZECH REPUBLIC

R. Moutelíková[1], J. Prodělalová[1]

[1]*Dept. of Infectious Diseases and Preventive Medicine, Veterinary Research Institute, Brno, Cechia*

Introduction
Porcine rotaviruses (RV) are known for their complex epidemiology, pathogenicity and great genetic diversity. RVA represent one of the most important causes of acute diarrhoea of young animals. Apart from the first days of life, piglets are at great risk of RV infection during the weaning period.

Materials and Methods
A total of 127 stool samples from domestic pigs of different age categories were collected during from July 2023 to June 2024. The samples were examined in real-time PCR with a hydrolysis probe for the presence of RVA (own modification)1, RVB and RVC.2 Porcine RVA genes coding whole VP7 and partial VP4 were sequenced and genotypes were determined.

Results
The results of rotavirus screening are summarized in Table 1. 78.7% of all samples (100/127) were positive for at least one rotavirus species. Rotavirus A was detected most often, either alone or in co-infection with other types of RV, occurring in 67.7% of all examined samples and in 86% of the rotavirus-positive samples. In the category of piglets after weaning, a high rate of rotavirus co-infections was detected - 89.6% of positive samples. RVC was detected more often in the group of piglets after weaning (64.6% of RVC-positive samples) than in suckling piglets (21.5% of samples positive for RVC). The most common porcine G-genotypes were G9 and G3 (each in 29% of all RVA-positive samples), G5 (23%), and G11 (12%) in combination with different P-types (mostly with P[13] and P[6]).

Discussion and Conclusion
The epidemiology of porcine rotaviruses is very complex and highly dynamic, which is confirmed by the discovery of new porcine RV species as well as evidence of high prevalence and genetic diversity of rotaviruses. Our results show that RVA-only infection occurred just

in suckling piglets. RVB and RVC began to appear as co-infections around the time of weaning which is in agreement with previously published research.3 Continuing surveillance studies as well as genotyping analyses of detected rotaviruses make it possible to verify the effectiveness and completeness of the diagnostic methods used.

ACKNOWLEDGEMENT
This study was financially supported by the NaCeBiVet project of the Technological Agency of the Czech Republic (No. TN02000017) and by the Concept of Research, Development and Innovation of the Ministry of Agriculture (No. RO0523).

References
1. Moutelíková, R., Dvořáková Heroldová, M. et al. Epidemiol Mikrobiol Imunol 2018;67(3):110-113.
2. Jothikumar, N., Kang, G., Hill, V. R. J Virol Methods 2009;155(2):126-31.
3. Baumann, S., Sydler, T., Rosato, G. et al. Viruses 2022;14:1117.

	Suckling piglet	Weaned piglet	Total
RVA+	20	1	21
RVB+	4	2	6
RVC+	5	2	7
RVA+B+C+	3	28	31
RVA+B+	11	14	25
RVA+C+	9	0	9
RVB+C+	0	1	1
Negative	27	0	27
Total	79	48	127

Table 1. Rotavirus in different age categories detected during July 2023-June 2024.

P137

Epidemiology / Risk analysis and communication

HOW THE LABORATORY CAN SUPPORT SWINE PRACTITIONERS IN UNDERSTANDING THE DIFFUSION OF *MYCOPLASMA HYORHINIS*

V. Righetti[1], G. Nai[1], A. Morales-Arce[1], E. Stefani[1], M. Gastaldelli[1], D. Prataviera[1], M. Bottazzari[1], I. Rossi[1], M. Merenda [1]

[1]*Mycoplasma Unit, WOAH reference laboratory for avian mycoplasmosis (M. gallisepticum, M. synoviae), SCT-1 Verona, Istituto Zooprofilattico Sperimentale delle Venezie, Buttapietra, Italy*

Introduction
Mycoplasma hyorhinis (MHR) infects the upper respiratory tract of pigs and spreads throughout the body causing polysierositis and polyarthritis in weaning pigs. It is also involved in conjunctivitis, otitis, meningitis but its role in respiratory disease is still unclear. Genotyping methods can be a helpful tool to deepen the dynamics of MHR spreading in the swine industry.

Materials and Methods
The MLST protocol developed by Tocqueville and Trüeb (2014) allows the unique identification of MHR strains through the global public database PubMLST (https://pubmlst.org/). This database assigns a Sequence Type (ST) to each strain based on the combined allelic sequence of six housekeeping genes. At the *Mycoplasma* Unit of IZSVe, we genotyped MHR isolates applying MLST to different organic matrices in distinct pig farms from northern Italy over the years.

Results
Farrow-to-finish breeding farms revealed a farm-specific ST among MHR isolates sampled over time. The continuous circulation of the same MHR in such types of farms can be explained by the limited introduction of new individuals and by the co-presence of animals of all production categories (breeders, weaning and fattening) in the same farm. Further analyses of MHR diffusion in different types of farming, such as farrow-to-weaning, weaning or fattening units, are ongoing. Moreover, we did not observe a relation between STs and sampling matrix. In addition, the comparison between Italian isolates and PubMLST global entries revealed a lack of genetic correlation among isolates from the same geographical area.

Discussion and Conclusion
In conclusion, the use of MLST method represents a powerful tool for investigating the intra-farm and inter-farm dynamics of MHR in swine populations and for improving the management and control of associated clinical forms.

References

Tocqueville, V., Ferré, S., Nguyen, N. H., Kempf, I., & Marois-Créhan, C. (2014). Multilocus sequence typing of *Mycoplasma* hyorhinis strains identified by a real-time TaqMan PCR assay. Journal of clinical microbiology, 52(5), 1664–1671.

Trüeb, B., Catelli, E., Luehrs, A., Nathues, H., & Kuhnert, P. (2016). Genetic variability and limited clonality of *Mycoplasma* hyorhinis in pig herds. Veterinary microbiology, 191, 9–14.

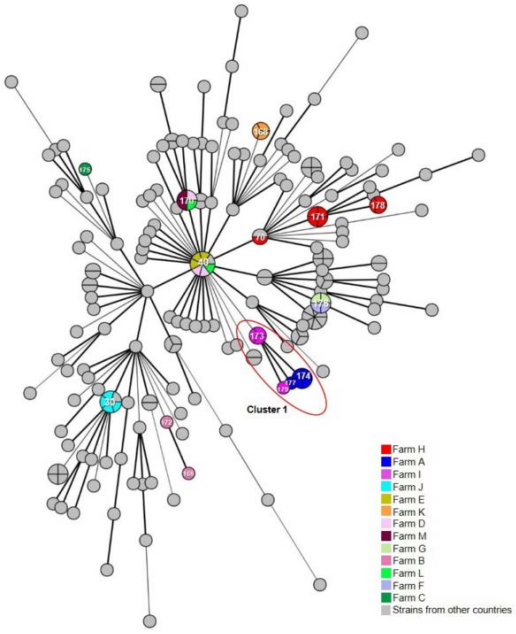


Figure 1. MST (Minimum Spanning Tree) of strains uploaded in PubMLST and Italian strains. Each circle and numbers correspond to a ST. Colors and size represent the different Italian farms and the number of isolates, respectively. Strains from other countries are encircled in grey. The lines joining the circles are represented with different thicknesses depending on the number of allelic differences between the respective STs (bold = 1 allelic difference, plain = 2 allelic differences). Cluster 1 highlights the genetic relationship between STs from Farm A and Farm I also shown in the figure 2.

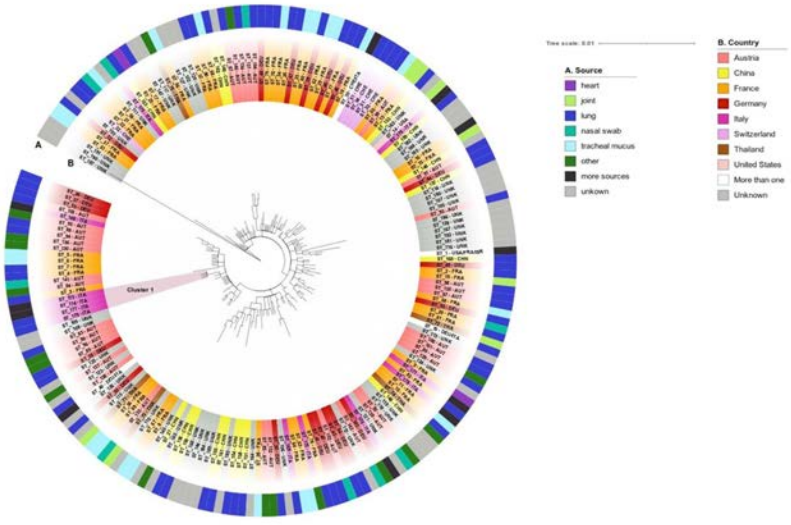


Figure 2. Global phylogenetic tree based on the concatenated allelic sequence (ST) of the six MHR housekeeping genes. The evolutionary history was inferred by using the Maxi3m2u1m Likelihood method based on the Tamura 3-parameter model7. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0,1162)). A. Source: classification of organic matrices shown in the external ring. B. Country: assignment of STs by countries available on PubMLST. Cluster 1 highlights the close phylogenetic relationship of STs from Farm A and Farm I shown in the figure 1. Evolutionary analyses were conducted in MEGA78. Visualization was made using.

P138

Epidemiology / Risk analysis and communication

ENVIRONMENTAL CONTAMINATION IN THE ANIMAL VETERINARY FACILITIES: RELATIONSHIP BETWEEN GOOD HYGIENE PRACTICES AND MESOPHILIC BACTERIAL COUNT OF THE SURFACES

A. Pinto[1], M. Corrò[1], M. Cocchi[1], L. Viel[1], D. Dellamaria[1], I. Drigo[1], L. Clapiz[1], P. Danesi[1], K. Capello[1], E. Spagnolo[1], G. Mascarello[1], C. Mantovani[1], S. Crovato[1], M. Zago[1], M. Vascellari[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

Introduction

In human health care, hospital-acquired infections (HAIs) are increasing worldwide due to the rise of multidrug- resistant bacteria. However, data regarding the prevalence of HAIs in the veterinary hospitals are largely unknown. These infections can arise from commensal or pathogenic bacteria and spread via environmental surfaces, posing risks to both hospitalized animals and veterinary staff and owners [1]. This study aims to collect data to highlight the relationship between hygienic practices and the mesophilic counts of different surfaces in the small animal veterinary facilities (VFs).

Materials and Methods

Social research techniques and laboratory analyses were used. Fifteen clinics were enrolled. A focus group with medical directors from six randomly chosen VFs in northeastern Italy defined the parameters to be measured. Additionally, an online semi-structured questionnaire investigating hygiene behaviours was administered to all the clinics. Samples were collected using sponges and dip-slides from several critical points in different rooms within the clinics, and the total mesophilic count was performed. Generalized linear mixed models were used to statistically analyse quantitative parameters.

Mesophilic count	<1		<4		4-25		26-50		51-100		101-300		>300	
Rooms	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Waiting rooms	245	25.68%	187	19.60%	310	32.49%	75	7.86%	52	5.45%	63	6.60%	22	2.31%
Examination areas	311	24.94%	250	20.05%	407	32.64%	103	8.26%	65	5.21%	77	6.17%	34	2.73%
Pre-operative room	342	31.70%	242	22.43%	302	27.99%	62	5.75%	35	3.24%	56	5.19%	40	3.71%
Surgery room	629	59.06%	233	21.88%	126	11.83%	19	1.78%	19	1.78%	24	2.25%	15	1.41%
Ward areas	260	24.14%	143	13.28%	280	26.00%	69	6.41%	76	7.06%	117	10.86%	132	12.26%

Table 1. Distribution of sampling results according to UFC classes and rooms.

Results

The questionnaire revealed that disinfectants were the primary hygiene product used, followed by ozone in pre- operative, surgery, and ward areas. Examination and ward areas were cleaned multiple times daily, while other rooms were cleaned at least once daily. A total of 5,513 environmental samples were analyzed. A significant association was found between UFC class values and room types ($p<0.001$, Table 1). The ward area represents the most critical area, whereas the waiting room and examination area should be kept under control.

Discussion and Conclusion

Collecting microbiological data about environmental contamination is crucial to prevent infections spread within VFs. Based on our findings, a protocol is being developed to guide infection prevention practices, including sampling points, times, frequencies and recommendations on hygiene products and cleaning procedures.

References

1. Walther B, Tedin K, Lübke-Becker A. Multidrug-resistant opportunistic pathogens challenging veterinary infection control. Vet Microbiol. 2017;200:71-78.

P139

Epidemiology / Risk analysis and communication

PREVALENCE AND GENETIC ANALYSIS OF BOVINE HERPESVIRUS 1 (BOHV-1) IN A DAIRY CATTLE FARM FROM ANKARA TURKEY

S. Şurak[2], F. Garİp[2], K. Dabur[2], Z. Akkutay-Yoldar[1]

[1]*Department of Virology, Faculty of Veterinary Medicine, Ankara University, Ankara, Turkey*
[2]*Graduate School of Health Sciences, Ankara University, Ankara, Turkey*

Introduction

Bovine Herpesvirus 1 (BoHV-1), widespread across the globe, primarily affects cattle and can cause decreased milk production and infertility. This study aimed to detect the presence of the virus in cattle and gather up-to-date information on the prevalence and genetic characteristics of BoHV-1 in Ankara.

Materials and Methods

A total of 46 animals (45 asymptomatic) were sampled on a dairy cattle farm in Ankara, Turkey. 137 recently processed samples including 46 nasal swabs, 45 genital swabs, and 46 blood were used to extract the virus. The phenol-chloroform extraction method was applied for viral DNA isolation [1]. The protein-coding region of the gB gene was partially amplified by PCR. Three out of nine positive samples were sequenced, followed by molecular characterization using the maximum likelihood method.

Results

The prevalence of BoHV-1 among 46 animals was detected as 20%. Virus was detected in 11% of nasal and 9% of genital swab samples. The blood samples showed no positivity. The prevalence of BoHV-1 acquired from nasal swabs in pregnant animals was 9%, whereas the prevalence obtained from genital swabs was 4%. The study revealed that the BoHV-1 infection rate was 11% in Holstein cattle and 9% in Simmental cattle. The amino acid sequences of the BoHV-1 gB protein gene showed that two of the strains found in this study were closely related to each other and formed a separate cluster. Additionally, one of our strains was located on a different branch, distinct not only from the two detected strains but also from other BoHV-1 strains, indicating its uniqueness.

Discussion and Conclusion

The presence of BoHV-1 in nasal and genital swab samples confirms the virus's presence in the herd and shows relation with a fertility issue in a Simmental heifer. By conducting future studies, the findings of this study can be interpreted to suggest that genetic factors may contribute to resistance in certain breeds. A distinct strain detected in this study could impact disease control strategies and vaccine development, as it may be a new variant with different pathogenic traits.

Breeds	Nasal Swab (46) (+) (%)	Genital Swab (45) (+) (%)	Blood Sample (46) (+) (%)	Cattle (46) (+) (%)
Holstein (20)	3 (7)	2 (4)	0 (0)	5 (11)
Simmental (26)	2 (4)	2 (4)	0 (0)	4 (9)
Total (46)	5 (11)	4 (9)	0 (0)	9 (20)

Table 1. Distribution of sampling results according to UFC classes and rooms.

Pregnancy Status	Nasal Swab (45) (+) (%)	Genital Swab (45) (+) (%)	Blood Sample (45) (+) (%)
Pregnant	4 (9)	2 (4)	0 (0)
Open Cow	0 (0)	2 (4)	0 (0)
Inseminated	0 (0)	0 (0)	0 (0)
Repeat Breeder	1 (2)	0 (0)	0 (0)
Total	5 (11)	4 (9)	0 (0)

Table 2. The PCR results for the sampled materials according to pregnancy status.

Figure 1. Phylogeographic analysis of BoHV-1 sequences based on partial gB gene. Newly identified Turkish BHV-1 strains are indicated by a solid red square.

References

1. Chomczynski, P., & Sacchi, N. (2006). The single-step method of RNA isolation by acid guanidinium thiocyanate– phenol–chloroform extraction: twenty-something years on. Nat Protoc, 1(2), 581-585.

P140

Epidemiology / Risk analysis and communication

UPDATES ON BOVINE TUBERCULOSIS IN ITALY

M. Tamba[1], S. Salvato[1], G. Casadei[1], G. Galletti[1], A. Rosamilia[1], R. Lomolino[2]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna, Brescia, Italy*
[2]*Ministry of Health. Directorate-General Animal Health and Veterinary Medicines, Rome, Italy*

Introduction

Bovine tuberculosis (bTB) is an infectious disease caused by *Mycobacterium tuberculosis* complex (MTBC), subject to eradication programs throughout the European Union. In Italy, the bTB situation varies by region, with some regions having both disease-free (DF) and non-disease-free (NDF) provinces at the same time. We analysed data from the eradication activities in the period 2018-2023 to identify critical issues.

Materials and Methods

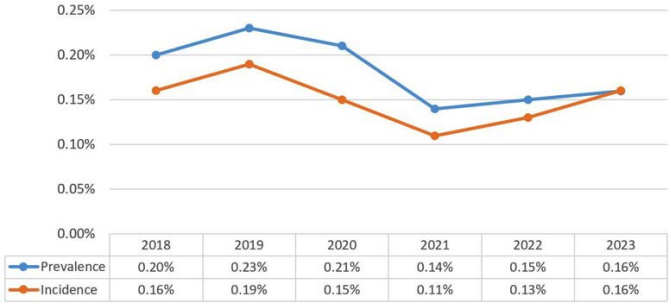
Data were downloaded from the Veterinary Information System, hosting data on bTB outbreaks, official controls and cattle population. We computed the main indicators of effectiveness: prevalence and incidence at herd level, confirmation and recovery times of outbreaks, culling times for infected heads. We also performed a univariate analysis focused on bTB outbreaks occurred in 2022.

Results

Starting from 2021, incidence and prevalence have increased, reaching values of 0.16% in 2023 (Figure 1). Case definition was amended by law, and since April 2021, cattle positive for immunological tests shall be subject to further investigation. This change has extended the time needed for confirmation, from 1.2 (2021) to 2.3 months (2023) in the NDF provinces (1.3 in DF provinces). The recovery time of outbreaks in the period 2018-2022 averaged around 8.8 months in NDF provinces and 4.8 months in DF provinces. Finally, the national timescales of 15 days for culling positive heads was respected. Considering 167 bTB outbreaks occurred in 2022, these risk factors were identified: location in a NDF province; herd size; a history (last 5 years) of MTBC; being a suspect case in the previous year; proximity (2 km) to infected farms; being a beef breeding farm; grazing; transhumance (Table 1).

Discussion and Conclusion

Currently, bTB mainly involves beef breeding farms that practice grazing, a common practice in NDF provinces. In NDF provinces, the longer times for confirmation and recovery of outbreaks increase the risk of MTBC spread due to the persistence of infection. We registered also a reduced use of complete herd depopulation and a larger use of test-and- cull policy. Due to the low sensitivity of tests, this practice favours a disease recurrence in subsequent years. The new national eradication program addresses these issues with the goal to achieve MTBC-free status by 2030.



	2018	2019	2020	2021	2022	2023
Prevalence	0.20%	0.23%	0.21%	0.14%	0.15%	0.16%
Incidence	0.16%	0.19%	0.15%	0.11%	0.13%	0.16%

Figure 1. Trends of bTB incidence and prevalence at herd level, Italy 2018-2023.

term	Non cases	Cases	OR	std. error	statistic	p value	OR CI95%	
(Intercept)			0	0.25	-33.68	0	0	0
Disease-free Province	72,716	16	*REF*					
Non-disease free Province	35,045	151	19.58	0.26	11.31	0	12.08	34.11
(Intercept)			0	0.24	-30.49	0	0	0
Type_herd=MILK	23,842	18	*REF*					
Type_herd=BEEF Fattening	33,391	13	0.52	0.36	-1.82	0.07	0.25	1.05
Type_herd= BEEF w Breeders	35,810	125	4.62	0.25	6.07	0	2.9	7.84
Type_herd=MIXED	14,718	11	0.99	0.38	-0.03	0.98	0.45	2.07
(Intercept)			0	0.25	-29.81	0	0	0
Housing=INDOOR	27,628	16	*REF*					
Housing=OUTDOOR	21,924	81	6.38	0.27	6.77	0	3.84	11.31
Housing=TRANSHUMANT	3559	7	3.4	0.45	2.7	0.01	1.3	7.96
(Intercept)			0	0.29	-28.78	0	0	0
Herd_size[1,10]	48,684	12	*REF*					
Herd_size[10,100]	44,885	119	10.76	0.3	7.84	0	6.2	20.55
Herd_size[>100]	14,192	36	10.29	0.33	6.99	0	5.51	20.64
(Intercept)			0	0.08	-82.29	0	0	0
Suspect_case2021=NO	107,563	160	*REF*					
Suspect_case2021=YES	198	7	23.77	0.39	8.07	0	9.99	47.65
(Intercept)			0	0.09	-77.31	0	0	0
bTB_History2017-21=NO	106,979	134	*REF*					
bTB_History2017-21=YES	782	33	33.69	0.2	17.8	0	22.52	48.99
(Intercept)			0	0.11	-64.08	0	0	0
bTB_Outbreaks in a 2km radius=NO	100,397	81	*REF*					
bTB_Outbreaks in a 2km radius=YES	7364	86	14.48	0.16	17.21	0	10.68	19.65

Table 1. Results of univariate analysis for bTB risk factors, Italy 2022.

P141

Epidemiology / Risk analysis and communication

A SYSTEMATIC REVIEW AND META-ANALYSIS ON *CAMPYLOBACTER* SPP. PREVALENCE IN CHICKEN IN ITALY

A.A.K. Zarea[1], M.F. Iulietto[1], T. Mandel[2], P. Scaramozzino[1], R. Condoleo[1]

[1]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Italy*
[2]*National Centre for Laboratory Research and Risk Assessment (LABRIS), Estonia*

Introduction
Campylobacter is responsible for campylobacteriosis, the most reported foodborne gastrointestinal infection in humans in the European Union, and broiler meat was the food primarily associated with outbreaks with stronger evidence (1). This study aimed to revise the literature and estimate the pooled prevalence of *Campylobacter* spp. in chicken in Italy from 2003 to 2023, as well as potential subgroup moderators.

Materials and Methods
Three electronic databases (i.e. Web of Science, PubMed and Scopus) were screened to retrieve the relevant articles. In addition, national zoonoses country reports (NZCR) submitted to EFSA were explored. A random-effects model was employed to calculate pooled prevalence estimates from three matrices from chicken (i.e. faeces, skin and meat), and
heterogeneity was assessed using Q-statistic and the I^2 index (2).

Results
The total number of eligible studies was 48. The meta-analysis revealed a similar pooled prevalence of *Campylobacter* spp. in faeces 0.65 and skin 0.67 matrices, whereas meat showed a lower proportion of 0.20, with high heterogenicity detected (I^2= 97.1–98.8%). As for the moderator subgroup analysis, *Campylobacter* spp. prevalence in meat was higher in the published bibliography (0.38) than in the NZCR (0.10). Moreover, *Campylobacter* prevalence in meat matrix was twice higher at slaughterhouse (0.51) than at retail (0.22). In the case of faeces, no significant difference (p-value = 0.5) was detected between the studies conducted at farms or slaughterhouses and sample types (caecal and cloacal samples).

Discussion and Conclusion
Our results showed that this microorganism is frequently detected in all the chicken matrices tested. Moreover, cross- contamination occurring after the primary production steps can impact the prevalence of *Campylobacter* spp. in meat sold at retail. The findings of this study can be used to develop quantitative microbial risk assessment and to assess the exposure of the Italian population through chicken products consumption.

References
1. EFSA and ECDC, (2023). The European Union One Health 2022 Zoonoses Report. EFSA Journal, 21(12), e8442.
2. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med. 2002 Jun 15;21(11):1539-58.

P142

Food Safety

OCCURRENCE OF POTENTIALLY PATHOGENIC *VIBRIO PARAHAEMOLYTICUS* IN BLUE CRABS (*CALLINECTES SAPIDUS*) FROM THE NORTHWESTERN ADRIATIC SEA

S. Rubini[3], F. Barsi[3], B. Bertasi[2], E. Galuppini[2], L. Mangeri[2], S. Todeschi[2], F. Righi[2], A. Scarazzato[2], M. Sampieri[1], R. Taddei[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Bologna, Italy* [2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy* [3]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Ferrara, Italy*

Introduction
The Atlantic blue crab (*Callinectes sapidus*), endemic to the eastern coast of America, colonized all the Italian coastal areas [1]. This invasive species poses threat to shellfish farming and biodiversity. A potential mitigation strategy is the use of blue crab as a food source. However, crustaceans can be a source of human exposure to pathogenic bacteria, such as *Vibrio* spp. Among these, *V. parahaemolyticus* (Vp) is a common cause of seafood-borne infections. Thermostable Direct Haemolysin (TDH) and Thermostable Related Haemolysin (TRH) are the main determinants of virulent strains [2,3]. The aim of this study was to estimate the prevalence of potentially pathogenic Vp in blue crabs from the northwestern Adriatic Sea.

Materials and Methods
From July 2023 to May 2024, 26 samples, consisting of approximately 30 *C. sapidus* individuals each, were collected in the waters bordering Rovigo, Ferrara and Ravenna provinces. For each sample, crabs' tissues were pooled and submitted to microbiological exam for the detection of Vp, according to ISO 21872-1:2017. Vp species confirmation was performed by a PCR targeting the Toxin operon gene (TOX-R). TDH, and TRH genes were detected by end-point PCRs using specific primers designed for each gene. The PCR products and 100 bp ladder were loaded into a 2.5% agarose gel to assess positive samples and the results were observed through a UV transilluminator.

Results
Vp was isolated from 17 of 26 samples. All 17 strains were confirmed to be Vp by TOX-R gene-PCR. One of these isolates was shown to carry the TDH gene and one the TRH gene.

Discussion and Conclusion
Vp was present in 65% (17/26) of the blue crab samples analyzed and two strains carried virulence factors. The use of blue crab for human food consumption may be a way to contain its population in the Italian seas, but it is necessary to consider the risks to the consumer due to the possible presence of pathogenic microorganisms such as Vp and other vibrios, whose proliferation is known to be favored by the warming of the aquatic environment [3], exactly as occurs with *C. sapidus.*

References
1. Tiralongo F., et al. Acta Adriatica. 2021
2. Austin B. Vet Microbiol. 2010
3. Smalls J., et al. Front. Microbiol. 2023

P143

Food Safety

FIRST DETECTION OF NOROVIRUSES IN BLUE CRABS (*CALLINECTED SAPIDUS*) COLLECTED IN ADRIATIC SEA (ITALY)

B. Bertasi[4], F. Righi[4], A. Scarazzato[4], E. Galuppini[4], L. Mangeri[4], F. Meletti[4], M.N. Losio[4], R. Taddei[2], G. Dell'Orfano[1], S. Rubini[3]

[1]*AUSL Ferrara – Dipartimento di Sanità Pubblica – U.O.C. Igiene Alimenti di Origine Animale – Az. USL di Ferrara, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna – Bologna, Italy*
[3]*Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna – Ferrara, Italy*
[4]*Istituto Zooprofilattico Sperimentale della Lombardia ed Emilia Romagna – Reparto Controllo Alimenti, Brescia, Italy*

Introduction
The recent appearance and spread of blue crab in Italy has already created a serious problem in shellfish farming, related to their habits and feeding. They are able to ingest large quantity of mussels and clams and, as many invasive species, can host or accumulate new or endemic pathogens. The aim of this study was to investigate Noroviruses presence in blue crabs in cold season, given that its prevalence generally increases during the autumn and winter months.

Materials and Methods
Samples collection of blue crabs and mollusks (from planned monitoring controls) started from November 2023 to March 2024 in Adriatic Sea, Emilia Romagna region, Italy (Table 1). The analysis of shellfish and blue crab was carried out in accordance with ISO 15216-2:2019 guidelines.

IZSLER Registration ID	Date of collection	Type of sample	Virus detected	Ct mean	Province
367763/1	20/11/2023	Mussels	NoV GI	37,35	Ferrara
367763/2	20/11/2023	Mussels	NoV GI	39,12	Ferrara
367763/3	20/11/2023	Mussels	NoV GI	38,41	Ferrara
389905	05/12/2023	Clams	NoV GI	40,56	Ferrara
395913	11/12/2023	Clams	NoV GI	39,51	Rimini
397852	12/12/2023	Clams	NoV GI	37,45	Rimini
389905	05/12/2023	Clams	NoV GII	40,32	Ferrara
397852	12/12/2023	Clams	NoV GII	40,42	Rimini
24675	23/01/2024	Mussels	NoV GII	34,73	Forlì-Cesena
21187	23/01/2024	Blue crab	NoV GII	38,57	Ferrara
30909	02/02/2024	Blue crab	NoV GII	40,82	Ferrara
46785	19/02/2024	Blue crab	NoV GI	38,57	Ferrara
64958	04/03/2024	Blue crab	NoV GII	39,22	Ferrara
73961	12/03/2024	Blue crab	NoV GII	37,69	Ferrara
77558	14/03/2024	Blue crab	NoV GII	39,39	Rovigo

Table 1. Details of samples of shellfish and crabs collected from November 2023 to March 2024: date of collection, type of sample, virus detected, Ct mean and province of origin

Results
Shellfish and crabs collected from the same sites of Emilia Romagna region were both positive for Noroviruses presence (table 1), although there was no match between genotypes identified by Real-time PCR based on ISO 15216-2. In details Norovirus was detected in 20.6% of molluscs samples; Noroviruses were detected in all blue crab samples, taken from the South part of Po Delta.

Discussion and Conclusion
results obtained suggest the possibility of detecting Noroviruses in crabs, when viral circulation has already been demonstrated at the sampling site. Organized controls and the evaluation of various factors, such as crab handling and cooking procedures, would allow us to verify the presence of a potential human health risk from the diffusion of this species in national waters. The current study presents the first detection of Norovirus in blue crab in Italy.

References
Zotti M., et al. H NMR metabolomic profiling of the blue crab (Callinectes sapidus) from the Adriatic Sea (SE Italy): A comparison with warty crab (Eriphia verrucosa), and edible crab (Cancer pagurus). Food Chem 2016, 196, 601-609

Mangeri L., et al. Monitoring and Genotyping of Norovirus in Bivalve Molluscan Shellfish from Northern Italian Seas (2018–2020). Foodborne Pathog Dis 2024, 21 (1), DOI: https://doi.org/10.1089/fpd.2023.0078

P144

Food Safety

HIGH PRESSURE PROCESSING (HPP) TREATMENT TO KILL *ANISAKIS* IN FISH FILLET AND NEAR INFRARED SPECTROSCOPY TO VERIFY ITS APPLIANCE: PRELIMINARY DATA

G. Esposito[3], E. Biasibetti[3], R. Provera[3], F. Pennisi[3], A. Brugiapaglia[2], M. Pezzolato[3], A. Greco[1], E. Bozzetta[3]

[1]*Azienda Unità Sanitaria Locale di Parma Dipartimento di Sanità Pubblica Parma, Italy*
[2]*Department of Agriculture, Forest and Food Sciences (DISAFA) University of Torino, Grugliasco, Italy*
[3]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy*

Introduction
To meet the demands of the seafood market, companies must necessarily develop new processing techniques aimed at offering adequate shelf life and safety of raw products. The main problem linked to raw fish is the presence of bacteria and/or parasites which can cause serious effects on consumers' health. One of the most dangerous parasite infecting raw fish is *Anisakis* spp (A). Recently, an EFSA opinion identified high pressure treatment (HPP) as an effective method for killing this parasite [1], thus providing for its adoption among effective treatments under current legislation (Regulation 853/2004 and 1276/2001). We will explore whether NIR coupled with chemometrics approach can discriminate between fish samples subjected or not to HPP treatments.

Materials and Methods
Fish fillets (n=20) of farmed Sea bream (*Sparus aurata*) were divided in two groups, identified as infested (T) with three larvae of A and not infested (NT). All samples, except one T were subjected to HPP for 290 sec at 5930 bar. NIR spectra acquisitions were performed with a portable NIR device (VIAVI), PRE and POST HPP treatment. The raw data were analysed with Unscrambler software. The LDA classification model was built using the spectral datasets between 1300-1440 nm.
After HPP treatment the vitality of Anisakis was evaluated by using a specific protocol.

Results
All larvae extracted from fish fillets subjected to HPP died, while larvae taken from a sample infested and not subjected to HPP were still alive. Good classification between the samples PRE and POST HPP was found with both principal component analysis (PCA) and linear discriminant analysis (LDA). In details, the PCA score plot was able to efficiently classify PRE and POST samples based on PC1 (94% of explained variance) and PC2 (4%). The LDA classification model adopted showed 100% classification accuracy.

Discussion and Conclusion
The growing consumer demand for high-quality raw products becomes an opportunity to develop new strategies to ensure product safety in short time suggesting HPP as a valuable strategy to kill A. In addition, the preliminary results from NIR spectroscopy coupled with chemometrics suggest the ability of this approach to discriminate fish fillets treated with HPP from those not treated.

References
EFSA Journal. 2024;22:e8719

P145

Food Safety

DETERMINATION OF 2-DODECYLCYCLOBUTANONE IN WHOLE EGGS FOR THE IDENTIFICATION OF IONIZING RADIATION TREATMENT

M. Campaniello[1], M. Tomaiuolo[1], R. Zianni[1], A. Chiappinelli[1], A. Mentana[1], M. Iammarino[1], V. Nardelli[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata – Laboratorio Nazionale di Riferimento per il trattamento degli alimenti e dei loro ingredienti con radiazioni, Foggia, Italy*

Introduction

The consumption of eggs and egg products has increased in recent years due to big request of high protein foods. On the other hand, eggs can be vehicles of foodborne diseases. Ionizing radiation is a non-thermal, safe and effective technology used to inactivate pathogens and make food safer. Nowadays, the irradiation of whole eggs is not permitted in Europe [1], so, control methods to identify any illicit treatments, especially on imported products, are essential for Member States.
In this study, the chemical method for the identification of the radiolytic marker 2-dodecylcyclobutanone (2-DCB), already used for meat products inspection [2], was optimized for the analysis whole eggs irradiated at 0.5 kGy.

Materials and Methods

The analytical method involves headspace solid phase microextraction (HS-SPME) of 2-DCB coupled with gas chromatography-mass spectrometry (GC/MS) analysis. The identification of 2-DCB was performed monitoring the 98 m/z and 112 m/z ions and the analyte was confirmed if their area ratio was in the range of 22.5-25.0%.

Results

In order to optimize the HS-SPME-GC/MS method, the quantity of matrix was evaluated using 5 g and 2 g of both non-irradiated and irradiated samples of homogenized whole egg, obtaining the best result using 2 g of product. Subsequently, a preliminary validation was carried out evaluating method selectivity, linearity in solvent (water) and in matrix. About selectivity, no peaks attributable to 2-DCB were identified in non-irradiated samples and the presence of matrix interferents was not highlighted, confirming method selectivity (Figure 1). The linearity was evaluated in the concentration range between 5.0 and 40.0 ng/mL of 2-DCB in water and between 5.0 and 80.0 ng/mL in matrix, obtaining a value of $R^2 \geq 0.99$ for both.

Discussion and Conclusion

The HS-SPME-GC/MS method, already accredited for meat products analysis, was successfully applied to non-irradiated and irradiated whole egg samples at a dose of 0.5 kGy. Moreover, a preliminary validation confirmed the possibility to use the HS-SPME-GC/MS method within official laboratories for routinely control activity. Thanks to the Italian Ministry of Health who financed the Research Project IZS PB 05/23 RC.

References

1. Official Journal of the European Union 2009/C, 283/02
2. Campaniello M, et al. Int. J. Food Sci. Tech. 55 (2020) 961–969

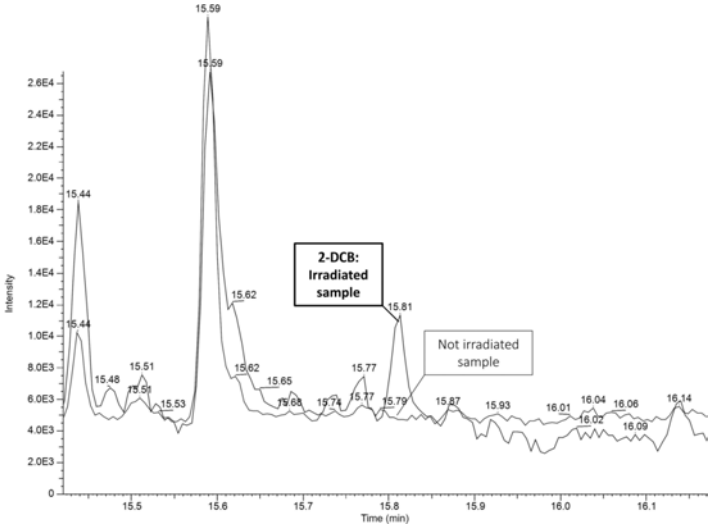


Figure 1. Overlay of two chromatograms (Total Ion Current) of egg samples not irradiated and irradiated at 0.5 kGy.

P146

Food Safety

RISK ASSESSMENT RELATED TO ANTIMICROBIAL-RESISTANT PATHOGENS IN READY-TO-EAT FOODS

L. Capozzi[1], A.M. Derobertis[1], M. Solito[1], L.M. Di Fato[1], S. Castellana[1], R. Fraccalvieri[1], L. Del Sambro[1], A. Bianco[1], E. Catalano[1], A. Caffò[1], A. Giannico[1], D.A. Raele[1], P. Di Taranto[1], M. Caruso[1], A. Parisi[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*

Introduction

Food can play a key role in the transmission of AMR microorganisms, and antibiotic-resistant bacteria can contaminate food at any stage of production. There is a real health risk for consumers of RTE foods, especially for the most susceptible patient groups, as genes responsible for resistance can be transferred to bacterial strains in the gut microbiota. The purpose of this study was to evaluate the presence of antimicrobial-resistant bacterial strains in RTE foods.

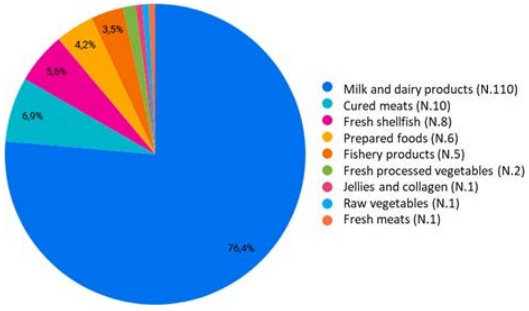


Figure 1. Distribution of the 144 matrices sampled in 5 months into RTE food categories.

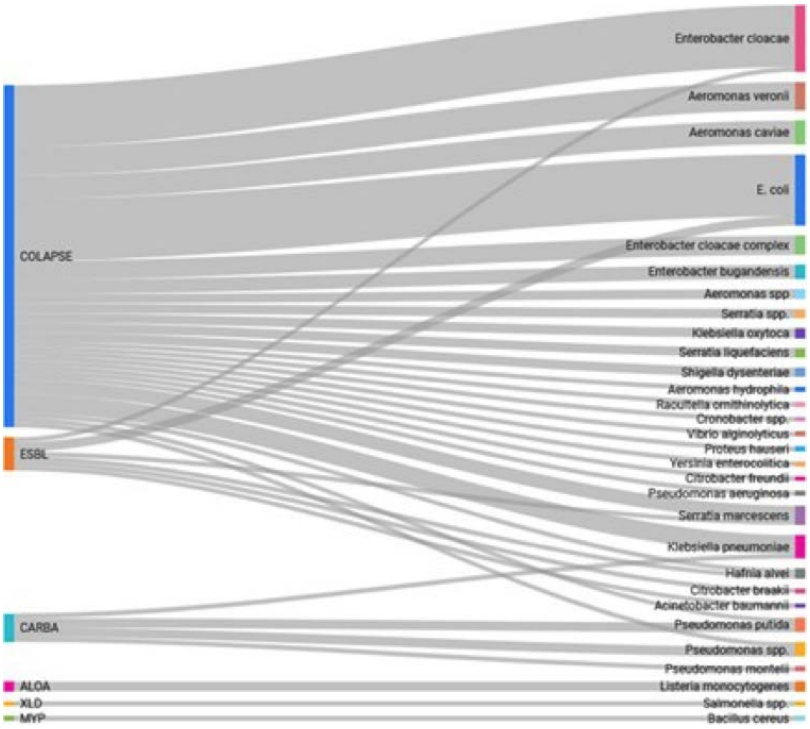


Figure 2. Displaying the selective media (COLAPSE, ESBL and CARBA) that allowed the growth of the following bacterial species.

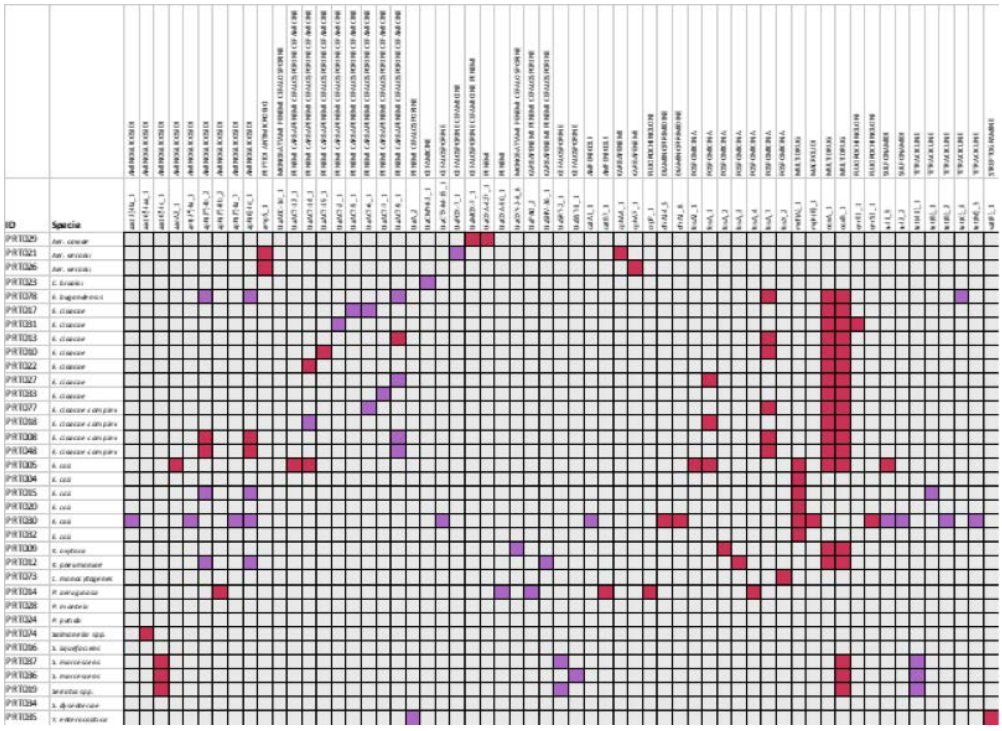


Figure 3. Schematic view of the resistome related to the 35 bacterial strains (ID) subjected to NGS, whose species is indicated in the second column. Resistance genes, grouped by drug class, detected in the genomic sequence are listed in the column. Predicted genes are indicated in magenta; those not detected or not tested in gray; genes whose resistance was also found *in vitro* are indicated in purple.

Materials and Methods

From November 2023 to April 2024, n.144 matrices of RTE foods were sampled (Figure 1) under different surveillance and control plans. Isolation was carried out on chromogenic and selective media for bacteria resistant to major antibiotics: CARBA, ESBL and COLAPSE agar. For isolated bacterial strains, an early taxonomic identification was conducted by MALDI-TOF and antimicrobial susceptibility was assessed by determination of MIC values. A selection of strains was subjected to DNA extraction and preparation of genomic libraries. WGS was performed on Illumina Miseq platform (1) and data analysis allowed for species identification (rMLST), genotyping (MLST, cgMLST) and resistome analysis (ResFinder).

Results

Potentially resistant bacteria were isolated from 61.8% of the analyzed matrices. Several genera of bacteria were isolated on the selective media (Figure 2). To date, n. 80 bacterial strains have been isolated. Of these strains, 86.2% were resistant *in vitro* to at least one antibiotic. Thirty-five bacterial strains were subjected to WGS, and resistome analysis detected the presence of AMR genes in 88.6% of the strains. For 35.4 % of these isolates, AMR genes not corresponding to *in vitro* resistance were found in silico (Figure 3).

Discussion and Conclusion

Although the convenience sampling conducted on RTE foods seems to be affected by the type of matrix mainly sampled in the target area (milk and dairy products), we are isolating several resistant bacteria from RTE matrices. To date, the data obtained are important and could provide the basis for risk assessment in the consumption of RTE foods.

References

1. DOI: 10.3389/fmicb.2020.599524

P147

Food Safety

THE VETERINARY DRUG FROM THE ANIMAL TO THE ENVIRONMENT: LC-HRMS ANALYTICAL INVESTIGATION ON THE CYCLIC FATE OF TULATHROMYCIN

F.T. Cherchi[1], N. Rubattu[1], S. Salis[1]

[1]*Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*

Introduction
As part of a research project on the evaluation of the environmental impact of veterinary drugs, we followed the "pathway" of tulathromycin after administration to a group of calves from a farm located in the Marghine subregion in Sardinia. Tulathromycin (trade name Draxxin, produced by Pfizer) is a macrolide antibiotic used for the treatment of bovine respiratory disease (BRD) associated with several pathogenic species and also in the treatment of infectious bovine keratoconjunctivitis (IBK) associated with Moraxella bovis [1]. We developed and validated an LC-Orbitrap-HRMS analytical method for the quantitative detection of tulathromycin residues in three livestock breeding matrices, namely animal feces, storage slurry and hay produced on the farm.

Materials and Methods
The method involves the same treatment procedure for all sample types, which consists of an extraction step with ACN/ H2O followed by SPE purification and final concentration/redissolution before instrumental analysis. Instrumental analyses were performed using the Dionex Ultimate 3000 UHPLC system coupled with the Q-Exactive Orbitrap HRMS (Thermo Fisher Scientific, MA) with a thermal electrospray ionization (HESI-II) source, operating in positive ion mode and using a Poroshell 120 EC-C18 3.0 x 100 mm, 2.7 µm chromatographyc column (Agilent, CA).

Results
The method has been validated according to the document SANTE/11312/2021 and has sensitivity specifications (LoQ) of 1.3 µg/kg in manure and faeces and 4 µg/kg in forage. Residues of tulathromycin were detected in all the matrices analyzed, demonstrating its cyclical fate: it arrives from the animals to the environment through feces and returns to the animals as a contaminant of the forage on which they feed.

Discussion and Conclusion
The analytical results show that feces and sewage analyses can be used as a useful non-invasive investigation tool suitable for the collection of experimental data for the overall evaluation of the incidence of veterinary pharmacological residues in the environment.

References
1. Berendsen B.J.A, Wegh R.S., Memelink J., Zuidema T., Stolker L.A.M. Talanta (2015), 132: 258-268.
2. Draxxin /European Medicines Agency (europa.eu)
3. Sarmah, A. K., Meyer, M. T., Boxall, A. B., Chemosphere (2006), 65(5), 725–59.

P148

Food Safety

DEVELOPMENT AND EVALUATION OF A DNA METABARCODING METHOD FOR SPECIES IDENTIFICATION IN MEAT PRODUCTS

L. Del Sambro[1], S. Castellana[1], A. Bianco[1], L. Capozzi[1], A.M. Derobertis[1], M.E. Gallitelli[1], C. Trisolini[1], D. Ridolfi[1], E. Buzzi[1], D. Farina[1], V. Rondinone[1], P. Di Taranto[1], A. Donatiello[1], A. Parisi[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*

Introduction
Food fraud is an increasing problem worldwide; the products most susceptible to adulteration include meat foods. Globally, food fraud cases in the meat sector have also been reported, including the sale of mislabeled red meat and poultry products (1). In this research project we aimed to implementing a molecular investigation based on the metabarcoding approach making use of NGS for the identification of species from meat food.

Materials and Methods
Sixteen pure and mixed meat samples were included in this study. The extraction of genomic DNA was carried out with the TANBead Food and Feed DNA Kit, and two 120-bp mammalian and poultry fragments of the 16S mitochondrial ribosomal DNA gene were used as the DNA barcode (2). Amplicons were prepared following the protocol for the Library preparation of metagenomics sequencing (Illumina)

and sequenced on Illumina MiSeq platform. After paired- end sequencing and amplicons alignment, the fragments were submitted to BLASTn and compared with the species declared on the label.

Results

The results related to the analyses conducted by metabarcoding approach are shown in Table 1. Based on genetic analysis, all eight samples labeled as pure chicken were found to contain >99% chicken meat. As regards the 2 samples of chicken and turkey kebabs, the analysis confirmed the presence of both species, but with a higher percentage of chicken than turkey. Beef and pork meat showed >90% identity percentages, while the equine sausage sample (MBC6) showed 15% of Sus scrofa meat contamination.

Discussion and Conclusion

The genetic strategies proposed in this research project are showing highly efficient results for the identification of commercialized biological tissues of meat samples. Additional samples will be processed to standardize the metabarcoding technique; experimental mixtures of known samples (previously sequenced) will be prepared and sequenced on Illumina Miseq platform, in order to establish the accuracy of the experimental procedure in quantifying species and the possible application of this method to detect adulteration of meat species in routine analysis.

References

1. DOI: 10.1016/B978-0-12-817242-1.00012-9
2. DOI: 10.1016/j.foodchem.2018.08.032

Sample ID	Product description	Primer (M= mammalian; P= poultry)	Declared species	Species identified	Total numer of Amplicon Sequence Variant	Target
MBC1	cooked ham	M	Sus scrofa	Sus scrofa (99.95%)	283842	16S
MBC2	chicken cutlet	P	Gallus gallus	Gallus gallus (>99.99%)	436803	16S
MBC3	Hamburger	M	Bos taurus, Sus scrofa	Bos taurus (60%), Sus scrofa (37%)	349439	16S
MBC4	Kebab	P	Gallus gallus, Meleagris gallopavo	Gallus gallus (99.6%) , Meleagris gallopavo (0.4%)	392048	16S
MBC5	Kebab	P	Gallus gallus (75%), Meleagris gallopavo (10%)	Gallus gallus (99.69%) , Meleagris gallopavo (0.31%)	287064	16S
MBC6	horse sausages	M	Equus caballus	Equus caballus (85%), Sus scrofa (15%)	269700	16S
MBC7	chicken meat	P	Gallus gallus	Gallus gallus (100%)	656880	16S
MBC10	bovine minced meat	M	Bos taurus	Bos taurus (90%), Sus scrofa (8.22%), other (1.78%)	303440	16S
MBC11	carpaccio of cattle	M	Bos taurus	Bos taurus (97.65%), Bos taurus nonspecific (1.04%), other (1,31%)	340053	16S
MBC12	chicken meat	P	Gallus gallus	Gallus gallus (99.67%), other (0.33%)	424266	16S
MBC13	chicken meat	P	Gallus gallus	Gallus gallus (99.72%), other (0.28%)	296031	16S
MBC14	slices of veal	M	Bos taurus	Bos taurus (98.88%), other (1.13%)	232344	16S
MBC15	chicken meat	P	Gallus gallus	Gallus gallus (>99.99%)	377824	16S
MBC16	chicken meat	P	Gallus gallus	Gallus gallus (>99.99%)	352344	16S
MBC17	chicken cutlet	P	Gallus gallus	Gallus gallus (99.83%), other (0.17%)	393589	16S
MBC18	chicken meat	P	Gallus gallus	Gallus gallus (99.95%), other (0.05%)	353102	16S

Table 1. results obtained by NGS sequencing

P149

Food Safety

PERSISTENCE OF HEPATITIS E VIRUS (HEV) SUBTYPES 3C AND 3E: LONG-TERM COLD STORAGE AND HEAT TREATMENTS

G. Ianiro[2], M. Monini[2], L. De Sabato[2], M. Bivona[1], G. Vaccari[2], F. Ostanello[1], I. Di Bartolo[2]

[1]*Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Italy*
[2]*Dipartimento di Sicurezza Alimentare, Nutrizione e Sanità Pubblica Veterinaria, Istituto Superiore di Sanità, Italy*

Introduction

Hepatitis E virus (HEV) is the causative agent of foodborne infections in high income countries mainly transmitted by consumption of undercooked and raw pork products [1]. Several studies proved the presence of HEV-RNA in pork products [2]. However, the detection of HEV nucleic acids does not necessary correspond to presence of infectious virus and information on the persistence of the virus in food is still limited.

Materials and Methods

Viral stocks of HEV-3c and -3e strains isolated from naturally infected pigs [3] were used in this study. For long-term storage, viral stocks were stored at 4°C or -20°C for 3, 6 and 12 weeks. Heat treatments were conducted at 56°C, 65°C, 72°C, and 93°C for different treatment

times (3, 6, 12 and 60 minutes). After treatments, viruses were inoculated on A549 cells for 21 days to evaluate the residual infectivity of HEV.

Results
Results confirmed that low temperature storage or freezing do not influence the survival of the virus, and only a moderate reduction of presence of its RNA after 12 weeks at 4°C. Heating at temperatures of 56°C, 65°C, and 72°C for 3, 6, 12, and 60 min resulted in progressively higher levels of inactivation. Viruses were completely inactivated when treated at 93°C.

Discussion and Conclusion
The proper manipulation of food plays a central role in preventing foodborne infections. Concerning HEV, the main risk of foodborne transmission, is represented by pig liver and pork products containing liver, and the manipulation procedure. At the basis of the risk reduction is the use of the correct cooking time and temperature to inactivate adequately the infectious virus. Understanding the effect of temperature on HEV inactivation is also important to evaluate virus environmental persistence and consequently its effect on viral transmission.

References
1. Chelli, E., et al. 2021. Hepatitis E Virus Occurrence in Pigs Slaughtered in Italy. Animals 11.
2. Colson, P. et al. 2010. Pig liver sausage as a source of hepatitis E virus transmission to humans. J Infect Dis 202.
3. Ianiro, G., et al. 2023. In Vitro Replication of Swine Hepatitis E Virus (HEV): Production of Cell-Adapted Strains. Animals 13.

P150

Food Safety

USE OF BDELLOVIBRIO AND LIKE ORGANISMS (BALOS) AS A FOOD SAFETY DEVICE

S. Di Lullo[1], G. Angelico[1], S. Pieralisi[1], G. Talevi[1], S. Nardi[1], E. Rocchegiani[1], S. Fisichella[1], D. Ottaviani[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Italy*

Introduction
The study is part of a project carried out in collaboration with a company which produces sprouting seeds. The research investigates a possible biological approach, as an alternative to the use of physical and chemical means (e.g. biocides), to contain undesirable bacteria in a food product. In particular, the project aims to use bacteria harmless to humans, BALOs (Bdellovobrio and like organisms), like predators capable of kill and/or contain gram-negative pathogenic bacteria such as *E. coli* STEC and *Salmonella*, possible contaminants of sprouts, in line with Commission Regulation (EC) No 2073/2005 which sets analytical limits for these microorganisms in this type of food matrix.

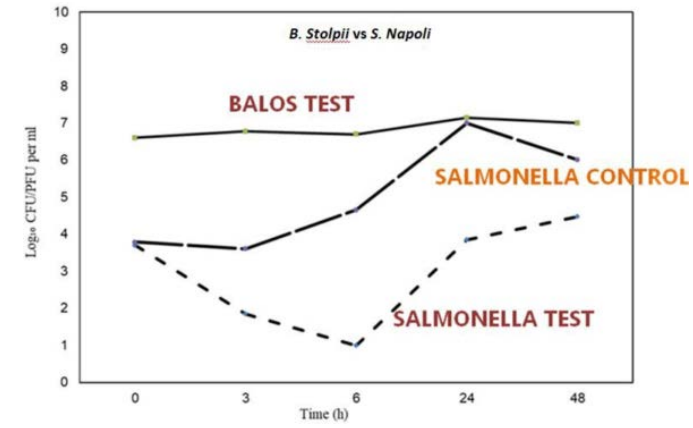
Materials and Methods
BALOs were isolated from seawater and freshwater by the plaque assay, using E.coli, *E. coli* STEC, *Salmonella* as prey; they were then identified by PCR and characterised subsequently by whole genome sequencing (1). A BALOs belonging to the species *Bacteriovorax* stolpii was selected to perform prey/predator challenge test (2) to assess predation capacity and predation dynamics over 48 h. BVB (BdelloVibrioBroth) tubes were prepared and divided into two series: TEST - tubes contaminated with 0.5 ml O/N enrichment in BVB of *B. stolpii* (filtered through a 0.20 µm syringe filter) + 0.5 ml O/N enrichment of the prey in BHI (Brain Heart Infusion), starting with a predator/prey ratio $10^7/10^5$ PFU/CFU/ ml. CONTROL - tubes prepared in the same condition of test without B. stolpii. The tubes were incubated at 25°C and analysed at 0, 3, 6, 24 and 48h to assess the level of prey and predator.

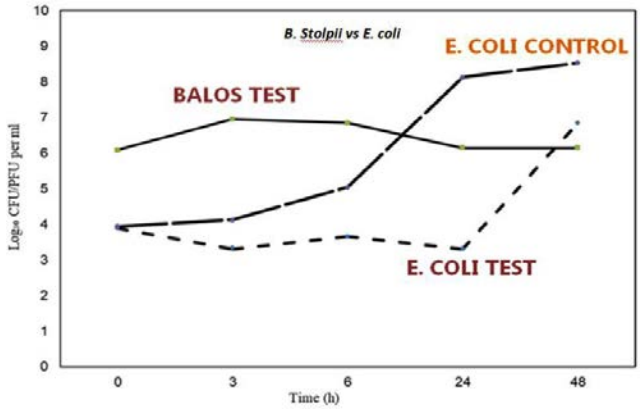
Results
The results showed the ability of BALOs to contain the proliferation of target preys, with a difference in the microbial load between TEST (balos against prey) and CONTROL (prey only) of up to 5 log CFU/ml.

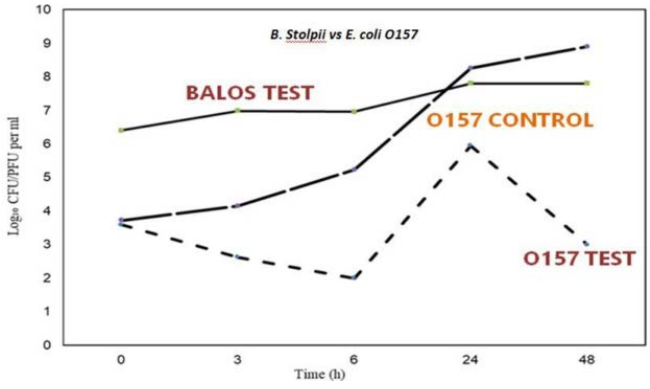
Discussion and Conclusion
B. stolpii could find application in food to reduce the microbial load of target bacteria and therefore increase its healthiness. In the following, experiments will be conducted on food matrix using *B. stolpii* and indigenous microbial communities.

This work was financed by the Ministero della Salute (RC07/2023)

References
1. Davidov, Y. et al (2006). doi:10.1111/j.1462-2920.2006.01101.x.
2. Pieralisi, S., et al (2023). doi.org/10.15586/IJFS.V35I2.2324







P151

Food Safety

ENHANCED IMMUNE RESPONSE AND TOLERANCE MECHANISMS IN *CAMPYLOBACTER JEJUNI*-INFECTED CHICKENS WITH PROBIOTIC TREATMENT

C. Di Pancrazio[2], F. Marotta[3], C. Merola[2], A. Cocco[2], V. D'Innocenzo[2], A. Petrini[2], F. Sacchini[2], C. Villani[3], F. Perletta[2], M. Maggetti[2], I. Krasteva[2], E. Felicioni[3], S. Salucci[2], M. Luciani[1], G. Garofolo[3]

[1]*Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Teramo, Italy*
[2]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy*
[3]*National Reference Laboratory for Campylobacter, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy*

Introduction

Campylobacter jejuni causes significant gastroenteritis in the EU, with severe inflammation in humans. Poultry, often asymptomatic, har-

bor *C. jejuni*, impacting their immune system. This study examined broiler immune responses, showing a decrease in anti-inflammatory cytokines and an increase in certain chemokines. Probiotic treatment significantly affected IgY levels, suggesting enhanced immune responses in treated chickens.

Materials and Methods

Sixty broiler chicks one-day-old were infected with *C. jejuni* strain M1 and fed either a conventional diet or a diet supplemented with a prebiotic and probiotic mixture. Additionally, a control group of uninfected chicks was established. The *C. jejuni* load in the caecum was monitored at 13, 27, 35, and 41 days post-infection. A MILLIPLEX® Chicken Cytokine/Chemokine Panel quantified 12 analytes, and IgY levels were measured using the Abcam IgY Chicken ELISA Kit. Significant differences between groups were evaluated using a t-test.

Results

Upon arrival, the chicks tested negative for *Campylobacter*. By day 5, *C. jejuni* colonization reached 7.23 ± 0.74 log10 CFU/g, decreasing gradually over time. The chicks showed no significant clinical signs, maintained good weight gain, and had an expected feed conversion ratio, with no mortality. The avian cytokine/chemokine profile indicated a significant decrease in anti-inflammatory cytokines. IgY levels were compared at each time point between the probiotic-treated group G5 and control group G6. A significant decrease in IgY levels was observed at t3 (p=0.023) in probiotic treatment group.

Discussion and Conclusion

The avian cytokine/chemokine profile analysis showed a significant reduction in most of the anti-inflammatory cytokines over time. Overall, cytokine expression was significantly downregulated compared to the control group. Despite high-level cecal colonization of *C. jejuni*, no noticeable pathology occurred, indicating a shift towards immune tolerance. Probiotic treatment in-group G5 modulated the immune response to *C. jejuni*, leading to significant IgY level differences at t3, suggesting specific immunological modulation.

References

doi.org/10.3389/fmicb.2020.583429
doi: 10.1371/journal.pone.0247080. PMID: 33720955; PMCID: PMC7959354

Time point	Cage 5 (Probiotics) IgY (mg/ml)	Cage 6 (Controls) IgY(mg/ml)
t0	1,48	1,52
t1	4,02	4,82
t2	6,02	5,95
t3	4,55	8,54

Table 1. Levels of IgY antibodies in cages treated with probiotics (Cage 5) and control cages (Cage 6) measured in mg/ ml at different time points (t0, t1, t2, t3).

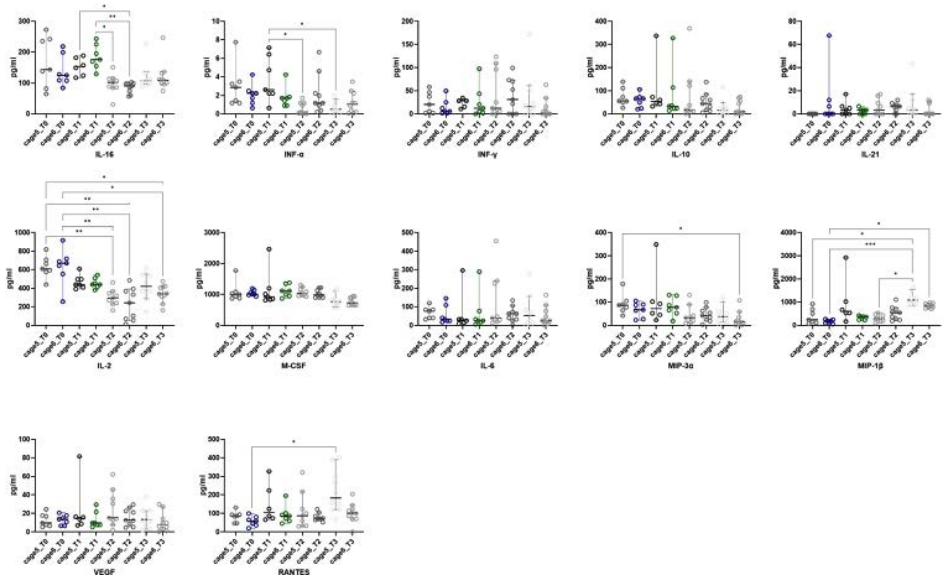


Figure 1. Levels of cytokines/chemokines in cages treated with probiotics (Cage 5) and control cages (Cage 6) measured in mg/ml at different time points (t0, t1, t2, t3).

P152

Food Safety

MOLECULAR APPROACH IN EPIDEMIOLOGICAL INVESTIGATIONS: AN OUTBREAK OF LISTERIOSIS FROM BLACK OLIVES

P. Di Taranto[3], L. Capozzi[5], S. Castellana[5], M. Solito[5], A. Castellana[5], S. Morabito[2], L. Addante[5], C. Trisolini[5], M. Caruso[4], C. Pedarra[3], V. Manzulli[3], V. Rondinone[3], G. Normanno[1], A. Parisi[3]

[1]*Dipartimento di scienze agrarie, alimenti, risorse naturali e ingegneria, Università degli Studi di Foggia, Italy.*
[2]*Istituto Superiore di Sanità, Rome, Italy*
[3]*Istituto Zooprofilattico della Puglia e della Basilicata, Foggia, Italy.* [4]*Istituto Zooprofilattico della Puglia e della Basilicata, Matera, Italy*
[5]*Istituto Zooprofilattico della Puglia e della Basilicata, Putignano (BA), Italy*

Introduction

Listeria monocytogenes is the causative agent of listeriosis, one of most serious foodborne diseases characterised by severe clinical pictures and high mortality rates (1). Several human cases of listeriosis were recorded in Italy in 2023 and the related isolates were genetically characterised for the purpose of epidemiological investigations.

Materials and Methods

From February to September 2023, 20 human *L. monocytogenes* isolates of Apulian clinical cases were subjected to DNA extraction and Whole genome sequencing (WGS) (Illumina Miseq platform) at the Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata (2). Genotyping of the strains by MLST and cgMLST was conducted on the IRIDA-ARIES platform (Istituto Superiore di Sanità). From September 2023 to June 2024, following the epidemiological surveys conducted on clinical cases, 130 environmental swabs and 4 food samples were collected from a suspected olive company and analysed according to the ISO 11290-1:2017 and ISO 11290-2:2017. Screening analysis (MLVA) was conducted on all strains isolated from these samples and one strain from each genetic profile was subjected to WGS.

Results

The cgMLST analysis identified 15 of the 20 Apulian human isolates as belonging to ST1 and genetic cluster 291. Matching with shared data on reference databases allowed to associate these isolates with an outbreak of listeriosis that involved 51 Italian cases (Figure 1), related to the consumption of baked black olives produced by an Apulian company. None of the food samples was positive. All strains isolated from swabs were found to belong to the same MLVA genetic profile. Therefore, WGS was conducted only on one strain isolated from swabs and cgMLST analysis allowed to genetically relate it to all 15 clinical isolates (Figure 2).

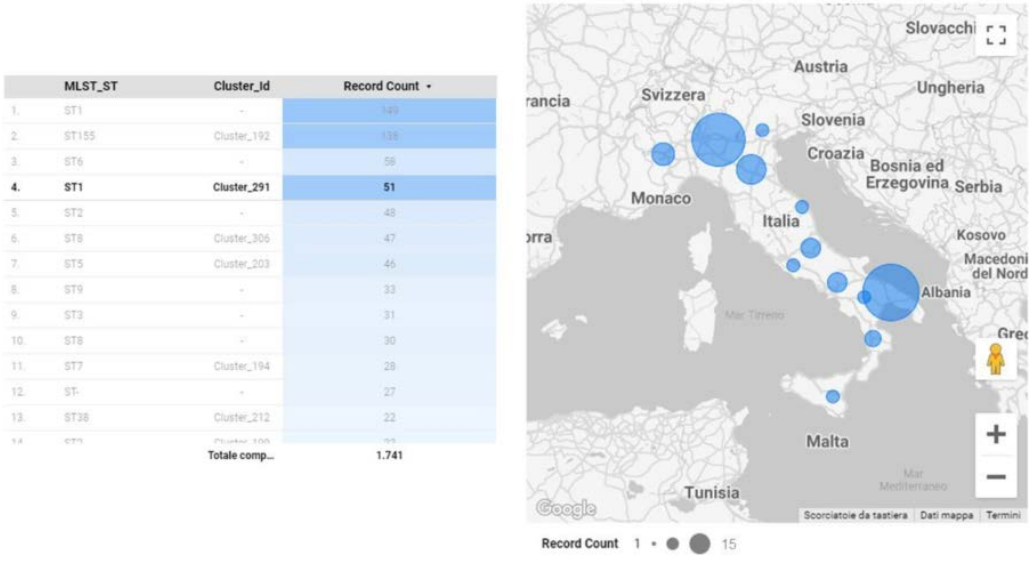


Figure 1. Out of 1741 genomes of human isolates of *L. monocytogenes* available on IRIDA-ARIES, representation of the geographical distribution of Italian clinical cases (n. 51/1741) referable to cluster 291. The 15 strains isolated in Apulia and sequenced by IZS della Puglia e della Basilicata are also included.

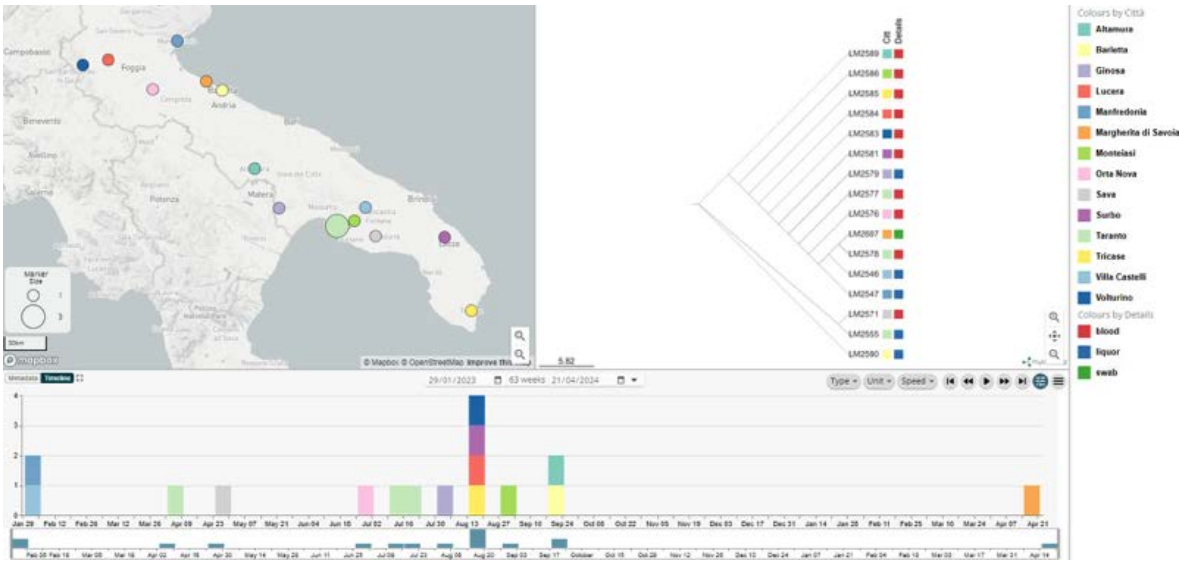


Figure 2. Image created with Microreact, geographically representing the cities of residence of the Apulian patients involved in the listeriosis outbreak (Cluster 291). The timeline shows the dates of sampling of clinical (blood, CSF) and environmental (swab) samples. A dendrogram obtained from the cgMLST data has been generated in the right panel.

Discussion and Conclusion

The results demonstrate how the WGS-based molecular approach and data sharing enable correlation with high specificity (comparison of 1748 gene loci) of *L. monocytogenes* strains isolated from human clinical specimens, the environment and food.

References

1. The European Union One Health 2022 Zoonoses Report. EFSA Journal. 21(12).
2. Bianco, A., et al. Characterization of *Bacillus* cereus group isolates from human bacteremia by WGS. Frontiers in microbiology, 2021,11:599524.

P153

Food Safety

CANNABINOIDS AND FOOD SAFETY: STRATEGY FOR MONITORING AND PERSPECTIVES

P. Gallo[1], A. Gallo[1], L. Ambrosio[1], M. Esposito[1], V. Urbani[1], G. Guadagnuolo[1], O. Arace[1], A. Siciliano[1]

[1]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy*

Introduction

From 2016 the Italian law [1] supports hemp cultivation, for many applications, also as food. Hemp is even authorized as feed. Hemp seeds, as well as the oil and flour derived, contain natural cannabinoids, showing nutritional effects. In 2016 an EU Recommendation [2] stated to monitor the natural levels of some cannabinoids in hemp derived food, to get data for health risk assessment by the EFSA and to set maximum limits for the psychoactive Δ-9-tetrahydrocannabinol (Δ9-THC) and its acidic precursor THCA-A, then issued in 2023 [3]. The Recommendation indicated to use methods based on mass spectrometry (MS) detection and to study possible carry over of cannabinoids from feed to animal derived food. A study about this topic is actually carried on in our Institute.

Materials and Methods

Hemp seeds, oil, flour, derived food, milk, drinks and feeds were collected between 2016 and 2023 in Italy, to monitor the levels of 9 cannabinoids, including Δ9-THC and THCA-A. The samples were analysed by accredited methods based on LC-MS/MS on QTRAP and LC-HRMS/MS on Q-Exactive Orbitrap. The experimental study about possible carry over from feed to animal derived food was based on 4 groups of goats and a negative control group. Milk samples were collected and analysed by LC-MS/MS.

Results

The data about Δ9-THC and THCA-A content were evaluated by the Italian Ministry of Health to set national maximum limits in 2019, that were updated in 2023 by the EU regulation [3]. The monitoring data were sent to the EFSA as the Italian contribution for risk assessment, issued in a Scientific Opinion in 2020. The data about the possible carry over of cannabinoids in goat milk are currently being evaluated.

Discussion and Conclusion
Our Institute was appointed to collect data for the Italian and the EU monitoring of cannabinoids in food and feed and to support detective actions. The perspective is to support the Italian production chain of hemp and derived food, to ensure effective official control and to update data for risk assessment and further evaluation by the EFSA.

References
1. Legge 2 dicembre 2016 n. 242, GU n.304 del 30.12.2016.
2. Commission Recommendation (EU) 2016/2115, OJ L 327, 2.12.2016, p. 103–104.
3. Commission Regulation (EU) 2023/915, OJ L 119, 5.5.2023, p. 103–157

P154

Food Safety

DETECTION OF NON-COMPLIANT CONCENTRATIONS OF CADMIUM, MERCURY AND LEAD IN SEAFOOD PRODUCTS OVER A TEN-YEAR PERIOD

L. Garofalo[1], M.G. Sala[1], C. Focardi[2], P. Pasqualetti[3], D. Delfino[1], P. Scaramozzino[1], K. Russo[1]

[1]*Istituto Zooprofilattico del Lazio e della Toscana M. Aleandri, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri. Via di Castelpulci, San Martino alla Palma (Firenze), Italy*
[3]*Sapienza Università di Roma, Dipartimento di Sanità Pubblica e Malattie Infettive. Piazzale Aldo Moro 5, Roma, Italy*

Introduction
The European Food Safety Authority (EFSA) continuously monitors food contaminants to mitigate health risks. Commission Regulation (EC) No. 2023/915, replacing Regulation (EC) No. 1881/2006, sets maximum levels of contaminants, including cadmium (Cd), mercury (Hg) and lead (Pb) in seafood, due to their high toxicity. To ensure compliance with regulations and protect consumer health, Italian veterinary services and Istituto Zooprofilattico del Lazio e della Toscana M. Aleandri (IZSLT) routinely conduct surveillance and diagnostic tests to monitor the levels of these heavy metals in molluscs, fish, echinoderms and crustaceans.

Materials and Methods
Over a ten-year period (2014-2023), 5˙874 seafood samples underwent 9˙831 chemical analyses on Cd, total Hg and Pb.

Results
Overall, 144 samples (2.45% out of those analysed) were non-compliant (n.c.) with regulations (Table 1). These samples were primarily collected during official inspections at ports, airports, warehouses and fish markets by border control agents and local health authorities. Besides sporadic combinations of taxon/analyte (Table 1), concentrations of Cd exceeding the limits were mainly found in cephalopod molluscs (N=17; [Cd] range 1.40-5.60 mg/kg, mean 2.96±1.46 mg/kg), especially in squids and flying squids (Figure1). Mercury concentrations above permitted limits were found predominantly in marine fish (N=118; Figure2), mainly in swordfish Xiphias gladius (N=59, i.e. 11.30% n.c. samples out of the total analysed for this species; [Hg] range 1.30-2.90 mg/kg, mean 1.78±0.38 mg/kg), shark species (N=16, i.e. 6.45% n.c. samples out of the total analysed for this group; [Hg] range 1.30-3.80 mg/kg, mean 2.01±0.85 mg/kg) and tuna species (N=14, i.e. 3.06% n.c. samples out of the total analysed for this group; [Hg] range 1.50-3.00 mg/kg, mean 1.88±0.41 mg/kg). An Indo-Pacific marlin, another marine top-predator, exhibited an extreme [Hg] value of 13.00 mg/ kg, well above the 1.0 mg/kg limit. Additionally, N=26 fish exceeded their [Hg] 0.5 mg/kg specific limit.

Discussion and Conclusion
Contamination patterns here emerged align with previous literature findings. Further analyses of the entire dataset will focus on identifying potential geographic and temporal hotspots of contamination and assessing health risks associated with the consumption of these seafood products.

TAXONOMIC GROUP	n.c. samples (RANGE [Cd])	n.c. samples (RANGE [Hg])	n.c. sample [Pb]	Total n.c. samples	% of n.c. samples
CRUSTACEANS	**2** (0.77-0.85)[a]	0	0	**2**	0.44%
BIVALVE MOLLUSCS	**3** (1.30-1.60)[b]	0	**1** (3.20)[c]	**4**	0.39%
CEPHALOPOD MOLLUSCS	**17** (1.40-5.60)	**1** (1.90)[d]	0	**18**	1.17%
FRESHWATER FISH	0	**2** (1.80-1.90)[e]	0	**2**	1.30%
MARINE FISH	0	**118** (0.74-13.00)	0	**118**	4.37%
TOTAL	**22**	**121**	**1**	**144**	**2.45%**

Table 1. Number of non-compliant (n.c.) samples for each element and percentage of n.c. samples for each taxonomic group; in parentheses the range of concentrations of each analyte (mg/kg) for n.c. samples. Note: a. American lobster (USA); b. Chilean mussels (Chile); c. Truncate donax (Italy); d. Squid (Peru); e. Barbels (Italy).

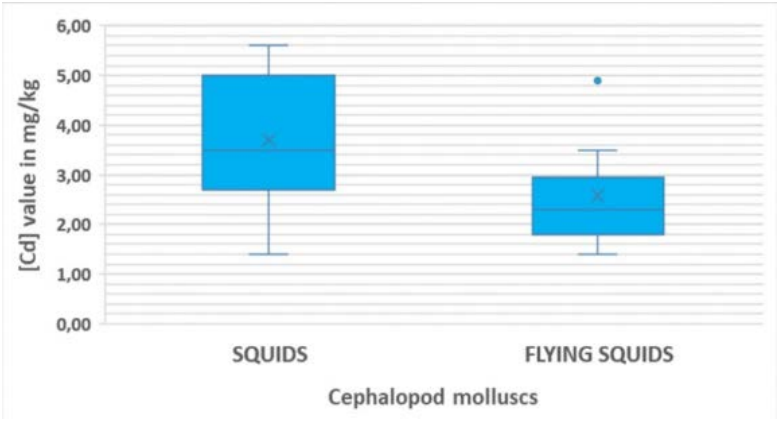


Figure 1. Box-plot of [Cd] values in mg/kg of non-compliant samples for the two main group of cephalopods (Squids: N=7; Flying squids: N=7). Values for octopuses (N=2) and cuttlefish (N=1) were not represented. Median value is marked with a horizontal line, while an X indicates the mean value.

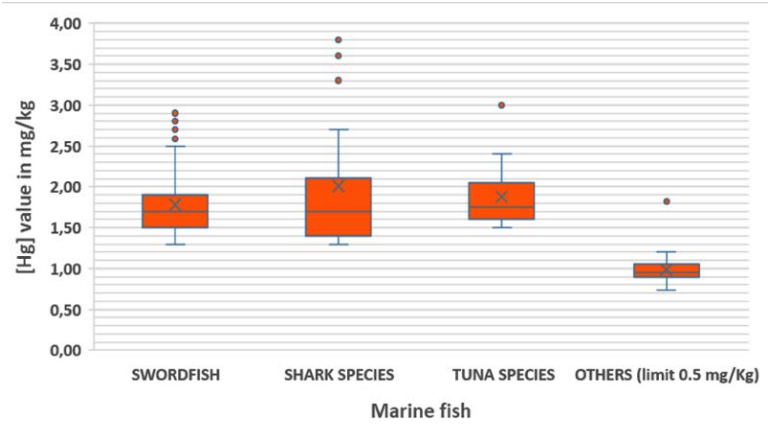


Figure 2. Box-plot of [Hg] values in mg/kg of non-compliant samples for each group of marine fish species (Swordfish: N=59; Shark species: N=16; Tuna species: N=14; others with limit 0.5 mg/kg: N=26). Values of samples (N=3) from other species with [Hg] limit 1.0 mg/kg were not represented. Median value is marked with a horizontal line, while an X indicates the mean value.

P155

Food Safety

MOLECULAR CHARACTERIZATION, VIRULENCE GENES AND ANTIMICROBIAL RESISTANCE OF *AEROMONAS* ISOLATES FROM FOOD AND FRESHWATER IN LOMBARDY REGION, ITALY

A. Grassi[1], E. Olivieri[1], S. Rigamonti[1], N. Vicari[1], F. Guadagno[1], L. Gintoli[1], S. Faccini[1], L. Marocchi[1], M. Gradassi[1], M.P. Sommariva[1], G. Sala[1], I. Bertoletti[1], A. Gazzola[1], M. D'Incau[1], P. Prati[1], G. Andreoli[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Italy*

Introduction

The genus *Aeromonas* includes 36 bacterial species, some of these can cause diseases in both humans and aquatic animals and have been recognized as emerging pathogens for over a decade. *Aeromonas* infections may have been underestimated due to the lack of microbiological methods and identification.

Materials and Methods

A total of 473 samples were collected from freshwater source (n=81), poultry meat (n=65), red meat (n=105), fish products (n=139), dairy products (n=68), gastronomy products (n=8), and vegetables (n=7) in Lombardy region and analyzed for the presence of *Aeromonas*. The strains were identified using microbiological investigation, and confirmed by MALDI-TOF and PCR. Virulence genes act, ast, alt, aerA,

hylA, stx-1, were also investigated by PCR, and AST was performed using agar diffusion method. WGS was performed with Oxford nanopore technology.

Results
Aeromonas was isolated in 211 (44%) samples, with highest prevalence in poultry meat (75%), followed by fish products (60%), freshwater (46%), and red meat (37%). All gastronomy preparations and vegetables tested negative. Among the eight bacterial species identified *A. salmonicida* (37%), *A. veronii* (15.6%) and *A. media* (11.8%) were the most common species. The virulence genes detected in *Aeromonas* strains were: alt 80%, aer 59.7%, act 55.9%, hylA 42.18%, stx-1 14.69%, ast 4.26%. The highest antibiotic resistances were observed for trimethoprim/sulfamethoxazole (36.6%), followed by ceftazidime (11.5%), levofloxacin (8.9%), ciprofloxacin (4.7%), cefepime (2.6%), and aztreonam (0.5%). WGS analysis, performed on 9 strains, confirmed the phenotypic results for AMR. The families of resistance genes detected included the beta lactamases (MOX, FOX, AQU, OXA and CphA), MCR, and tet(E) efflux pump.

Discussion and Conclusion
This study shows a high prevalence of *Aeromonas* in water and food, and provides the first evidence of virulence genes, such as stx-1, of significant importance for public health and never reported before in Italy. The data obtained confirmed the potential of this bacterial genus as a reservoir of drug resistance factors.This research was supported by Ministry of Health PRC 2021013.

References
1. Alperi et al. 2010
2. Fernández-Bravo et al. 2020
3. Hoel et al. 2019

P156

Food Safety

FOOD BORNE PATHOGENS IN VEGETABLES AND ANIMAL FOOD PRODUCTS

V. Blanda[1], I. Giacchino[1], V. Vaglica[1], V. Milioto[1], S. Migliore[1], S. Di Bella[1], F. Gucciardi[1], R. D'Agostino[1], C. Sciacca[1], C. Cardamone[1], I. Mancuso[1], L. Scatassa[1], G. Purpari[1], G. Chiarenza[1], A. Guercio[1], V. Cannella[1], F. Grippi[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia, Italy*

Introduction
Food borne diseases result from the consumption of foods contaminated with pathogens or their toxins and represent a serious public health problem worldwide. Toxoplasmosis is caused by the protozoan Toxoplasma gondii. The infection may occur as a result of ingestion of oocysts in contaminated water and foods or through the consumption of undercooked or raw meats or milk. The obligate intracellular bacterium Coxiella burnetii can infect different animal species and it is excreted in the milk, urine, and feces. This study aimed to assess the presence of T. gondii and *C. burnetii* DNA across various food matrices in Sicily.

Materials and Methods
The analysis concerned 504 samples: 85 samples of bulk milk from sheep or cattle; 34 samples of meat from farm animals (cattle, pigs, and chickens); 51 mussel samples; 16 vegetable samples and 318 samples derived from game meat, specifically wild boars.
Following appropriate pre-treatment, DNA extraction was carried out using kits based on affinity column technology and lysis tubes filled with ceramic, silica, and glass beads. T. gondii DNA was amplified by both real-time PCR targeting a specific region of the 529 bp repeat element and nested PCR amplifying part of the B1 repetitive sequence. *C. burnetii* DNA was amplified by Real-Time PCR targeting a multicopy insertion element IS1111.

Results
Toxoplasma gondii DNA has been detected in two bovine milk samples (2.4% positivity rate), one meat sample from the farm animal category (2.9%), and 18 wild boar game meat samples (5.7%). Other food matrices were negative. In addition, 16 of 85 samples of bulk milk tested positive for *C. burnetii* with a prevalence rate of 17.7%; all the other tested matrices were negative.

Discussion and Conclusion
The study highlights the variability in contamination risk of different food matrices, confirming the importance of vigilance in the consumption of potentially contaminated food products. The detection of food-borne pathogen DNA in milk samples and in game meat and offal highlights the need to avoid the consumption of unpasteurized milk and derivatives, as well as undercooked game meat, particularly wild boar, or its use in preparation of unseasoned cured meats.

Funded by Ministry of Health RC IZS SI 02/21.

References
Burg et al., 1989; Edvinsson et al., 2006; Schets et al., 2013

P157

Food Safety

THE ILLEGAL TREATMENT OF RED TUNA WITH NITRITES: FOOD SAFETY IMPLICATIONS AND ANALYTICAL METHODS OF IDENTIFICATION

S. Summa[1], M. Iammarino[1], S. Lo Magro[1], P. D'Antini[1], G. La Bella[1], G. Nobili[1], M.G. Basanisi[1], G. La Salandra[1], R. Romaniello[2], A.E. Barrasso[2], M. Muscarella[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy*
2University of Foggia - Dept. Of Agricultural Sciences, Food, Natural Resources and Engineering, Foggia, Italy

Introduction

The illegal treatment consisting of nitrites addition in red tuna to prolong the shelf-life is a significant concern in food safety [1]. Other than nitrite presence, the possible increase of histamine and microbial load, also pathogens, together with possible formation of N-nitrosamines, represent other concern for public health.
In this study, the treatment of red tuna (*Thunnus thynnus*) with nitrite solutions was simulated and chemical/ microbiological analyses were performed to evaluate the overall food safety level. Moreover, a non-destructive analytical approach for detecting the addition of nitrite in red tuna samples, based on a hyperspectral method and chemometrics, is presented [2].

Materials and Methods

Biogenic amines, volatile basic nitrogen, nitrite/nitrate, ascorbic acid and sulphites, total microbial count at 30 °C, *Enterobacteriaceae, Vibrionaceae,* coagulase-positive *staphylococci, Salmonella*, *Escherichia coli* were determined comparing the results obtained by analysing a fresh sample and the same treated with nitrite, after 5 days of storage at 4 °C. Regarding novel analytical method by hyper-spectral analysis, samples added with different levels of nitrite were analysed by both ion chromatography and the presented approach, in order to define its detection capability (CCβ).

Results

The results indicate that, starting from products characterized by good hygienic/sanitary quality, the chemical parameters and microbial load of treated samples are still within the normal ranges and do not constitute a health risk. Therefore, this study confirmed that no other food safety concern subsists, apart from nitrite amount, and the possible development of N-nitrosamines. Regarding novel analytical tool under development, at least five wavelengths in the visible region were identified as able to detect the illegal treatment of tuna with nitrite solutions at concentration of 50 ppm or more. This first result could be the way to develop and optimize an industrial on-line application to inspect tuna in objective and non-contact manner.

Discussion and Conclusion

Thanks to the Italian Ministry of Health who financed the Research Project IZS PB 05/21 RC.

References

1. European Commission. Illegal treatment of Tuna: from canning grade to Sushi grade, https://food.ec.europa.eu.
2. Romaniello et al. (2021) Chem Eng Trans, 87, 217-222.

Figure 1. Red tuna sample treated with nitrite (A) and not treated (B) after 5 days of storage at 4°C.

P158

Food Safety

COMPARATIVE EVALUATION OF THREE CAPTURE ENRICHMENT KITS FOR THE VIROME ANALYSIS BY SHOTGUN METAGENOMIC: A PILOT STUDY ON OYSTERS

V. Indio[1], J. Schaeffer[2], F. Troja[1], M. Desdouits[2], A. De Cesare[1], S. Le Guyader[2]

[1]*Department of Veterinary Medical Sciences, Alma Mater Studiorum University of Bologna, Ozzano dell'Emilia (BO), Italy*
[2]*Ifremer, Unité Microbiologie Aliment Santé et Environnement, RBE/LSEM, Nantes, France*

Introduction
Enteric viruses can be accumulated into filter feeders organisms and infect consumers [1]. Recently, metagenomics approaches, have emerged as a suitable methodology to detect and subtype viruses in several complex matrices, including foods [2]. In this pilot study we compared three capture enrichment kits for the virome analysis in oysters.

Materials and Methods
Oyster (n=5) were collected in two French regions known for being subjected to faecal contamination. Total RNA was extracted from each sample, reverse transcripted to cDNA and then submitted to library preparation using three different kits (i.e., Twist Bioscience, Roche and Illumina) for virome enrichment before shotgun metagenomic.. Bioinformatic and biostatistic analysis were aimed to comparatively evaluate the overall performances of the three library preparation kits and their feasibility to detect and quantify four viral families of food safety interest, namely the *Caliciviridae, Mamastroviridae, Reoviridae* and *Picornaviridae.*

Results
The results showed that the Twist Bioscience kit performed better in identifying various norovirus genotypes within the Caliciviridae family. Moreover, this kit allowed to detect two virus contigs from the *Picornaviridae* family while the other two kits did not retrieve any contig. For the *Astroviridae* family, Roche kit produced a higher number of contigs but with a lower average coverage in comparison to the other kits. Furthermore, the calculation of the α-diversity index at species level showed a significant increase in richness for Twist Bioscience ($p=0.018$), indicating that this library preparation protocol outperforms the others in terms of capability to capture viral richness in the tested oysters.

Discussion and Conclusion
The results of our pilot study demonstrated that the Twist Bioscience kit performed better than the Roche and Illumina kits in the identification of viral genotypes potentially relevant for human health investigated in oysters. The results of the comparison of the three library preparation kits provide practical insights for future investigations in food virome.

References
1. Le Guyader FS et al. Appl Environ Microbiol. 2009 Feb;75(3):618-24.
2. Buytaers FE et al. Foods. 2022 Oct 25;11(21):3348.

P159

Food Safety

EFFECT OF DIFFERENT COOKING TREATMENTS ON THE FORMATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN PORK MEAT

M. Ingegno[1], M. Iammarino[1], G. Berardi[1], I. Della Rovere[1], R. Colangelo[1], A. Calitri[1], V. Nardelli[1]

[1]*Istituto Zooprofilattico Sperimentale Della Puglia E Della Basilicata, Italy*

Introduction
Polycyclic aromatic hydrocarbons (PAHs) are a class of chemical contaminants, comprising over 200 organic compounds, originated from the incomplete combustion or pyrolysis of organic matter by natural and anthropogenic processes. PAHs have several negative effects on human health. For this reason, the Regulation (EU) 2023/915 sets maximum levels for 4 PAHs in foods. These contaminants can be found in meats especially after cooking.

Materials and Methods
In this study, 10 different types of cooking treatment have been tested on 2 meat cuts (neck and loin), in order to evaluate their possible effects on 4 PAHs (namely benzo[a]anthracene, benzo[b]fluoranthene, benzo[a]pyrene (BaP) and chrysene) formation. The analyses were performed by using a validated and accredited analytical method based on QuEChERS extraction optimized and quantitative determination by GC-MS/MS [1]. The overall evaluations have been made by taking into account the concentrations of both the sum of 4 PAHs and the BaP, since legal limits have been established.

Results

The first finding concerns the well-known relationship between PAHs formation and fat percentage of sample. More than the fat percentage of 2 different meat cuts (variable between 5% of loin and 40% of neck) the PAHs formation was more influenced by the use of oil during cooking. The highest PAHs levels were detected in samples cooked by frying, with no substantial difference between the use of EVO oil (loin PAHstot: 0.310 µg/kg, neck PAHstot: 0.408 µg/kg) and sunflower oil (loin PAHstot: 0.494 µg/kg, neck PAHstot: 0.385 µg/kg). If compared to the PAHs levels detected for other cooking types which do not use oil, these values were well higher. Cooking time also influences the PAHs formation. Indeed, the mean concentrations of PAHs increased by 22%, 155% and 324% for sunflower oil frying, EVO oil frying and griddling, respectively, when cooking time was doubled.

Discussion and Conclusion

The lowest levels of PAHs were then obtained by using cooking methods that do not use oil, and are completed in a relatively short time, as in the case of air frying (10') and griddling (15').

This study was supported by the Italian Ministry of Health (Rome, Italy) who financed the Research Project code IZSPB 06/21 RC.

References

1. Ingegno et al (2024) Front. Nutr. 11:1403541.

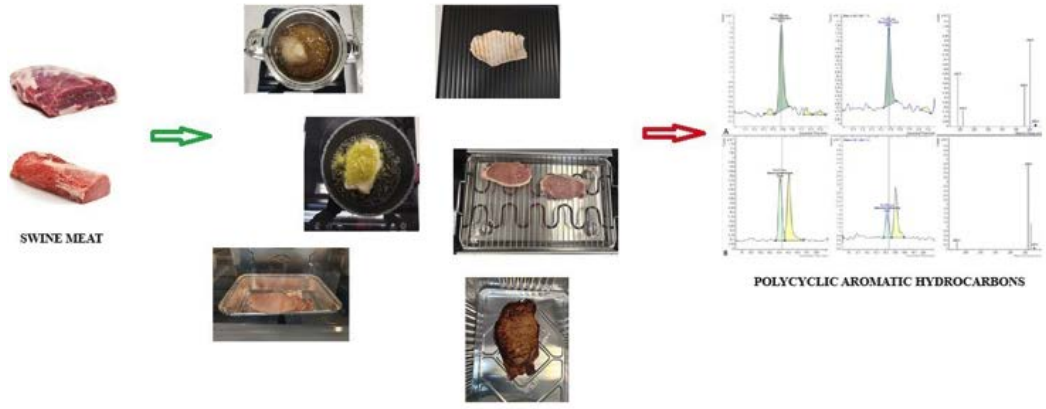


Figure 1. PAHs IN PORK MEAT AFTER COOKING.

P160

Food Safety

DETECTION OF CIGUATOXINS: LC-MS/MS METHOD AND IMPLEMENTATION OF CYTOTOXICITY ASSAY NEUROBLASTOMA (N2A)

D. Lenzo[2], D. Ippoliti[2], T.M. Vollaro[2], S. Dall'Ara[1], F. Pino[1], S. Milandri[1]

[1]*Fondazione Centro Ricerche Marine, National Reference Laboratory for Marine Biotoxins, Cesenatico, Italy*
[2]*Food Chemical Department, Istituto Zooprofilattico Sperimentale della Lombardia e dell' Emilia Romagna (IZSLER), Bologna, Italy*

Introduction

Ciguatera (CFP) is a syndrome of ciguatoxin poisoning, the most widespread non-bacterial poisoning associated with the consumption of large predatory fish, and it is considered a tropical disease, which is spreading northwards due to climate changes. Ciguatoxins (CTXs) in fish derive from the biotransformation of gambiertoxins, produced by benthic dinoflagellate algae of the genus *Gambierdiscus* and *Fukuyoa*. Currently, in Europe there aren't regulatory limits for CTXs in fish but Implementing Regulation 2019/627 requires that no fish products containing CTXs are placed on the market. To ensure food safety, EFSA has recommended a limit of 0.01ng P-CTX-1 eq/g tissue in fish intended for human consumption. CFP is characterized by gastrointestinal, neurological and cardiovascular symptoms. Italy imports fish from several non-EU countries, some identified as at risk of CTXs in the European EUROCIGUA Project I (which aims to develop a detection method for CTXs, identified as an emerging risk by international competent authorities).
The purpose of this study is aimed at implementing a method based on CBA-N2a for the search and identification of CTXs in fish and to prepare an extraction and analysis procedure that allows carrying out screening investigations on imported fishery produtcs.

Materials and Methods

To date, the cytotoxicity assay (CBA) method, which uses N2a type neuroblastoma cells, is used for screening purposes to verify the pres-

ence of CTXs. Positivity is then confirmed by LC-MS/MS analysis, characterized by first extraction (Fig1) followed by a phase of search for [M+ Na]+, [M+H-nH20]+ and [M+NH4]+ adducts for the quantification of CTXs.

Results

Until today, in Italy the laboratory of the Marine Research Center Foundation, part of EUROCIGUA II, carried out analysis on 12 fish samples from 6 official control points, reporting negative results after LC-MS/MS analysis.

Discussion and Conclusion

This project has the goal of implementing the CBA-N2a assay in Italy, to create a quicker identification system for CTXs, allowing the activation of an "early warning" system at Customs Offices in collaboration with the competent Authorities.

References

Canals, A. et al.;2021
Loeffler, C.R., et al.;2021
Spielmeyer, A., et al.;2021
Castro, D., et al.;2022

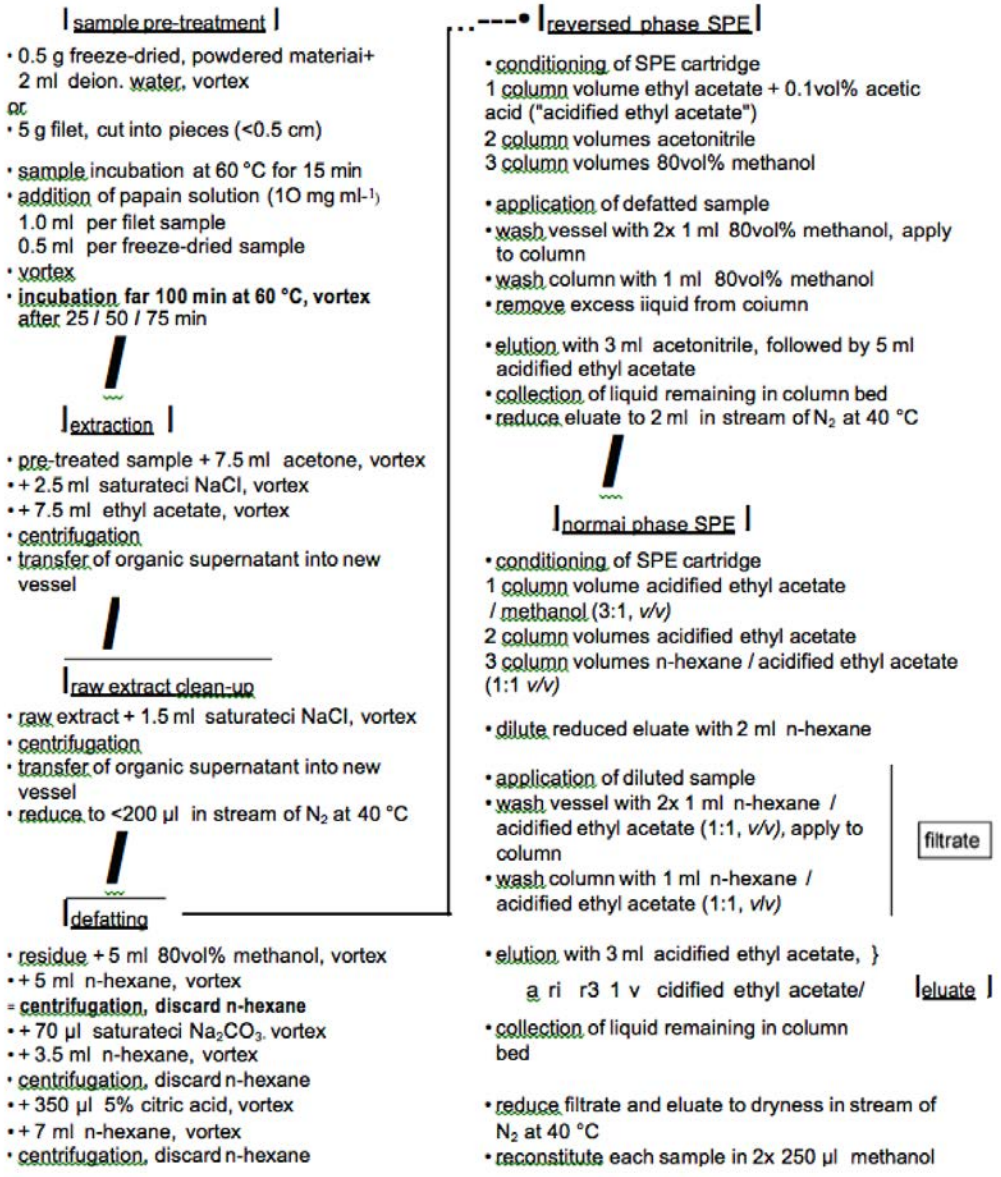


Figure 1. Extraction protocol for CTX (Castro et al., 2022).

P161

Food Safety

QUALITATIVE ANALYSIS OF OCHRATOXIN A LEVELS IN SOLUBLE AND ROASTED COFFEE SAMPLES BY LC-MS/MS

G.M. Lo Dico[1], A. Calogero[1], L. Pantano[1], A. Macaluso[1], E.M.D. Messina[1], M.D. Buscemi[1], F.G. Galluzzo[1], V. Ferrantelli[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia, Italy*

Introduction
Coffee is one of the most widely consumed beverages globally in various forms, ground roasted coffee and instant coffee being among the most common. One of the primary contaminants of concern in coffee is Ochratoxin A (OTA), a mycotoxin that can be produced by fungi of the genus *Aspergillus*. These fungi can contaminate the production chain, especially during the drying phase. The EU 2023/915 sets the maximum allowable level of OTA in instant coffee at 5.0 µg/kg, and in roasted coffee beans at 3.0 µg/kg [1].

Materials and Methods
A total of 40 samples of soluble coffee (STC=5) from four different brands and 40 samples of roasted coffee (STC=3) from four different brands were analysed using a screening method. Analyses were conducted in triplicate. Sample extraction and purification were performed using Ochraprep® immunoaffinity columns. Instrumental analysis was carried out by HPLC-MS/MS with a Hypersil Gold® column, 50 mm, 2.1 mm ID, 1.9 µm (Thermo Fischer), and a TSQ Vantage (Thermo Fischer).

Results
All analysed samples showed OTA levels below the maximum limits imposed by European Regulation 2023/915, indicating full compliance with current food safety regulations.

Discussion and Conclusion
Despite the absence of values exceeding the permissible limits, a detectable presence of OTA was found in a considerable proportion of the samples. For ground coffee samples, the detectable OTA percentage ranged from a minimum of 20% (2 out of 10) to a maximum of 40% (4 out of 10) of the samples analysed per brand. Soluble coffee samples, on the other hand, showed a percentage of detectable OTA ranging from 30% (3 out of 10) to a maximum of 50% (5 out of 10) of the samples analysed per brand. Considering the total number of samples (n=40 for each type), soluble coffee samples exhibited a higher frequency of detection (17/40) compared to ground coffee (15/40). This data underscores the importance of maintaining stringent quality control practices throughout the entire coffee production chain to reduce exposure to such contaminants.

References
1. European Commission Commission Regulation (EU), No. 915/2023 of 25 April 2023 on Maximum Levels for Certain Contaminants in Food and Repealing Regulation (EC) No. 1881/2006. Available online: https://eur-lex.europa.eu/legal- content/EN/TXT/?uri=CELEX%3A02023R0915-20230810 (accessed on 1 June 2024).

P162

Food Safety

ORIGANUM SPP. SPECIES IDENTIFICATION: COMPARISON BETWEEN SANGER SEQUENCING AND METABARCODING

G. Magagna[2], B. Maestrelli[1], M. Tilola[2], G. Fedrizzi[2], M.N. Losio[2], V. Filipello[2]

[1]*Department of Civil, Environmental, Architectural Engineering and Mathematics, University of Brescia, Brescia, Italy*
[2]*Food Safety Department, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Brescia, Italy*

Introduction
Food fraud has become a critical issue in our food system and is gaining significant attention from global authorities. Herbs and spices standards are some of the most stringent in the EU, and being high-priced commodities, they are persistently vulnerable to adulteration [1]. DNA barcoding has been employed to identify plant species. However, distinguishing plant species is challenging since multiple DNA regions in plants are used as markers for DNA barcoding which have different performance in different families. Internal transcribed spacer (ITS) region is one of the most used [2]. The aim of this study was to identify the species of 4 shredded dried oregano samples using Sanger sequencing and metabarcoding.

Materials and Methods
For Sanger sequencing, the target gene (ITS2, 160-320 bp) was sequenced on a SeqStudio Genetic Analyzer (Thermo Fisher Scientific) and species identification was carried out using GenBank. For metabarcoding, ITS2 libraries were prepared using the Nextera XT DNA

Library Preparation Kit (Illumina) and run on an Illumina MiSeq platform. Relative species abundances were determined using QIIME 2 with a custom reference database.

Results

For Sanger sequencing, it was possible to identify the species for only one sample, while for the other samples, only the genus was determined. In particular, sample 1, 3, and 4 were found to belong to either *O. onites* or *O. vulgare* species, while sample 2 was identified as *O. vulgare*. Metabarcoding allowed instead the identification of different oregano species in each sample along with their relative abundances (Figure 1).

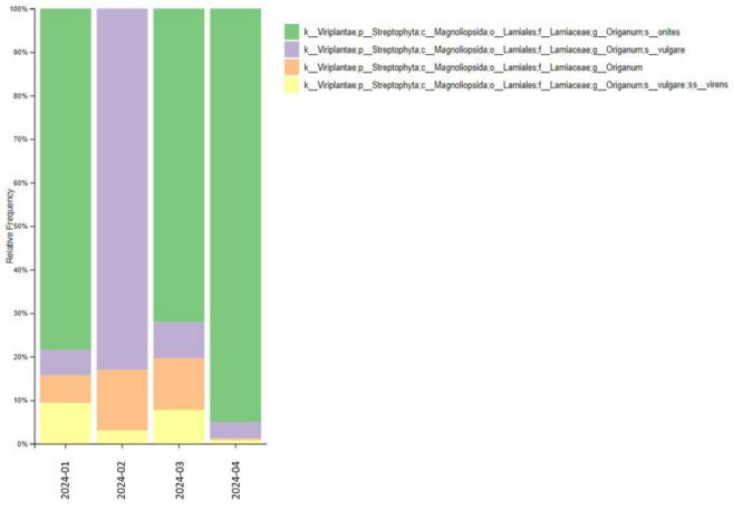


Figure 1. Stacked barplot of relative abundance of the species that make up the 4 oregano samples.

Discussion and Conclusion

The results of this study suggest that metabarcoding is a better alternative for species identification, when not dealing with intact specimens. Indeed, it was possible in a single analysis to uniquely distinguish the different species contained in the samples, along with their relative abundance. This information is relevant since different oregano species have very different cost and content of toxic alkaloids.

References

1. Van De Steene J, et al. Food Res Int. 2022. doi:10.1016/j.foodres.2022.111962
2. Dawan J, Ahn J. Food Sci Biotechnol. 2022. doi:10.1007/s10068-022-01143-7

P163

Food Safety

DEVELOPMENT OF NEW MONOCLONAL ANTIBODIES AGAINST FOOD ALLERGENS

M. García-Durán[2], E. Soria[2], R. González[2], E. Hevia[2], A. Camuñas[2], J. Martínez-Cano[2], P. Boehler[1], C. Romero[2], S. Peransi[3], J. Salcedo[3], P. Rueda[2]

[1]*Gold Standard Diagnostics Kassel, Kassel, Germany*
[2]*Gold Standard Diagnostics Madrid SA (GSD Madrid), Madrid, Spain*
3Lumensia Sensors SL, Valencia, Spain

Introduction

Food allergy has become a major public health problem. As there are unknown allergen components in novel food and hidden allergens caused by cross contamination in food processing, it is important to develop sensitive systems for allergen detection. Immunoassays are widely used for that purpose and monoclonal antibodies (MAb) are highly valuable tools. In this context, MAb are preferred over polyclonal Ab (pAb) due to the higher specificity, better reagent characterization and thus batch-to-batch reproducibility. In this work, MAb against mustard, fish and almond were developed as tools for improving allergen detection.

Materials and Methods

Four groups of mice were immunized with native or recombinant (rec) immunogens. The functionality of the obtained MAbs was determined by indirect ELISA with the antigens described in the Table 1.

Results

MAb obtained:

- 7 MAbs against Almond, 3 of them not cross-reacted with apricot seeds.
- 4 MAb against Mustard, 3 of them recognized one or several mustard ext but also rapeseed ext although with lower sensitivity; the fourth MAb only recognized Sina 1.
- 11 MAb against Fish: all recognized cod and/or salmon rec ßparv and extracts in different extent, 7 recognized the mix of native ßparv.

Preliminary tests with MAb against almond and mustard in a Photonic Integrated Circuit biosensor platform (SAPHER project, EU funding, EIC-FTI-2018-2020, ID: 958855) gave promising results.

Discussion and Conclusion

The MAb panel described represents a valuable tool for improving allergen detection tests, which may provide useful solutions for food safety. Further work to test the MAb for multiplexing or for use in combination instead of a pAb could improve the reproducibility of different batches of reagents, one of the main concerns when the pAb used runs out.

MAb Specificity	Immunogen	Target extract	Cross extract	Negative extract	Negative Ag
Almond	PrudU6	Almond	Apricot seeds	Cashew	-
Mustard	Sina 1	Yellow, red and brown mustard	Rapeseed	Cinnamon	Ag1
Fish (cod)	Cod-1, 2, 3	Cod (or salmon)	Meat	-	Ag2
Fish (salmon)	Sal-s1	Salmon (or cod)	Meat	-	Ag2

Table 1. Antigens in the functionality assays. Cod-1,2,3 and Sal-s1: rec βparvalbumins (βparv) from cod or salmon. Ag: antigen, Ag1,2: non-related antigen processed similarly to immunogen. Extract (ext) of yellow and red mustard, rapeseed, cod, salmon and meet provided by GSD Kassel.

Table 1.

P164

Food Safety

VALIDATION OF SENSITIVE AND RAPID IMMUNOASSAY METHOD TO DETECT COCONUT ALLERGEN IN FOODS AND BEVERAGES

S. Morello[1], S. Lupi[1], C. Tramuta[1], E. Barcucci[1], D.M. Bianchi[1]

[1]*Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta - Centro di Referenza Nazionale per la Rilevazione Negli Alimenti di Sostanze e Prodotti che Provocano Allergie e Intolleranze (CReNaRiA), Turin, Italy*

Introduction

Coconut (*Cocos nucifera*) is a fruit widely consumed all over the world in different food preparations. It contains proteins (7S and 11S globulin) that can trigger allergic reactions in sensitized individuals. Clinical symptoms include hives, skin rushing, and, in severe case, anaphylactic reactions. According to the Food Allergen Labelling and Consumer Protection Act (FALCPA) enforced in USA and Canada, coconut is included among the big-8 major allergenic foods, and it must be reported as an ingredient on labels. Due the increased consumption of coconut-containing foods imported by extra-EU countries the potential presence of "hidden" coconut allergen could represent a rising health concern to allergic consumers. The aim of this study is to validate an ELISA qualitative method to detect coconut allergen in food and beverage.

Materials and Methods

The SENSISpec Coconut ELISA kit (Gold Standard Diagnostics) for qualitative determination of coconut in food (baked and dairy products) and beverage. The validation performances evaluated were specificity, sensitivity, ruggedness. Specificity analyses were performed on 24 negative samples (bakery products n=8; dairy products n=8; fruit juices n=8). Sensitivity was tested on the 24 negative samples fortified to a final coconut concentration of 2.5 ppm. Ruggedness was evaluated according to the Youden approach, modifying incubation temperature, rpm of centrifugation, and conjugate volume.

Results

Validation performances (specificity, sensitivity, and ruggedness) were verified. Specificity analyses carried out on 24 samples showed β error below 5%. Sensitivity analyses revealed that the test is able to detect analyte at 2.5 ppm, set as limit of detection (LOD). Slight variations (±5%) applied on nominal value of incubation temperature (T=60°C), time of incubation (10 min) and conjugate volume (100 µL), do not significantly affect assay efficiency, indicating that the test is robust.

Discussion and Conclusion

The ELISA method for the detection of coconut in foods and beverage presented in this study was successfully validated; it could be a reliable tool to guarantee allergic consumers' protection proving high analytical specificity and sensitivity. This study was funded by the Italian Ministry of Health IZSPLV 03/22 RC.

P165

Food Safety

SPECIES AUTHENTICATION OF INSECT-BASED FOOD BY DNA METABARCODING

A. Mottola[1], L. Lorusso[1], L. Ranieri[1], C. Intermite[1], A. Di Pinto[1]

[1]*Department of Veterinary Medicine – University of Bari Aldo Moro – Valenzano (Bari), Italy*

Introduction

Given that edible insects are a sustainable source of food protein and have a lower ecological footprint than conventional sources, entomophagy is gaining interest in Europe[1]. To date, the European Union has authorised the marketing of insect-based foods (IBFs) containing *Tenebrio molitor, Locusta migratoria, Acheta domesticus* and *Alphitobius diaperinus*. In order to reduce food disgust sensitivity, such products are mainly sold in the form of conventional food (e.g., snacks, pasta). The absence of morphological features makes such products vulnerable to species substitution. Therefore, this study aimed to assess the species authenticity of processed IBFs and to evaluate mislabelling rates.

Materials and Methods

A total of 18 processed IBFs samples were purchased through European and Asian e-commerce platforms (Table 1). Samples were subjected to DNA extraction, and subsequently a 200 bp fragment of the 16S rRNA mt gene [2] was sequenced by a metabarcoding approach on the Illumina MiSeq platform with a paired-end approach. Then, raw reads were pre-processed to generate Amplicon Sequence Variants. Taxonomic assignment was performed using standalone blast in the blast + suite against a 16Smt custom database [3].

Sample ID	Type of product	Declared species	Country	Brand
I3	Crackers	*Acheta Domesticus*	Belgium	A
I4	Flour	*Acheta Domesticus*	Thailand	E
I5	Snack	*Acheta Domesticus*	Belgium	A
I10	Premix	*Alphitobius diaperinus*	Netherlands	D
I2	Bar	*Alphitobius diaperinus*	France	C
I6	Flour	*Alphitobius diaperinus*	Netherlands	D
I7	Premix	*Alphitobius diaperinus*	Netherlands	D
I8	Muesli	*Alphitobius diaperinus*	France	A
I9	Bar	*Alphitobius diaperinus*	France	C
I11	Whole insect	*Locusta migratoria*	France	C
I12	Flour	*Locusta migratoria*	Thailand	E
I13	Whole insect	*Locusta migratoria*	France	C
I1	Chips	*Tenebrio molitor*	Italy	B
I14	Cookies	*Tenebrio molitor*	Italy	B
I15	Crackers	*Tenebrio molitor*	Italy	B
I16	Chips	*Tenebrio molitor*	Italy	B
I17	Flour	*Tenebrio molitor*	Italy	A
I18	Cookies	*Tenebrio molitor*	Italy	B

Table 1. Sampling details

Results

The study highlighted the presence of 38 Insecta, 1 Aves, 1 Magnoliopsida and 3 mammalian taxa, with an overall mislabelling rate of 55.6% including some insects not authorised as food consumption in Europe, as well as unexpected species (i.e.,bovine, swine, poultry and mouse) (Figure 1). Such presences raise consumer safety concerns and the violation of ethical or religious rights.

Discussion and Conclusion

The outcomes confirm that IBFs are vulnerable to cases of mislabelling [4]. The presence of unexpected species could pose serious safety concerns, since insects are consumed in their entirety and could carry biological, chemical and physical hazards that can affect consumer health [1]. Overall, the study shows that the application of metabarcoding is a promising approach to successfully ensure the traceability and safety of IBFs and support both producers and official control activities.

References

1. FAO, 2021 openknowledge.fao.org/handle/20.500.14283/cb4094en
2. Hillinger 2023 doi.org/10.3390/foods12051086
3. Mottola 2023 doi.org/10.1016/j.foodcont.2022.109459
4. Giusti 2024 doi.org/10.1016/j.foodres.2024.114268

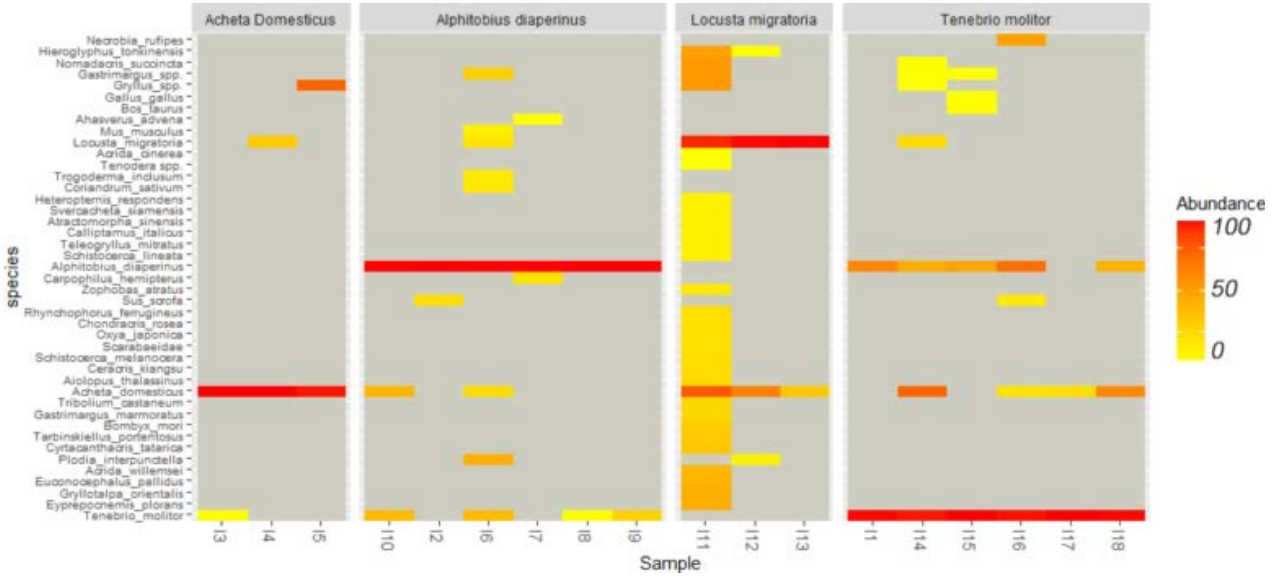


Figure 1. Heat map of taxa in IBFs. Colour gradients correspond to the relative percentage abundance within each sample.

P166

Food Safety

MICRO-RNAS IN MILK EXOSOMES AS BIOMARKERS FOR DETECTION OF RECOMBINANT SOMATOTROPIN MISUSE IN DAIRY COWS

N. Giaccio[2], M. Arigoni[1], R. Calogero[1], M. Pezzolato[2], E. Biasibetti[2], E. Bozzetta[2], A. Lamas[3], P. Regal[3], A. Benedetto[2]

[1]*Dipartimento di Biotecnologie e Scienze della Salute, UNITO*
[2]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria and Valle d'Aosta, IZSPLV*
[3]*University of Santiago de Compostela, UCS*

Introduction

Recombinant bovine somatotropin (rbST) is a synthetic hormone designed to boost milk production in dairy cow by mimic natural growth hormone (GH). Its use is approved in several countries, but in Europe it is banned due to animal welfare and health concerns [1]. Targeted mass-spectrometry methods for detecting rbST are challenging because some commercial rbST forms have identical amino acid sequence of natural GH [2]. This study aims to develop an indirect biomarker-based method to identify rbST treatment by profiling miRNA perturbations in milk exosomes.

Materials and Methods

A retrospective analysis was conducted from a previous animal trial [3]: Nine Holstein cows at 67-75 ± 4 days of lactation were divided into two groups, one treated with rbST (500 mg every 14 days for 6 months) and a control group. Exosomes were isolated using Exoquick Precipitation Solution (SBI) from 63 milk samples, collected from 7 time points covering 90 days after treatment start. After exosomes characterization (Malvern Panalytical), RNAs were purified using the miRNeasy serum/plasma kit (Qiagen). Small RNA libraries were prepared by NEB-Next kit (NEB) and sequenced on an NextSeq 1000 sequencer (Illumina). Data analysis by dedicated bioinformatics pipelines [4] allowed to filter out non-treatment-related miRNA variations (EdgeR), and then to identify specific miRNA perturbations linked to rbST administration by differential expression (DE) analysis (DESeq2).

Results

The analysis identifies 35 DE miRNAs (Figure 1). According to recorded expression profiles bta-miR-10167-3p was strongly up-regulated by rbST treatment during all considered sampling points, showing interesting potential as transcriptional biomarkers to develop alternative screening test in the frame of official control plans.

Discussion and Conclusion

A conclusive field study for biomarker validation will be therefore needed to check specificity of all 35 miRNAs identified as rbST related, especially for bta-miR-10167-3p.

References

1. Council Decision 1999/879/EC of 17 December 1999.
2. Report on Animal Welfare Aspects of the Use of Bovine Somatotropin. 1999.http://ec.europa.eu/food/fs/sc/scah/ out21_en.pdf.
3. Lamas A et al. Sci Rep.9:4788, 2019. doi: 10.1038/s41598-019-41343-6
4. Kulkarni N et al.BMC Bioinformatics 19, 349, 2018. doi: 10.1186/s12859-018-2296-x

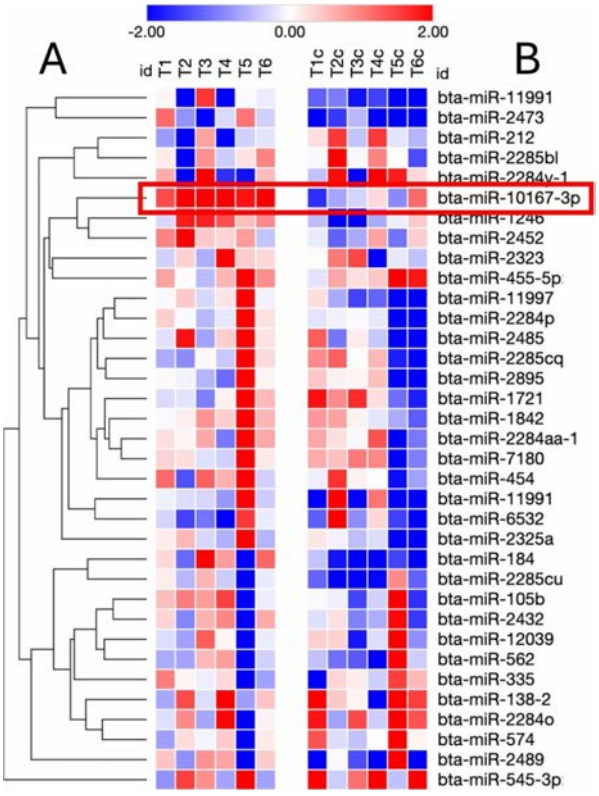


Figure 1. Comparison of expression levels of 35 DE miRNAs identified by sequencing analysis on milk exosomes from treated (T1-T6, A) and control cows (T1c-T6c, B). Bta-miR-10167-3p shows significant up-regulation in rbST-treated animals.

P167

Food Safety

ASSESSMENT OF PERSISTENCE AND VIABILITY OF SARS-COV-2 WUHAN B.1 AND SARS-COV-2 ERIS EG.5.1 ON PLANT- BASED FOODS: IMPLICATIONS FOR TRANSMISSION AND FOOD SAFETY

G. Picazio[2], G. Fusco[2], S. Brandi[2], L. Cardillo[2], L. Cozzolino[2], M. Viscardi[2], A. Pucciarelli[2], F. Di Maggio[1], M. Nunziato[1], E. De Carlo[2], C. De Martinis[2], F. Salvatore[1]

[1]*CEINGE-Biotecnologie Avanzate Franco Salvatore, Napoli, Italy*
[2]*Dipartimento di Sanità Animale, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Napoli, Italy*

Introduction

Recent research indicates that the survival of SARS-CoV-2 on food items and packaging materials can range from several hours to days, depending on various factors such as temperature and humidity levels, representing a critical concern for the indirect transmission of the virus (1).

Materials and Methods

Ten food samples were artificially contaminated with viral solutions at viral titre of 105 TCID50/mL and a concentration of 1.56E+07

copies/µl for SARS-CoV-2 Wuhan B.1, and 2.30E+07 copies/µl for SARS-CoV-2 Eris EG.5.1, respectively. Samples were sectioned into 7 areas of 4-5 cm, contaminated by spraying a 50 µL viral aliquot, and finally incubated at 22.5°C and 58.6% of relative humidity. Sampling was done at specific post-contamination times (0.5, 4, 8, 24, 48, 72 and 96 hours) and UTM medium was used for RT-qPCR analysis, as cellular inoculum for viability assessment, and finally, for the evaluation of TCID50/mL. Finally, cellular supernatants, after 5-7 days, were analyzed by RT-qPCR and monolayers were observed for cytopathic effect (CPE) assessment (Figure 1).

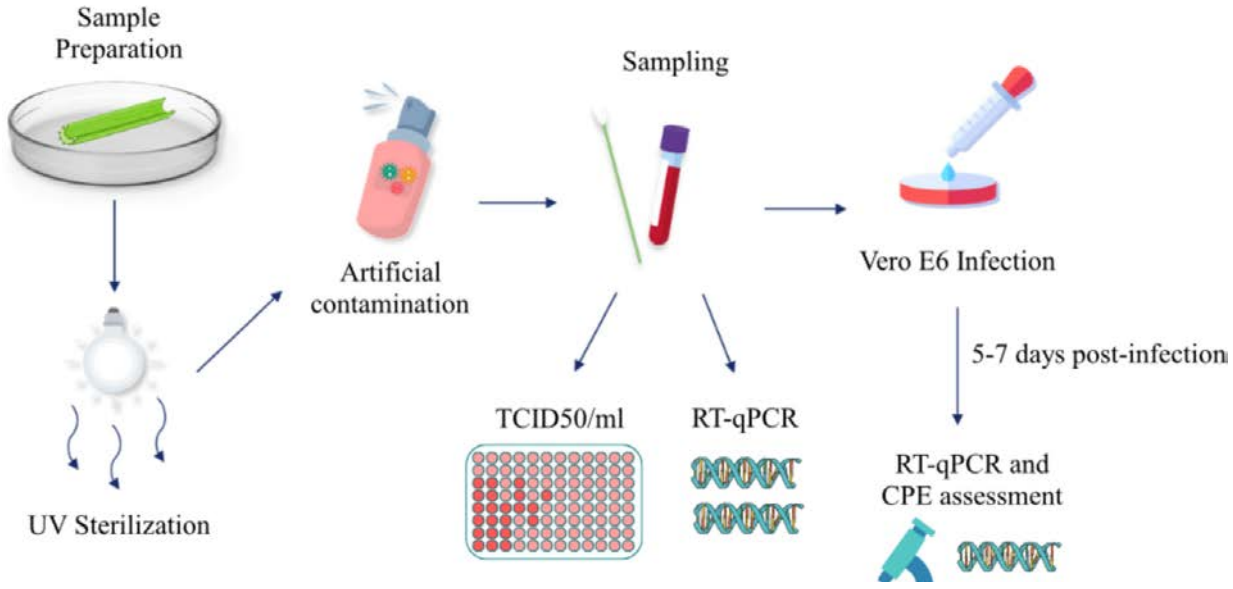


Figure 1. Schematic diagram of the main steps of the experiment. Samples were dissected and sterilised using UV light; SARS-CoV-2 lineage B.1 and EG.5.1 were used to perform the artificial contamination of the 10 different food matrices. From 0.5 h to 96 h post-contamination, swabs were rubbed from the matrices and deposited in 1 mL of UTM medium. Next, 50 µL of UTM were collected to perform viral titration by TCID50/mL, 200 µL for nucleic acids extraction and subsequent RT-qPCR analysis, and 100 µL were used as inoculum for Vero E6 cells. The cell cultures were daily inspected up to 7 days p.c. using an inverted microscope. When at least 50% CPE occurred, or at 5-7 days post-inoculum in the absence of CPE, the culture supernatant was collected and analysed by RT-qPCR.

Results

Our results revealed significant differences in the survival rates of the two viruses on various foods, indicating possible variant-specific survival capabilities. SARS-CoV-2 Wuhan B.1 appears to be viable for up to 4 hours on apple, grape, fennel, carrot, lettuce, celery, radish and rocket, and viable for up to 0.5 hours on tomato and cucumber while SARS- CoV-2 Eris EG.5.1 persisted in a viable state for up to 4 hours on apple, tomato, radish, rocket and cucumber, and for up to 0.5 hours on grapes, fennel, lettuce and celery. Finally, no viable virus was detected on carrots even at 0.5 hours.

Discussion and Conclusion

The increase in viral load, from UTM medium to cellular supernatant, TCID50/mL and CPE evaluation, allowed us to demonstrate the survival times of the two viruses. Understanding the survival dynamics of these virus variants is crucial for evaluating the potential role of contaminated food in the transmission chain of the virus through food consumption and production.

References

1. Xie, Chaojun et al. “The evidence of indirect transmission of SARS-CoV-2 reported in Guangzhou, China.” BMC public health vol. 20,1 1202. 5 Aug. 2020, doi:10.1186/s12889-020-09296-y

P168

Food Safety

ASIATIC PRODUCTS MONITORING TO VERIFY LABEL COMPLIANCE: ALLERGENS DETECTION IN IMPORTED FOOD SOLD IN NORTHERN ITALY MARKET

L. Ragni[1], R. Bertolassi[1], M. Ferrari[1], E. Pavoni[1], G. Finazzi[1], M.N. Losio[1], B. Bertasi[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER), Reparto Controllo Alimenti, Brescia, Italy*

Introduction
Food labelling is crucial to help consumers to make healthier and safer food choices, in particular regarding the presence of allergens. This could represent a problem in case of imported food. Indeed, consumption of imported Asian food is a growing reality in the EU; non-compliant labelling could enhance the probability of allergic consumers to get adverse events. According to EU Reg. 1169/2011 (Annex II), allergens must be labelled in bold and translated in the current language of the Member State. The present work investigates the label compliance to allergens on Asiatic products imported and sold in Northern Italy.

Materials and Methods
All the considered samples did not report on label the allergen they were tested for. Totally, 39 foodstuffs were tested for gluten, 118 for milk protein ß-lactoglobulin, 93 for white egg proteins, 81 for sesame, 12 for soy and 20 for crustaceans with commercial ELISA kits. Samples were considered non-compliant when the results were above 20 mg/ Kg, the law limit for gluten, or above the methods LOD (milk: 10 µg/Kg; egg: 0.4 mg/Kg; sesame: 2 mg/Kg; soy: 2 mg/ Kg; crustaceans: 20 µg/Kg).

Results
Data showed 18% of non-compliant samples for gluten: 6 samples > 80 mg/Kg (snacks and ready to eat meals) and 1 sample containing 48 mg/Kg (snack). In addition, 13.6% samples were irregular for undeclared milk (noodles and snacks), 19.4 % showed presence of egg proteins (noodles, snacks, patties and pasty), 3.7% were not compliant for sesame (snacks), 8.3% for soy (noodles) and finally, 5% for crustaceans (noodles).

Discussion and Conclusion
If the controls were based only on the ingredients labels, the presence of undeclared elements in food could increase the risk for consumers; furthermore, the presence of allergenic substances could cause severe pathological episodes in sensitive people. The present work underlines the great importance of food monitoring imported in the EU to verify the labels compliance respect to the food composition and to establish labelling rules internationally shared.

References
James C.A et al (2024): "Evaluation of food allergen information, labelling and unintended food allergen presence in imported prepacked foods and drinks purchased online in the UK" – Food Control 162 (2024) 110462

P170

Food Safety

TESTS FOR DETECTION OF ALLERGENS IN EDIBLE INSECT-BASED FOODS: ELISA AS A SCREENING TEST AND FOOD SAFETY INDICATOR

L. Biondi[1], B. Cioffi[1], A. Cutarelli[1], A.M.I. Montone[1], D. Cristiano[1], F.P. Serpe[1], F. Capuano[1], E. De Carlo[1], G. Fusco[1]

[1]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici (NA), Italy*

Introduction
In recent years, among the novel food, edible insects emerged as a valid and environment-friendly protein source. The adoption of insects as food requires the individuation of new risk factors about food safety; for example, allergic to crustaceans could manifest reactions after insect consumption. Such cross-reactivity also exists by the analytical point of view. Object of the present work is to investigate the cross-reactivity in insect-based foods, limited to the four insects permitted for human consumption in Italy (DM 6 April 2023).

Materials and Methods
Five foods for each insect were analyzed: snacks, biscuits, cereal bars, crackers and pasta based on the cricket (*A. domesticus*), the locust (*A. diaperinus*, the larva of the lesser mealworm), the grasshopper (*L. migratoria*), the larva of the yellow mealworm (*T. molitor*). Screening analysis for search of crustacean allergens was carried out with an ELISA sandwich test and confirmation by RT-PCR after extraction (R-Biopharm).

Results

Results aresummarized in Table 1.

Species	Food type	ELISA	RT-PCR
Cricket (*Acheta Domesticus*)	Curcuma cricket	+	-
	Crackers	-	-
	Whole cricket	+	-
	Red fruit cricket	+	-
	Cricket with smoked onion and BBQ	+	-
Locust (*Alphitobius Diaperinus*)	Apple and caramel bar	-	-
	Pasta with basil	-	-
	Bar with apricot and almonds	-	-
	Granola with honey and almonds	-	-
	Granola with dark chcocolate	-	-
Grasshopper (*Locusta Migratoria*)	Pepper grasshopper	+	-
	Grasshopper with thyme and oregano	+	+
	Grasshopper with pepper and dried tomato	+	-
	Cacao grasshopper	+	-
	Yellow curry grasshopper	+	-
Larva of the yellow mealworm (*Tenebrio Molitor*)	Whole larva	+	-
	Corn and flour biscuits	+	+
	Corn chips with cheese	+	-
	Larva with garlic and provencal herbs	+	+
	Larva with salted cinnamon	+	-

Table 1. Results of the analyses by ELISA and RT-PCR of permitted insect-based foods investigated.

Discussion and Conclusion

Cross-reaction between crustaceans and several species of insects is described in literature ([1][2][3][4] and others) and is known that cross-reactivity between allergens is present in laboratory (correctly declared by the companies producing the diagnostic tests).

Even if results obtained evidence that analytical cross-reactivity for insects cannot be addressed with immune- enzymatic techniques, basing on protein detection, because of the presence of false positives, the crustacean test may be a good screening test for insects for the absence of false negatives for insects. On the other hand, ELISA, recognised as gold standard in search of allergens, may also be useful for the evaluation of the potential risk to which consumers with full-blown allergy to crustaceans are exposed, after consumption of this novel food. The preliminary data reported in this study requires further investigation, especially with regards to research a greater number of insect species (This activity was carried within the scope of the RC IZS ME 03/2021).

References

1. De Marchi L., et al. Curr aller asth rep (2021) 21:35
2. Eischeid A., et al. Food add cont: Part A (2023) 40, 12, 1531:1540
3. Kamaemura N., et al. Moi Immunol 2019 feb: 106:127-134.
4. Srinroch C., et al. Food Chem 2015, oct 1:184:160-6.

P171

Food Safety

DETECTION OF VERTEBRATE DNA IN VEGAN FOOD BY REAL-TIME PCR METHOD

C. Tramuta[1], S. Morello[1], C. Avena[1], D.M. Bianchi[1]

[1]*Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta - Centro di Referenza Nazionale per la Rilevazione Negli Alimenti di Sostanze e Prodotti che Provocano Allergie e Intolleranze (CReNaRiA), Turin, Italy*

Introduction

In recent years is increasing the number of consumers who choose vegan or vegetarian diet for ethical, health, nutritional or environmental reasons. To date, the term vegan is not defined in food law and there are not a standardised criteria for a reliable labelling of vegan and vegetarian products. This can lead to incorrect labelling by the producers and consequently may pose a risk to people with allergies and intolerances due to potential cross-contamination with products of animal origin. Here we present validation of a real-time PCR assay for the detection of vertebrate DNA in food products.

Materials and Methods

The method was evaluated on three classes of food matrices (blank samples): i) bakery and pastry products, ii) gastronomic preparations, iii) sauces and vegetable drinks. The specificity of the PCR primers was tested by amplifying the DNA extracted from blanks processed in 30 replicates (10 for each of the 3 food matrices). The extracted DNA of vertebrates, plants and non-vertebrates was also tested (Table 1). To detect sensitivity, DNA extract from meat samples (*Bos taurus, Sus scrofa domesticus* and *Gallus gallus domesticus*) was diluted to a final concentration of 0,01% (100 mg/kg) by Sure Food PREP Advanced kit (r-Biopharm). DNA was then amplified by real-time PCR (SureFast Vegan kit, r- Biopharm): vertebrates detected in the Cy5-channel and plants in the FAM-channel.

Sample	Vertebrate animals (Cy5)	Real-time PCR cycle threshold (Ct)
Almond (*Prunus dulcis*)	negative	> 30
Barley (*Hordeum vulgare*)	negative	> 30
Beef (*Bos taurus*)	positive	18.7
Cacao (*Theobroma cacao*)	negative	> 30
Carrot (*Daucus carota*)	negative	> 30
Celery (*Apium graveolens*)	negative	> 30
Chicken (*Gallus gallus domesticus*)	positive	19.1
Horse (*Equus caballus*)	positive	20.4
Insect (*Acheta domesticus*)	negative	> 30
Oat (*Avena sativa*)	negative	> 30
Onion (*Allium cepa*)	negative	> 30
Peas (*Pisum sativum*)	negative	> 30
Pig (*Sus domesticus*)	positive	19.3
Plaice (*Pleuronectes platessa*)	positive	26.5
Rice (*Oryza sativa*)	negative	> 30
Scallop (*Pecten jacobaeus*)	negative	> 30
Soy (*Glycine max*)	negative	> 30
Shrimp (*Caridea Dana*)	negative	> 30
Tomato (*Lycopersicon esculentum*)	negative	> 30
Tuna (*Thunnus*)	positive	28.1
Turkey (*Meleagris*)	positive	21.1
Wild boar (*Sus scrofa*)	positive	20.1

Table 1. List of animal and plant species used in the study and specificity results of real-time PCR.

Results

All 30 blank samples were tested negative for the target vertebrate and no amplification signals in Cy5 were noted. The method was rated specific for the detection of vertebrates and no cross-reaction were observed (Table 1). About sensitivity, results demonstrate that the real-time PCR here validated proved to be sensible, showing a LOD of 100 mg/ kg (mean Ct 19.3) in all food matrices (Figure 1).

Discussion and Conclusion

Methods that are both specific and sensitive for the detection of vegan foods are needed to ensure the authenticity of vertebrate-free products and to protect food allergic consumers. The present real-time PCR method proved suitable for vertebrate DNA detection and demonstrated specificity, sensitivity, repeatability and robustness. The method provides reliable information to implement handling and cleaning protocols of production facilities.

This study was funded by the Italian Ministry of Health IZSPLV 08/23 RC.

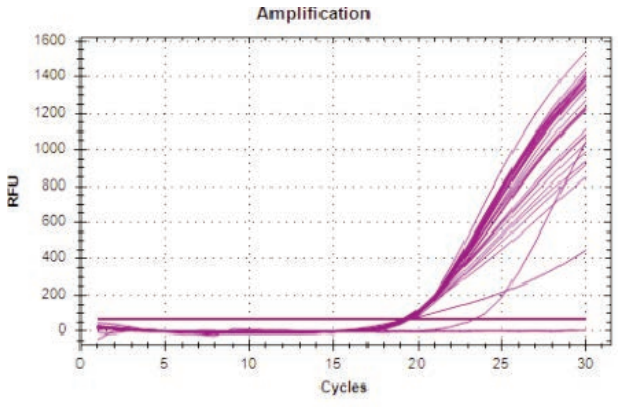


Figure 1. Amplification curves of the vegan real-time PCR analysis of DNA extracts from the samples to the LOD concentration corresponding to 0.01% (100 mg/kg).

P172

Food Safety

DETECTION OF *E. COLI* STEC IN DAIRY PRODUCTS: VALIDATION OF POOL ANALYSIS ACCORDING TO ISO 6887-1:2017

G. Weiss[1], G. Cento[1], E. Cristelli[1], K. Debiasi[1], M. Furlan[1], E. Ramon[1], S. Rodas[1], A.A. Lettini[1], R. Lucchini[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

Introduction

Shiga-toxin producing *E. coli* (STEC) has been the fourth most reported zoonosis in Europe in 2022, with milk and dairy products as one of the most frequent sources of infection[1]. In order to provide a cost-effective and reliable tool for analysis charged to the FBOp and to allow the possibility to analyze a greater number of self-controls, IZSVe proposed a pooled method of analysis of raw milk products, described by ISO 6887-1:2017. This study sets out to describe the evaluation of the method carried out both on artificially contaminated samples and positive samples isolated during routine analysis.

Materials and Methods

The wet pooling method provided by ISO 6887-1:2017 has been tested on both artificially contaminated dairy product samples with a level of contamination of 2 – 5 CFU and on routine samples that already tested positive through real time PCR based on ISO/TS 13136:2012. Then, test pools have been formulated by adding 1 ml of known positive pre-enriched to 9 ml of known negative pre- enriched samples. The obtained pools have been vortexed and then 1 ml has been used for DNA extraction and real time PCR.

Results

All five pools with a LOD of 5 CFU have been correctly identified as positive. Among the ten pools with a LOD of 2 CFU, three resulted negative. Regarding the 28 pools collected from routine samples, 26 resulted positive and 2 negative.

Discussion and Conclusion

The tested method gave great results among the pools with a level of contamination of 5 CFU. Regarding the pools contaminated with 2 CFU, the noncompliant outcomes concern one sample with a very high CT value (>37) and two samples that were probably not properly contaminated, since they didn't show any bacterial growth even in the single samples (no CT). Regarding routine samples, the pools found negative are those with a high CT of the individual sample obtained by ISO/TS 13136:2012 (35 and >45, respectively).
This validation showed that the wet pooling method, proposed by ISO 6887-1:2017 and applied to ISO/TS 13136:2012 is reliable for routine analysis. However, this method is recommended for a routinely use, with samples with a low expected prevalence of *E. coli* STEC.

References

1. EU One Health 2022 Zoonoses Report

P173

Forensic diagnostics

FORENSIC VETERINARY MEDICINE – ILLEGAL DISPOSAL OF PIGEON CARCASSES WITH MULTIPLE EPIZOOTICS

M. Alsaaod[3], S. Hermann[3], M. Sinniger[1], H. Marti[2], S. Albini[3]

[1]*Department for the Protection of Animals and the Environment (SPSA-TU), Cantonal Police Department, Zurich, Switzerland*
[2]*Institute of Veterinary Pathology, University of Zurich, Zurich, Switzerland*
[3]*National Reference centre for Poultry and Rabbit Disease, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland*

Introduction
Five carcasses of fancy pigeons, four adults and one juvenile, were found by a member of the public in an organic waste bin. The finder alerted the local police, which contacted the Department for the Protection of Animals and the Environment of the Cantonal Police for advice. The dead birds were then submitted to the National Reference centre for Poultry and Rabbit Disease (NRGK) for pathological forensic examination. The focus of this case was mainly on illegally disposed animal suffering from a notifiable animal disease and to a lesser extent on potential animal cruelty.

Materials and Methods
Testing was done for (all tests carried out individually): i) Avian Chlamydiosis (*Chlamydia psittaci*) by real-time PCR (qPCR)[1], ii) for Pigeon Paramyxovirus-1 by reverse transcriptase qPCR 2,3, iii) *Salmonella Typhimurium* by culture (ISO Method).

Results
Macroscopic examination showed two emaciated adults, two moderately nourished adults, and one moderately nourished juvenile. All pigeons were positive for all zoonotic agents tested, apart from the juvenile, which was negative for *C. psittaci.* The owner of the animals could be located through investigation by the police and the cantonal veterinary authority. To confirm the origin of the carcasses, official environmental samples were taken in the pigeon loft and sent for analysis. 15 out of 20 dust samples were weakly positive for *C. psittaci* by qPCR and one out of six environmental samples was positive for *Salmonella Typhimurium*, corroborating that the carcasses originated from this loft.

Discussion and Conclusion
Fancy pigeons can be vaccinated against pigeon paramyxovirus and *Salmonella Typhimurium*. Therapy against avian chlamydiosis is generally possible, but laborious, expensive, and not always successful. If a legally compliant treatment cannot be implemented, the only option is to cull the animals. The pigeons in this case were euthanized by the veterinary office in strict compliance with personal hygiene protection of the people involved for protection from the
zoonotic diseases, especially chlamydiosis[4].

References
1. Pantchev et al. 2009
2. Annaheim et al. 2022
3. Bhande et al. 2023
4. Imkamp et al. 2022

P174

Forensic diagnostics

DEVELOPMENT OF ANALYTICAL METHODS AND DIAGNOSTIC PROTOCOLS FOR THE DETERMINATION OF PLANT TOXINS IN ANIMAL POISONING CASES

F. Maccagnan[2], A. Mariacher[1], L. Di Benedetto[2], C. Eleni[1], A. Mastromattei[1], G. Bruni[1], V. D'Onofrio[1], F. Corrias[1], A. Piazza[1], S. Tonon[1], D. Deni[2], C. Focardi[1], G. Rosa[2]

[1]*Istituto Zooprofilattico Sperimentale Del Lazio E Della Toscana, Italy*
[2]*Istituto Zooprofilattico Sperimentale Del Lazio E Della Toscana - Centro Di Referenza Nazionale Per La Medicina Forense Veterinaria, Italy*

Introduction
Accidental toxic plant ingestion (TP) is estimated to be the second most common poisoning in ruminants after pesticides, and to account for 5-10% of carnivore poisoning cases[1,2]. TP is probably underestimated in clinical practice, due to lack of knowledge of poisonous essences and diagnostic methods. IZSLT 05/21 RC research project aimed to develop rapid analytical methods for the identification of plant toxins, through involvement of multidisciplinary units composed of veterinarians, botanists and chemists.

Materials and Methods
TP of interest were selected through a preliminary botanical census in Latium and Tuscany and collected in various stages between may and october in order to study an analytical protocol for molecules belonging to various classes, including alkaloids, glycosides, and non-proteogenic amino acids, both on plant and animal (liver and gastric content) matrices. A veterinary investigation based on clinical and anamnestic suspicion, and organic lesions was then conducted in order to draw up a diagnostic protocol.

Results
A panel of 26 phytotoxins was analytically identified from plants. In the diagnostic protocol, three main steps were identified: 1) clinical history and inspection with possible sampling of the suspected plant, 2) necropsy and collection and handling of the matrices to be analysed, 3) chemical and histological analyses. 31 samples were analyzed for phytotoxins and 3 tested positive from animal matrices. Of these, 1 calf for oleandrin and 1 donkey for evomine had a pathological picture consistent with TP, while 1 badger examined for road traffic accident was positive for coniine.

Discussion and Conclusion
The aim of this project was to develop a method for the identification of a panel of plant toxins and an operational protocol and TP database in order to guide veterinarians in diagnosis TP cases. The application of our protocol led the team to detect 3 clinical cases of plant poisoning in which clinical, pathological and chemical findings were described.

References
1. Cortinovis C., Caloni F. 2013. 10.1016/j.tvjl.2013.03.007
2. Albo G., Nebbia C. 2004. 10.1023/b:verc.0000045383.84386.77
3. SWGTOX. 2013. 10.1093/jat/bkt054

P175

Forensic diagnostics

OVERKILLING IN A DOG: A CASE REPORT

F. Pesce[2], E. Sannino[1], E. Ragosta[2], G. Picazio[2], M. Esposito[2], M. Di Matteo[2], B. Degli Uberti[2], S. De Luca[1], N. Di Caprio[1], D. Citarella[1], G. Fusco[2], G. Miletti[2]

[1]*Asl Napoli 3 Sud, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici, Italy*

Introduction
The term "overkilling" in Forensic Medicine is not clearly defined and is used in reference to homicides involving unusually massive injuries by far exceeding the extent necessary to kill the victim [1], that is a quite rare phenomenon in veterinary medicine [3]. This case report present findings in a dog found in Frebruary 2023, in a city near to Naples with a rope around the neck and in correspondence with the metacarpal region of the forelimbs (Figure 1).

Materials and Methods
The dog was admitted to the IZSM Portici where it was submitted to a total body radiographic study in right LL and VD projection (Figure 2) and to a complete necropsy collecting samples of organs that where analyzed by laboratories. The samples were fixed by immersion in 10% neutral buffered formalin for histological examination. Toxicological examinations were carried out for the research of rodenticides (by LC-MS/MS) and of pesticides (by HPLC and GC-EI/MS).

Figure 1. Rope foundend around the neck and metacarpal region.

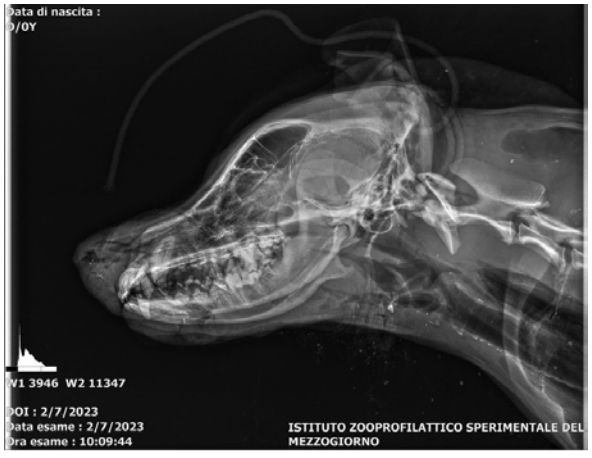


Figure 2. Radiographic study of the region of the head and neck.

Results
The autopsy revealed anatomopathological lesions compatible with strangulation and poisoning such as skin hyperemia of the neck region, pneumoderma of the neck, sternum and left scapular region, blood effusion in the trachea and laceration of a tracheal ring, serohaematic thoracic effusion, food material mixed with blackish microgranules and harmful substances in the stomach and unclotted blood in the heart. Histopathological exam revealed comminuted fracture of the hyoid bone, dyskeratosis and ischemic necrosis of traumatic origin of the epidermis.The suspicion of poisoning was confirmed by the positive outcome for dicoumarin anticoagulants.

Discussion and Conclusion
The forensic necropsy approach represents the first piece of a solid judicial investigation that leaves out no detail and leads to the conviction of the person responsible.

Funded project Ministry of Health IZS ME 05/22 RC.

References
1. Kopacz P, et al. Overkilling:A specific type of homicide – Constructing the definition:Injuries and victim. Legal Medicine,vol 59, 2022
2. Kopacz P, et al. Overkilling:a specific type of homicide – Constructing the definition:perpetrator, weapon and circumstances. Legal Medicine,vol 64, 2023
3. Solarino B, Punzi G, Di Vella G, Carabellese F, Catanesi R. A multidisciplinary approach in overkill: Analysis of 13 cases and review of the literature. Forensic Sci Int. 2019 May;298:402-407. doi: 10.1016/j.forsciint.2019.03.029. Epub 2019 Mar 22. PMID: 30953975.

P176

Forensic diagnostics

REPORTED MORTALITY AND PREVALENCE OF INFECTIOUS DISEASES OF FREE-LIVING GRIFFON VULTURE (*GYPS FULVUS*) OVER THE TWENTY-YEAR PERIOD 2003-2023 IN CENTRAL ITALY

S. Salucci[1], G. Scortichini[1], G. Di Teodoro[1], P. Badagliacca[1], G. Di Francesco[1], S. Pelini[1], M.C. Cantelmi[1], B. Costantini[1], S. Centi[1], M. Ciminelli[1], F. Iannino[1], A. Cocco[1], N.M. Sulli[1], E.E. Tieri[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise 'G. Caporale' (IZSAM), Campo Boario, Teramo, Italy*

Introduction
Vulture species worldwide play a key role in ecosystems as obligate scavengers and several populations have had a precipitous decline. In Italy, all griffon vulture (*Gyps fulvus*) populations became extinct between the nineteenth and twentieth century, with the exception of the Sardinian one. Currently many populations of *Gyps fulvus* are present in Sardinia, Friuli- Venezia Giulia, Abruzzo, Basilicata, Calabria and Sicily regions, mainly originating from reintroduction (Posillico et al, 2022). We reported the results of post-mortem examinations and analyses of griffon vulture carcasses collected in the Abruzzo region. The current population originated from 93 Spanish individuals, released between 1994 and 2002 mainly in the Monte Velino Reserve (Posillico et al, 2023). Understanding the clinical disorders and cause of mortality in *Gyps fulvus* is crucial for monitoring its Appennine population and for planning and enforcing appropriate conservation measures.

Materials and Methods
From January 2003 to December 2023, 109 griffon vultures, found dead predominantly in municipalities within the province of L'Aquila (Figure 1), were delivered at the IZSAM, and subjected to necropsy and related analytical activity, including in some cases histological examinations.

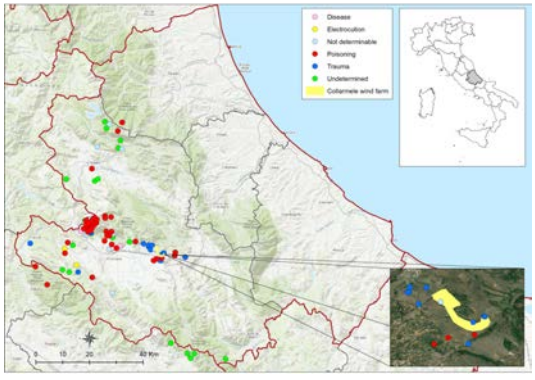


Figure 1. Abruzzo region, showing the municipalities of origin in wich tested vultures were found dead.

Cause of death	Number of vulture	Frequency of causes of death %
POISONING*	Total 46	42.20
Carbamates	34	
Carbofuran	20	
Aldicarb	11	
Carbaryl	2	
Methomyl	1	
Anticoagulant rodenticides (ARs)	1	
Bromadiolone	1	
Organophosphates	10	
Phorate	10	
Sulfotep	1	
Organochlorines	9	
Dieldrin	5	
Hexachlorobenzene	3	
HCH (beta)	2	
Linda ne	1	
P,P-DDD	1	
P,P-DDE	9	
Plant toxins		
Strychnine	2	
UNDETERMINED	25	22.95
NOT-DETERMINABLE	18	16.50
TRAUMA	Total 15	13.75
Collision with wind turbines	8	
Collision (cause	7	

Table 1. Distribution of causes of death.

Table 2. Results of the analytical activity

Chemical-toxicological analyses	Methods	Number vultures positive	Number vultures examined	Frequency %	Etiological agent identified
Carbamates	LC-HRMS	34	66	51.51%	
Anticoagulant rodenticides (ARs)	LC-HRMS	1	25	4.0%	
Organophosphates	GC-LRMS	10	62	16.13%	
Organochlorines	GC-LRMS	9	60	14.52%	
Plant toxins					
Strychnine	GC-LRMS	2	52	3.85%	
Inorganic Zinc					
Zinc phosphide	GC	0	13		
Heavy metals					
Lead (Pb) in bones (from 2017)	ICP-MS	40*	40	100%	
Molluscicides					
Metaldehyde	GC-LRMS	0	20		
Non-steroidal anti-inflammatory drugs (NSAIDs) (from 2017 by IZS del Mezzogiorno)	HPLC-MS-MS	0	13		
Infectious diseases					
West Nile Disease	RT-PCR Real Time	0	41		
Usutu virus	RT-PCR Real Time	0	32		
Newcastle disease	RT-PCR Real Time	0	32		
Avian influenza virus	RT-PCR Real Time	0	40		
Trichinella spp.	Artificial digestion	0	69		
Aerobic bacteria culture from liver, kidney, gut, brain, heart and lung	Culture	3	21	14.28%	1 *Staphylococcus aureus* 2 *Escherichia coli*
Anaerobic bacteria culture from liver, kidney, gut, brain, heart and lung	Culture	2	8	25.0%	2 *Clostridium perfringens*
Salmonella spp. from liver	Isolation	1	24	4.17%	*S. kasenyi*
Faecal eggs examination	Zinc sulphate flotation	11	22	50.0%	10 Ascaridídae 1 *Capillaria* spp.

*not toxic levels

Table 2.

Results
Poisoning was the prevalent cause of mortality (42.20%), mostly due to carbamates, followed by traumatic injury (13.75%), infectious diseases (2.75%) and electrocution (1.85%). In 18 cases (16.50%), the carcasses consisted of a few remains on which it was not possible to carry out a necropsy examination and were classified as "Not determinable". For 25 vultures (22.95%), the necropsy was not conclusive and so defined as "Undetermined" (Table 1). The results of analytical activity are reported in Table 2.

Discussion and Conclusion
Worldwide poisoning represents the most frequent threat for vultures and the central Apennines are no exception, based on the results of this study and the previous one by (Posillico et al, 2023). Data on vulture health are limited in the world (Ives et al, 2022) and this study was a contribution to reducing this gap.

References
Ives A.M. et al, 2022. Ecohealth, 19 (1):4054.
Posillico M. et al., 2023. Bird Conservation International, 33:e68. doi:10.1017/S0959270923000199

P177

Genomics

MOLECULAR IDENTIFICATION AND TARGET ENRICHMENT SEQUENCING OF PANCORONAVIRUS DETECTED IN ANIMALS

A. Bianco[1], S. Castellana[1], L. Del Sambro[1], L. Capozzi[1], A. Giannico[1], L. Pace[1], L. Marino[1], V. Manzulli[1], C. Ortello[1], M.L. Difato[1], G. Caldarola[1], V. Martella[2], D. Galante[1], A. Parisi[1]

[1]Istituto Zooprofilattico Sperimentale della Puglia e Basilicata [2]UNIBA-Facoltà di Medicina Veterinaria, Italy

Introduction
In the last few years, coronaviruses (CoVs) have become an emergency for public and animal health. During the COVID-19 pandemic, NGS sequencing was essential in the identification and monitoring of SARS-CoV-2 variants [1]. However, viral prevalence and diversity in animals are still poorly explored and surveillance studies are often performed using a serological approach. In this study we aim at testing a molecular approach for the identification and genomic sequencing of CoVs in wild and non-wild animals.

Materials and Methods
We collected 221 samples, from wild, livestock and companion animals in Puglia e Basilicata regions, between 2021-2024. Total RNA was extract and tested using a PanCoV semi-Nested-PCR[2]. Positive PCR products were purified and sequenced by ABIPRISM3130. Sequences were assembled, while taxonomical attribution was performed by BLASTn tool. The positivity to BCoV and FCoV was confirmed by real time assay. Positive samples were sequenced by applying targeted gene hybridization capture using the Pan-CoV panel by Illumina. Normalized libraries were pooled and sequenced on MiSeq platform. The raw data were analysed using the App DRAGEN Enrichment.

Results
Of the 221 samples tested, PanCoV semi-Nested-PCR and following Sanger sequencing identified a total of 47 positive samples. BLAST analysis of partial sequencing of the samples targeting RdRp gene showed the homology range to 94-99% with closed CoVs reference (Table 1). Samples positive for BCoV and FCoV were also confirmed by real-time assays, showing a ct value between 18 to 34. All samples were sequenced using PanCoronavirus panel enrichment. Bioinformatic analysis allowed a quite full reconstruction of the genome for 25 samples(Table 2; Figure1). For the remaining 22, reads did not map to CoVs genome.

Discussion and Conclusion
Among the samples analysed we identified 29 βCoVs(BCoVs), 14 αCoVs(CCoVs and FCoVs), 2 γCoVs and 1 □CoV. Although it was not possible to reconstruct the viral genome of all sequenced samples, the probe-based enrichment panel proved successful in capturing and sequencing the four genera of the family Coronaviridae. This user-friendly approach may enable comprehensive studies of known and potential spillover species in the future.

References
1. doi:10.3390/v13050731
2. doi:10.3390/v13101975

Host	Total N.	Source				Positive PanCoV (N.)	Taxonomy	% Identity (BLAST)	Genome (N.)
		Faecal swab	Respiratory swab	Organs	Peritoneal fluid				
Tawny owl	2	2	2	-	-	-	-	-	-
Duck	3	3	-	-	-	2	IBV	>98	2
Scops owl	1	1	1	-	-	-	-	-	-
Barn owl	2	1	1	-	-	-	-	-	-
Bos Taurus	38	10	38	-	-	29	BCoV	>98	9
Dog	11	11	3	-	-	8	CCoV	>98	9
Wild boar	44	44	44	-	-	1	-		-
Owl	1	1	1	-	-	-	-	-	-
Pigeon	2	2	2	-	-	-	-	-	-
Crow	1	1	1	-	-	-	-	-	-
Marten	2	2	2	-	-	-	-	-	-
Ferret	1	1	1	-	-	-	-	-	-
Chicken	1	1	-	-	-	-	-	-	-
Cat	19	17	-	2	6	6	FCoV	>98	3
Magpie	23	23	23	-	-	-	-	-	-
Jay	14	14	14	-	-	-	-	-	-
Porcupine	4	4	4	-	-	-	-	-	-
Otter	2	2	2	-	-	-	-	-	-
Wolf	16	16	16	-	-	-	-	-	-
Swine	2	2	1	-	-	-	-	-	-
Kite	4	4	4	-	-	-	-	-	-
Parrot	1	1	1	-	-	-	-	-	-
Sheep	1	1	1	-	-	-	-	-	-
Buzzard	1	1	1	-	-	-	-	-	-
Hedgehog	1	-	-	1	-	-	-	-	-
Coloeus monedula	15	1	1	-	-	1	DeltaCoV	94	1
Badger	2	2	-	-	-	-	-	-	-
Fox	6	6	6	-	-	-	-	-	-
Total	221	175	170	3	6	47	-	-	25

Table 1. Samples collected and analysis results The table shows the samples collected, including the type of sampling analysed, the number of PCR assay positive samples, the BLAST identification and the number of sequenced genomes. IBV: infectious bronchitis virus; BCoV: Bovine coronavirus; CCoV: Canine coronavirus; FCoV: Feline coronavirus; Delta CoV: deltacoronavirus.

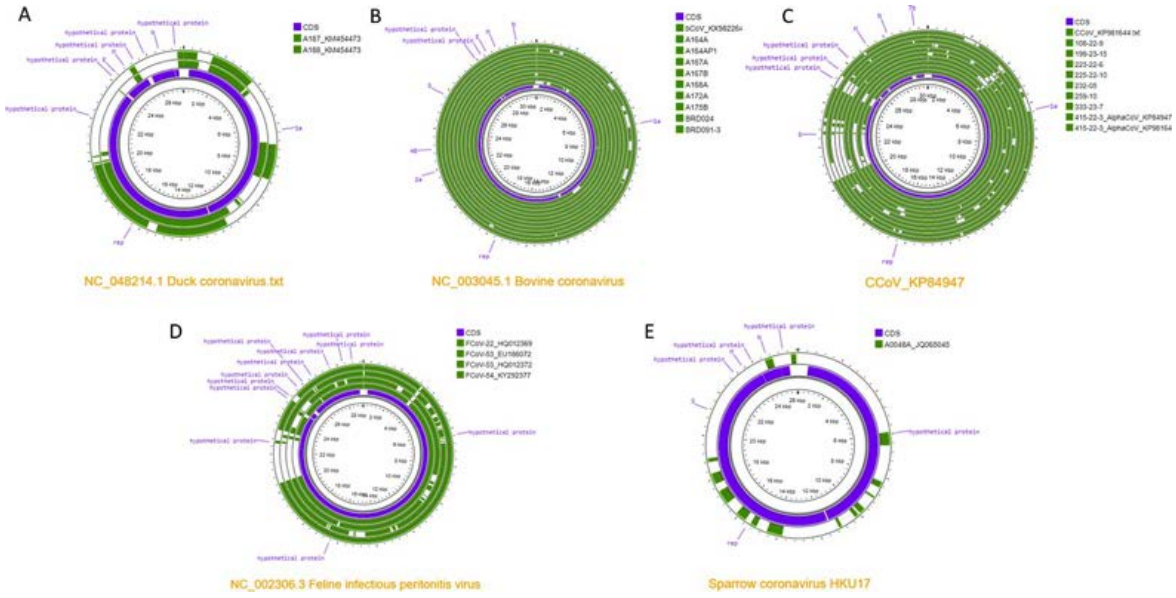


Figure 1. Genomes representation map and comparison. Reference genome (indicated in orange: A. Duck coronavirus comparison; B. Bovine coronavirus comparison; C. Canine coronavirus comparison; D. Feline coronavirus comparison; E. Sparrow coronavirus comparison) and complete genome/partial genome of CoVs identified in the study were compared using PROKSEE (https://proksee.ca/). In purple was indicated the Coding Sequences (CDS) generated on reference sequence. The genome sequenced by PanCoronavirus Panel were showed in green.

P178

Genomics

DIAGNOSTICS WITH OXFORD NANOPORE SEQUENCING: KEY IMPROVEMENTS AND INSIGHTS FROM YEARS OF ONT EXPERIENCE.

G. Croville[1], M. Walch[1], A. Secula[1], J. Guerin[1]

[1]*IHAP, Université de Toulouse, INRAE, ENVT, Toulouse, France*

Introduction
Animal health diagnostic predominantly rely on real-time PCR because of its specificity, low detection threshold, affordability, simple application, and robust interpretation. However, PCR's susceptibility to genetic evolution can result in false negative outcomes, and its focus on targeted pathogens limits its applicability for the detection of subpopulations or unknown pathogens. Consequently, diagnostic protocols are increasingly incorporating Next Generation Sequencing (NGS), which can rapidly produce high-throughput data. Currently, the main challenge in metagenomics is not generating data, but rather preanalytical treatment and bioinformatics analysis. After three years of routine use of Oxford Nanopore Technologies (ONT), we present our feedback based on multiple protocols for the analysis of three avian RNA viruses—Influenza A virus, avian coronavirus, and avian orthoreovirus.

Materials and Methods
The samples processed in this study included organs, environmental samples (dust) and FTA cards. The nucleic acids were extracted using magnetic bead extraction kit and were submitted to host and bacterial RNA depletiona. Then, a random amplification of RNA was performed following a modified SISPA protocolb. The sequencing libraries were prepared using Native Barcoding kit (ONT) and loaded onto MinION flowcells. Sequencing was run on the MK1C device with or without adaptive sampling.

Results
Whole genomes of the three viruses were successfully obtained from organ and environmental samples, with significant improvements after depleting host and bacterial RNA. However, the use of FTA cards resulted in reduced sequencing throughput and quality. A phylogenetic analysis was conducted to review circulating strains of Influenza A virus, avian coronavirus, and avian orthoreovirus.

Discussion and Conclusion
ONT metagenomics protocols applied to clinical samples enable whole genome sequencing of viruses. This workflow can contribute to the management of viral epizootics by facilitating early detection of emerging variants and enhancing our understanding of viral spread and evolution. However, continuous efforts are necessary to benchmark new innovations and assess their applicability to clinical samples.

References
1. Parris, Kariithi & Suarez, 2022
2. Chrzastek et al., 2017

P179

Genomics

GENETIC CHARACTERIZATION AND STUDY OF VIRULENCE AND AMR GENES OF *BACILLUS ANTHRACIS* STRAINS ISOLATED IN ITALY

D. Galante[1], S. Castellana[2], V. Manzulli[1], A. Parisi[2], L. Serrecchia[1], V. Rondinone[1], L. Pace[1], D. Farina[1], A. Fasanella[1], A. Scarinci[1], A. Bianco[2]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Putignano, Italy*

Introduction
Despite standardized genotyping methods for *B. anthracis* (e.g., SNPs, MLVA) have been available over the years, whole- genome sequencing (WGS) allows the complete genetic characterization and the study of genes related to virulence and antibiotic resistance. This project aims to genetically characterize *B. anthracis* strains collected in Italy over the last 40 years and to investigate the presence of virulence and antibiotic-resistance genes.

Materials and Methods
A total of 102 *B. anthracis* strains isolated from anthrax outbreaks in Italy were analyzed. DNA was extracted from bacterial colonies and sequenced using the MiSeq Illumina platform. Assembled genomes were analyzed using the BTyper v.3 tool (1), which identifies species based on ANIblast analysis, sequence type (ST), phylogenetic group, and ABRicate v1.0.1 for the prediction of virulence and antibiotic resistance genes.

Results

All strains were identified as *B. anthracis* belonging to *B. mosaicus* genomospecies. The seven loci necessary to define the ST revealed that 98% of the strains belonged to ST1, and 2% to ST3. Virulence factors, including the non-hemolytic enterotoxin NHE (nheABC gene cluster), sph, and bpsE genes, were identified in all the isolates. The co-presence of pXO1 (lef, pag, cya) and pXO2 (capABCDE gene cluster) plasmid genes, encoding toxins and capsule, was found in 85.29% of isolates. Based on AMR predicted genes, the presence of resistance genes for beta-lactams (bla1, bla2), clindamycin (LsaB), fosfomycin (fosB1, fosB2), and glycopeptide antibiotics (vanRM, vanZF) was found in almost all strains (Figure 1).

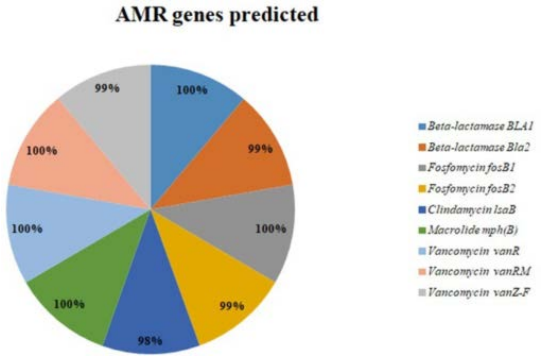


Figure 1. Graphic showing the different percentages of presence of predicted AMR genes performed by ABRicate v1.0.1.

Discussion and Conclusion

The presence of predicted AMR genes suggests the potential resistance to clinically relevant antibiotics, such as beta- lactams, including benzylpenicillin. Although the predicted antimicrobial resistance by WGS represents a good method to rapidly define eventual AMR phenomena, the phenotypic validation is also necessary to correctly address antibiotic treatments in case of anthrax outbreaks.

References

1. Carroll LM, et al. Rapid, High-Throughput Identification of Anthrax-Causing and Emetic *Bacillus* cereus Group Genome Assemblies via BTyper, a Computational Tool for Virulence-Based Classification of *Bacillus* cereus Group Isolates by Using Nucleotide Sequencing Data. Appl Environ Microbiol. 2017;83.

P180

Genomics

EXTENSION OF MULTIPLEX PCR FOR *STREPTOCOCCUS SUIS*

N. Kralova[1], O. Legradyova[2], M. Zouharova[3], J. Matiasovic[3]

[1]*Department of Experimental Biology, Faculty of Science, Masaryk University; Veterinary Research Institute, Brno, Cechia*
[2]*Department of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, University of Veterinary Sciences Brno*
[3]*Veterinary Research Institute, Brno, Cechia*

Introduction

Streptococcus suis is an important zoonotic pathogen of swine. 29 different capsular serotypes have been described and novel cps loci are constantly emerging [1-5]. To distinguish between serotypes, Multiplex PCR tests have recently been developed that target specific genes in the cps loci of *S. suis* [6]. Here, we have developed an extension of the multiplex PCR method from Kerdsin et al. for non-serotypeable strains collected in the Czech Republic.

Materials and Methods

After Whole genome sequencing of *S. suis* isolates collected in the Czech Republic and analyzing the cps loci of non- serotypeable isolates, we designed 19 pairs of specific primers targeting unique sequences to detect potentially novel cps loci. After verifying the functionality of Singleplex PCR reactions, we developed three Multiplex PCRs to detect multiple isolates simultaneously.

Results

Initially, we successfully tested all 19 PCR reactions. We developed Multiplex PCR schemes for 15 primers, while the remaining 4 primers, with similar PCR product lengths, have not yet been included in the Multiplex PCR. We successfully tested two Multiplex PCRs, each capable of distinguishing five types. Despite successful Singleplex PCRs, our attempt to verify the functionality of five additional primer pairs in the third Multiplex PCR was unsuccessful, indicating the need for optimization of the reaction conditions.

Discussion and Conclusion

We successfully developed two Multiplex PCRs, which can distinguish a total of 10 possible strains in two reactions. Although we were unable to design a third Multiplex PCR for the simultaneous differentiation of five strains, we believe that optimizing the conditions will allow us to achieve this. The extended Multiplex PCR allows for the identification of additional serotypes, thus reducing the number of untypeable *S. suis* strains.

This work was supported by grants RO0523 and TN02000017.

References

1 Okura M, et al. 2016. doi:10.3390/pathogens5030045
2 Zheng H, et al. 2015. doi.org/10.1128/AEM.00315-15
3 Qiu X, et al. 2016. doi.org/10.1128/AEM.02102-16
4 Zheng H, et al. 2017. doi.org/10.1186/s13567-017-0417-6
5 Huang J, et al. 2019. doi.org/10.1111/tbed.13123
6 Kerdsin A, et al. 2014. doi:10.1099/jmm.0.069757-0

P181

Genomics

POPULATION ANALYSIS OF YERSINIA ENTEROCOLITICA STRAINS CIRCULATING IN ITALY THROUGH WHOLE GENOME SEQUENCING

E. Ventola[2], M. Montalbano Di Filippo[2], P. Chiani[2], A. Knijn[2], M. Marra[1], V. Libri[1], G. Scavia[2], E. Delibato[2], S. Morabito[2], V. Michelacci[2]

[1]*Istituto Superiore di Sanità, Core Facilities Technical-Scientific Service, Italy*
[2]*Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health, Italy*

Introduction

Yersinia enterocolitica is the third most commonly reported foodborne zoonotic pathogen in humans in EU [1]. Conventional methodology for strains characterization involve PCR methods and phenotypical biotype (BT) classification, which is laborious, but relevant, as different BTs are associated with different pathogenicity levels in the human host: BT1B is considered as highly pathogenic, BT2, BT3, BT4 and BT5 as pathogenic and BT1A as non pathogenic [2]. This work aimed at studying the evolution of the BTs through Whole Genome Sequencing (WGS).

Materials and Methods

Twenty-seven strains belonging to different BTs and serogroups isolated in Italy from 1985 to 2022 from human, food and animal sources were subjected to WGS on an Ion Torrent S5. WGS analysis was performed on ARIES (https://aries.iss.it/platform) by assembling with SPAdes and performing core genome-MLST (cgMLST) analysis with chewBBACA [3] in comparison with 65 sequences from public repositories (21 BT1A, 11 BT1B, 17 BT2, 12 BT3, 24 BT4 and 7 BT5) [2]. A multivariate data analysis was performed on cgMLST allelic results and descriptive data of the strains, by conducting a Multiple Correspondence Analysis (MCA) followed by Hierarchical Clustering on Principal Components (HCPC) with R software.

Results

The cgMLST grouped the strains in BT-specific clades (Figure 1). The MCA showed that >50% of the variance observed in the dataset was ascribed to five dimensions and the following HCPC identified four well defined clusters and two outliers (Figure 2). In detail, the strains belonging to BT2, BT3 and BT4 were grouped in one cluster (cluster 1) and those belonging to BT5, BT1A and BT1B were grouped in different well defined clusters (clusters 2, 3 and 4, respectively).

Discussion and Conclusion

The population study clustered the strains on the pathogenicity level assigned to the different BTs, suggesting that BT1A and BT1B could have originally diverged due to a single event, driving the evolution towards non pathogenic and highly pathogenic strains, respectively. A deeper study of the variables describing the clusters may allow the identification of genetic markers for different pathogenicity levels.

References

1. EFSA and ECDC. 2023. EFSA Journal;21(12):8442
2. Reuter S et al. 2014. Proc Natl Acad Sci USA;111(18):6768-73
3. Silva M et al. 2018. Microbial Genomics,4(3): e000166

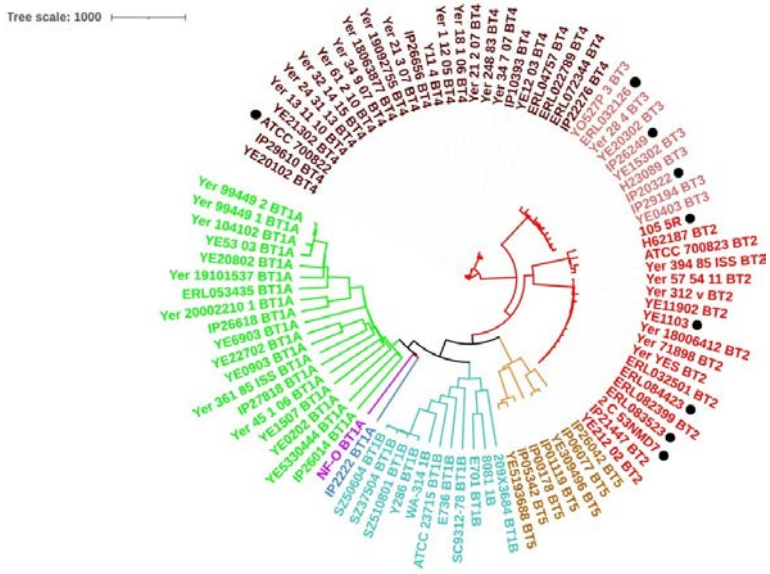


Figure 1. cgMLST phylogenetic tree. Information on the biotype characterization is appended to the strains' names. The black dots indicate strains for which the biotype assignment described in the literature is different from that of the strains part of the same clade. Each clade was assigned a different colour. The largest clade grouping BT2, BT3 and BT4 strains was coloured with different shades of red attributed to the different subclades corresponding to the different biotypes.

Figure 2. HCPC Factor Map. The clusters were coloured based on the clades detected in cgMLST analysis (Figure 1).

P182

Genomics

CIRCULAR ECONOMY AND AQUACULTURE: EVALUATING THE EFFECTS IN FARMED *SPARUS AURATA* OF DIETS SUPPLEMENTED WITH POLYPHENOLS FROM OLIVE OIL BY-PRODUCTS

M. Torricelli[3], A. Felici[3], R. Branciari[1], M. Trabalza Marinucci[1], M. Biagetti[3], A. Manfrin[2], R. Galarini[3], L. Boriani[3], E. Radicchi[3], C. Sebastiani[3], M. Ciullo[3], S. Primavilla[3], F. Agnetti[3]

[1]*Dipartimento di Medicina Veterinaria – Università degli Studi di Perugia - Perugia, Italy*
[2]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy*
[3]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy*

Introduction

Circular economy based on the recovery of by-products for the integration of feed with bioactive molecules, sustainable and with high nutritional value, is an ambitious issue to be explored in aquaculture. The aim of this work was to evaluate in farmed *Sparus aurata* the effect of diets integrated with polyphenols obtained from olive mill waste on gene expression level, through a nutrigenomic approach.

Materials and Methods

Gilthead seabream were divided into two groups: one control group (ctrl) fed with conventional feed, the other group (tr) treated with diet supplemented with polyphenols, chemically characterised. A portion of each liver district was sampled, from which mRNA was extracted to perform novel RT-qPCR assays. Target genes investigated were: interleukins (IL-10; IL-12) for immunity pathway, alkaline-phosphatase (ALP) and fatty acid binding protein 2 (FABP2) involved in fatty acid metabolic pathway, glutathione reductase (GR) and superoxide-dismutase (SOD) as mediators of oxidative stress [1,2]. Differential gene expression analysis was performed and differences between groups were calculated using Student's t-test ($p<0.05$), [Table 1].

Table 1. DGE (differential gene expression) analysis in liver between control and treated group, with level of significance (p value).

		RQ				RQ				
gene	matrix	mean_control	σ	ci-	ci+	mean_treated	σ	ci-	ci+	pvalue (ttest)
FABP2	**liver**	**1.07**	**0.39**	**0.77**	**1.37**	**0.6**	**0.44**	**0.26**	**0.94**	**0.0308**
SOD1	**liver**	**1.05**	**0.37**	**0.77**	**1.33**	**0.59**	**0.1**	**0.51**	**0.67**	**0.0022**
IL-12 β	**liver**	**1.13**	**0.63**	**0.64**	**1.61**	**0.63**	**0.26**	**0.43**	**0.83**	**0.0433**

RQ: Relative Quantification
σ: standard deviation
ci: confidence interval

Table 1. DGE (differential gene expression) analysis in liver between control and treated group, with level of significance ($p<0.5$).

Results

Relative quantification (RQ) outcomes showed a down-regulation of gene expression for FABP2 in the treated group (mean RQ_tr: 0.6 vs mean RQ_ctrl: 1.07) with p-value <0.05, confirming the role of polyphenols in reducing the synthesis, uptake and transport of fatty acids. A down-regulation was also observed for SOD1 gene in the treated group (mean RQ_tr: 0.59 vs mean RQ_ctrl: 1.05), with a high level of significance (p-value <0.01), supporting the hypothesis that the antioxidant power of polyphenols limits the enzyme synthesis. A significant (p-value<0.05) down-regulation was observed for IL-12β in the treated group (RQ: 0.63) compared to the control (RQ: 1.13), confirming the polyphenol's anti-inflammatory action. For the other target genes no statistically significant differences were observed.

Discussion and Conclusion

These preliminary data have to be confirmed, but they already suggest that a controlled use in aquaculture of bioactive molecules may have positive implications in terms of animal welfare, product safety and eco-sustainability.

Research funded by Italian Ministry of Health RC IZSUM 01/2021.

References

Pérez-Sánchez, J. et al. 2013
Naya-Català, F. et al. 2021

P183

Genomics

TRANSCRIPTOME ANALYSIS OF BRAIN, INTESTINE AND HYPOPHARYNGEAL GLANDS OF *APIS MELLIFERA* USING THE OXFORD NANOPORE THIRD GENERATION SEQUENCING TECHNOLOGY

L. Zulian[3], M. Pauletto[2], A. Vezzi[1], G. Sales[1], F. Bordin[3], M. Dacasto[2], F. Mutinelli[3], A. Granato[3]

[1]*Department of Biology, University of Padua, Padua, Italy*
[2]*Department of Comparative Biomedicine and Food Science BCA, University of Padua, Legnaro (Padua), Italy*
[3]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (Padua), Italy*

Introduction
Apis mellifera plays a crucial role in biodiversity and ecological balance. So far, honey bee transcriptome and its modifications induced by stress or pathogens have been poorly studied. Thus, the characterization of the transcriptional profiles of key organs in response to biotic and abiotic factors could fill these gaps and provide new knowledge.

Materials and Methods
Honey bees from four apiaries in Northern Italy were collected and immediately frozen in liquid nitrogen. Brain, hypopharyngeal glands, and intestine were excised and total RNA was extracted from four pools of each tissue (1 pool = 3 bees from a single apiary). Twelve tissue-specific cDNA libraries were constructed and sequenced using the Nanopore Mk1C. State-of-the-art software were used for raw data analysis, reads alignment, and splicing isoforms detection.

Results
About 27 million sequences per tissue were obtained. A total of 55,651 isoforms were identified, of which 21,501 were expressed in at least one of target tissues. Out of these, 6,719 transcripts were annotated, while 10,071 were identified as novel transcripts. The brain showed the highest number of transcripts (14,689), followed by the intestine (8,953) and hypopharyngeal glands (5,227). The analysis of differential expression among the tissues highlighted 2,371 differentially expressed transcripts (DETs), of which 1,236 with known biological function and 715 novel transcripts. The most abundant DETs were those 'activated' exclusively in the brain (some opsins, crucial for vision, and a gene with a fundamental role in longevity) or intestine (genes involved in apoptosis). Several DETs were moderately, but differentially, expressed in all the target organs. As an example, Glutathione S-transferase theta-3 and peroxiredoxin 6 genes, involved in oxidative stress response, were significantly more 'activated' in the intestine and hypopharyngeal glands.

Discussion and Conclusion
The present study, carried out within the RC IZS VE 09/20 project and funded by the Ministry of Health, has improved the knowledge of the transcriptome of three key organs of A. mellifera and investigated its complexity by identifying new transcripts. Therefore, it represents a valuable basis for further molecular investigations into honey bee response to environmental stress.

P184

Immunology and vaccines

AN ANNUAL PRIMARY VACCINATION OF REPLACEMENT EWES AGAINST COXIELLOSIS INDUCED A LONG-LASTING IMMUNE RESPONSE AND ALLOWED A CONTROL OF INFECTION

J. Böttcher[1], B. Bauer[4], C. Ambros[1], M. Alex[1], U. Domes[1], S. Roth[3], K. Boll[2], M. Korneli[3], K. Bogner[2], A. Randt[1], B. Janowetz[1]

[1]*Animal Health Service Bavaria, Germany*
[2]*Bavarian Health and Food Safety Authority, Erlangen, Germany*
[3]*Staatl. Veterinäramt, Landratsamt Main-Spessart, Germany*
[4]*University of Veterinary Medicine Hanover, Foundation, Clinic of Swine and Small Ruminants, Forensic Medicine and Ambulatory Service, Germany*

Introduction
Small ruminants are regarded as a major source of human infection with *Coxiella burnetii* (Cb). Q fever in humans was traced back to a sheep flock with 650 ewes in 2012. Since January 2013 replacement ewes were annually primary vaccinated with Coxevac® (Ceva Santé Animale) without any further revaccination.

Materials and Methods
Immune responses of sentinels (antibodies/IFN-γ; (1)), of vaccinated sheep before and after primary vaccination (PV) and shedding at

lambing (vaginal and nasal swabs) were monitored until 2024. The effect of a single revaccination (RV) was assessed in 2018 and 2023 on randomly selected animals. The immune response prior to and 3 (2018) and 4 weeks (2023) after RV was determined in animals primary vaccinated in 2015, 2016, 2017 (in 2018) and primary vaccinated in 2019, 2020, 2021, 2022 and 2023 (in 2023).

Results
Major shedding was detected until February 2014, the mean pathogen load in positive samples was 102.6 and 101.6 Cb for vaginal and nasal swabs, respectively. Thereafter one vaginal and one nasal swab tested weak positive in 2021 and one vaginal swab in 2023. PhII-seroconversion in sentinels faded out in 2017, but irregular and low level IFN-γ- reactivity persisted. A retrospective analysis of sentinels in 2021 confirmed a low-level and irregular seroconversion. The post-vaccination immune response (humoral/cellular) decreased since 2015 and the major serological pattern after vaccination switched from PhI+/PhII+ to PhI-/PhII+. A single RV induced a strong and complete (PhI, PhII, IFN-γ) immune response irrespective of the time between PV and RV. Post-revaccination antibody responses in 2018 and 2023 were not significantly different. However, a lower level IFN-γ-reactivity was observed in 2023.

Discussion and Conclusion
A PV of replacement ewes induced a long-term immune response and allowed the control of Coxiellosis. A subliminal infection persisted, but it did not result in a major outbreak. A single RV might be considered as an emergency measure.

References
1. Böttcher J, Bauer BU, Ambros C, Alex M, Domes U, Roth S, et al. The immune response to a Coxiella burnetii vaccine in sheep varies according to their natural pre-exposure. Vaccine. 2024;42(8):1993-2003.

P185

Immunology and vaccines

EFFECT OF VACCINE CANDIDATES ON THE IMMUNODIAGNOSIS OF ANIMAL TB AND ASSESSMENT OF DEFINED ANTIGENS AS DIVA REGENTS

P. Cuenca-Lara[3], M. Blay-Benach[3], Z. Cervera[3], C. Melgarejo[3], J. Moraleda[3], I.A. Sevilla[1], J.M. Garrido[1], M. Singh[4], G. Jones[2], B. Pérez De Val[3]

[1]Animal Health Department, NEIKER-Instituto Vasco de Investigación y Desarrollo Agrario, Basque Research and Technology Alliance (BRTA), Derio, Bizkaia, Basque Country, Spain
[2]Department of Bacteriology, Animal and Plant Health Agency (APHA), Surrey, NB, UK
[3]IRTA, Programa de Sanitat Animal, Centre de Recerca en Sanitat Animal (CReSA), Campus de la Universitat Autònoma de Barcelona (UAB), Bellaterra, Catalonia, Spain
[4]Lionex Diagnostics and Therapeutics GmbH, Braunschweig, Germany

Introduction
Tuberculosis (TB) in goats is mainly caused by *Mycobacterium caprae* and *M. bovis*. Without official caprine TB eradication programs in the EU, bovine TB strategies are usually adapted for goats in high-incidence areas. In addition, vaccination has been proposed when the test-and-slaughter strategy is not feasible. Official antemortem tests for TB diagnosis include the single and comparative intradermal (i.d.) cervical tuberculin tests (SICT and SICCT, respectively) and the Interferon-γ release assay (IGRA), all based on tuberculins. However, parenteral vaccination with live or inactivated mycobacteria compromises the tests' specificity. This study assessed the effects on the diagnosis of different vaccination protocols and the suitability of defined antigens as reagents to differentiate between infected and vaccinated animals (DIVA).

Materials and Methods
Thirty goats were divided into six groups: *M. bovis Bacillus* Calmette-Guérin (BCG, intranasal - i.n.); Heat-inactivated *M. bovis* (HIMB, i.n.); HIMB with mucosal adjuvant (HIMBmuc, i.n.); Prime-boost HIMB (subcutaneous - s.c.) with parenteral adjuvant (HIMBpar) and HIMBmuc (i.n.); Prime-boost BCG (s.c.) and HIMBmuc (i.n.). Two goats were used as nonvaccinated controls (NV). Skin tests were performed at 14 weeks after HIMBpar/BCG s.c. vaccinations and 8 weeks after intranasal vaccinations or boosting, by inoculating (i.d.) *M. bovis* PPD (PPDB), *M. avium* PPD (PPDA) and the fusion protein (DST-F) containing ESAT-6, CFP10 and EspC (6). The skin fold thickness was measured before inoculation and after 72h. Concurrently, IGRA was performed by stimulating whole blood with PPDB and PPDA and an antigenic cocktail of ESAT-6, CFP10 and EspC (DIGRA-C). Two assays were carried out using commercial kits.

Results
Qualitative and quantitative results of the immunodiagnostic tests are shown in Table 1 and Figure 1 and 2, respectively. NV goats were negative to all tests.

Discussion and Conclusion
The specificity of tuberculin-based diagnostic tests was compromised in vaccinated animals, especially in the BCG (i.n.) and prime-boosted groups. In contrast, the use of DIVA reagents minimized the diagnostic interferences on the skin tests and IGRAs, particularly in intranasal delivered vaccines.
This study was funded by the Spanish State Research Agency (Ref. PID2022-142939OR-C22).

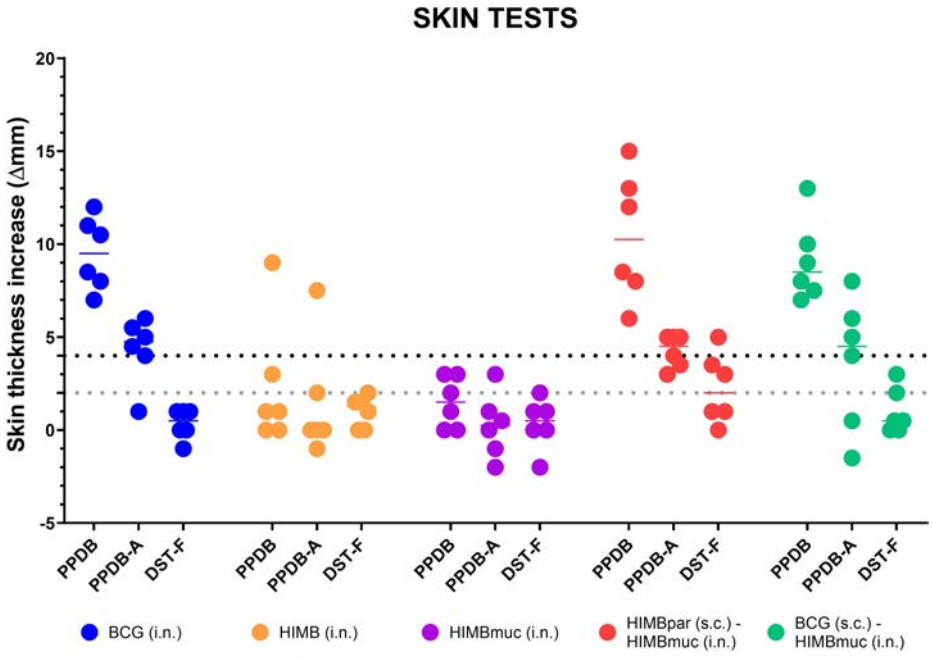
TABLE 1. Immunodiagnostic tests results.

Group (n)	SKIN TESTS						IGRAs [1]			
	Severe Interpretation			Standard Interpretation			ID Screen® Ruminant IFN-γ		BOVIGAM™ TB	
	SICT [2]	SICCT [3]	DST-F [4]	SICT	SICCT	DST-F	PPDB/PPDA [5]	DIGRA-C [6]	PPDB/PPDA	DIGRA-C
NV (2)	0 (+ [7])	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)
BCG i.n. (6)	6 (+)	6 (+)	0 (+)	6 (+)	4 (+)	0 (+)	5 (+)	0 (+)	3 (+)	0 (+)
HIMB i.n. (6)	2 (+)	2 (+)	0 (+)	1 (+)	1 (+)	0 (+)	1 (+)	0 (+)	0 (+)	0 (+)
HIMBmuc i.n. (6)	2 (+)	1 (+)	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)	0 (+)
HIMBpar s.c.-HIMBmuc i.n. (6)	6 (+)	6 (+)	3 (+)	6 (+)	3 (+)	1 (+)	6 (+)	2 (+)	2 (+)	1 (+)
BCG s.c. –HIMBmuc i.n. (6)	6 (+)	4 (+)	1 (+)	6 (+)	3 (+)	0 (+)	6 (+)	0 (+)	4 (+)	0 (+)

[1] Interferon-γ release assay; [2] Single Intradermal Cervical Tuberculin Test; [3] Single Intradermal Comparative Cervical Tuberculin Test; [4] Fusion protein containing ESAT-6- CFP10 and EspC antigens; [5] *M. bovis* and *M. avium* tuberculin purified protein derivatives; [6] Antigenic cocktail composed of ESAT-6, CFP-10 and EspC recombinant proteins; [7] Positive animals.

SICT and DST-F skin test were considered positive if Δ skin fold thickness to PPDB > 2mm (severe interpretation) or ≥ 4mm (standard interpretation). SICCT was considered positive if positive bovine reaction > avian reaction in ≥ 1 mm (severe interpretation) or > 4 mm (standard interpretation). Cut-off for positivity in the ID Screen® Ruminant IFN-g (ID, Grabels, France) kit: S/P ≥ 16%. Cut-off for positivity in the BOVIGAM™ TB (Thermo Fisher Scientific, Waltham, MA, USA) kit: ΔOD ≥ 0.1.

Table 1.



DGE (differential gene expression) analysis in liver between control and treated group, with level of significance (p-value < 0.5).

Figure 1. Skin fold thickness increase after PPDB and DST-F inoculation. PPDB: *M. bovis* tuberculin; DST-F: Fusion protein containing ESAT-6, CFP10 and EspC antigens. Each colour represents a different vaccination group. Horizontal lines in each group represent the median values. The dotted lines show the positivity cut-offs for the severe (grey) (> 2mm) and standard (black) (≥ 4mm) interpretations of the single intradermal tuberculin test (SICT) and DST-F skin test. Animals were considered positive to the skin test if their reaction was above the positivity cut-offs.

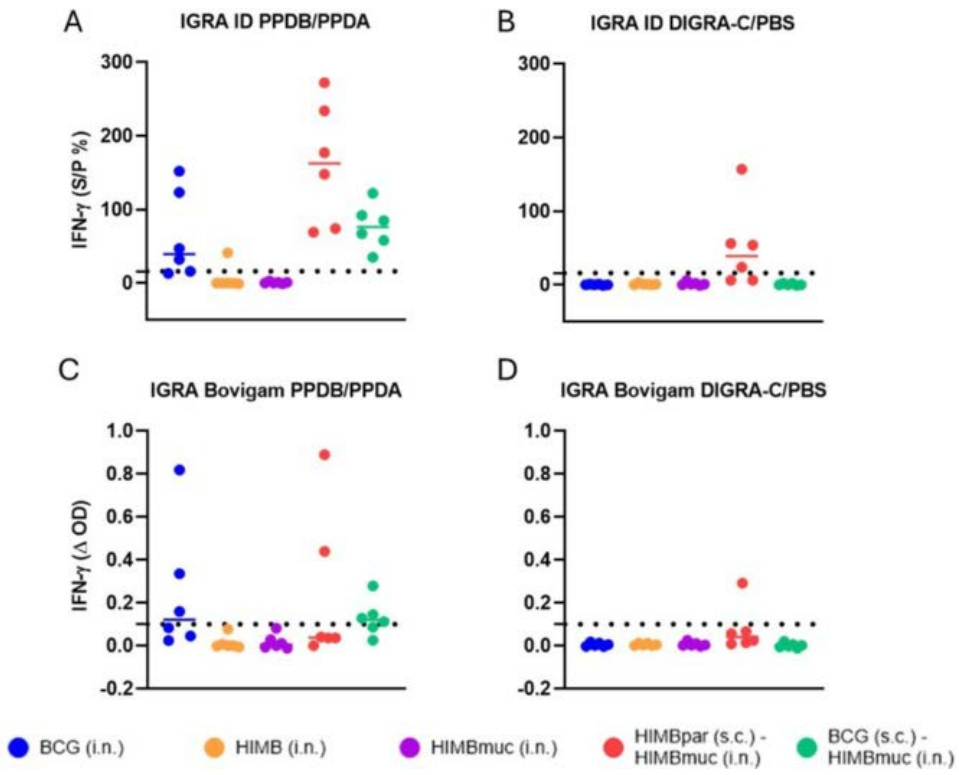


Figure 2. IFN-γ responses using each IGRA kit. (A, B) ID Screen® Ruminant IFN-g (ID, Grabels, France). The S/P (%) ratio was calculated dividing the Optical Density (OD) PPDB – PPDA or PBS/OD Positive Control-Negative Control x 100. Cut- off for positivity: S/P ≥ 16%. (C, D) BOVIGAM™ TB (Thermo Fisher Scientific, Waltham, MA, USA). The OD was calculated using PPDB OD - PPDA OD or DIGRA-C OD - PBS OD, respectively. Cut-off for positivity: OD ≥ 0.1. The interpretation of the results was done following the indications of the Spanish National Reference Laboratory for bovine TB. Horizontal lines in each group in (A), (B), (C) and (D) represent the median values.

P186

Immunology and vaccines

QUALITY CONTROL OF VETERINARY AUTOGENOUS VACCINES BY MONOCYTE ACTIVATION TEST (MAT) PERFORMED ON A MOUSE CELL LINE: PRELIMINARY DATA.

A. Di Paolo[1], C. Colabella[1], R. Liberti[1], L. Anzalone[1], I. Font Monclus[2], C. Raviolo[2], G. Severi[1], M. Cagiola[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" IZSUM Perugia, Italy*
[2]*Merck Life Science Srl – Milano, Italy*

Introduction

Veterinary vaccines play an important role in control and prevention of animal diseases, furthermore, autogenous vaccines (AVs) are an efficient tool against antibiotic resistance. AVs, as all injectable products, undergo to quality control process (QC) to ensure their safety and suitability. The acceptance of the 3Rs principle is leading to the adoption of *in vitro* methods (i.e. Monocyte Activation Test – MAT) in several QC processes. MAT is an alternative to animal- based methods and allows the detection of both endotoxic and non-endotoxic pyrogens in injectable pharmaceutical products and can be performed on immortalized murine monocyte-macrophage cell line and/or cryopreserved PBMCs. The inflammatory process elicited could be highlighted through specific cytokines release. Among the various pro-inflammatory interleukins assessed as potential biomarkers for the MAT test, IL-1β, IL-6 and TNF-α have been found to be statistically significant (1). In this work, we described the first application of MAT as a QC assay for AVs produced at IZSUM Pharmaceutical Unit.

Materials and Methods

A total of twelve Gram-positive and viral AVs diluted at 1:100 were tested on murine macrophages Raw 264.7 cells. After 24 hours of incubation, the concentration of the cytokines released in culture supernatants was analyzed by a customized mouse cytokine/chemokine multiplex panel Milliplex® with Luminex200 ™ system.

Results
All AVs showed increasing in cytokines production versus unstimulated control. Among the three biomarkers investigated TNF-α showed higher levels, followed by IL-6, while IL-1β displayed the lowest levels.

Discussion and Conclusion
Application of *in vitro* methods for AVs QC instead of *in vivo* pyrogen tests could determine a substantial replacement in the use of animals. These preliminary results, together with those previously obtained with *in vitro* MTS assay (2), need to be implemented and are necessary to get a correlation to lab animal evidence. The final perspective is the standardization of protocols and the application of validated alternative tests. Funded by the Italian Ministry of Health, IZSUM PALTEX22.

References
1. Valentini S et al. Vaccine 37 (2019) 3754–3760.
2. Colabella C et al. Estiv XXI International Congress. Book of abstract page 559.

P187

Immunology and vaccines

EVALUATION OF THE PERFORMANCE OF TWO COMMERCIAL INTERFERON GAMMA RELEASE ASSAY KITS FOR THE DIAGNOSIS OF TUBERCULOSIS IN WATER BUFFALO (*BUBALUS BUBALIS*): PRELIMINARY DATA

A. Martucciello[4], A. Donniacuo[4], E. De Carlo[4], P. Mazzone[2], L. Schiavo[4], M.B. Boniotti[3], P. Cerrone[1], C. Spoleto[1], G. Galiero[4], J. Bezos[5], B. Romero Martinez[5]

[1]*Azienda Sanitaria Locale, Salerno, Italy*
[2]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Perugia, Italy*
[3]*National Reference Centre for Bovine Tuberculosis, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy*
[4]*National Reference Centre for Hygiene and Technologies of Water Buffalo Farming and Productions, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Salerno, Italy*
[5]*VISAVET Health Surveillance Centre, Complutense University of Madrid, Madrid, Spain /Facultad de Veterinaria, Departamento de Sanidad Animal, Universidad Complutense de Madrid, Madrid, Spain*

Introduction
The in vivo diagnostic methods used for tuberculosis (TB) in cattle and buffaloes are the tuberculin skin test (TST) and the interferon-gamma realease assay (IGRA), as provided by EU Delegates Regulations 2020/689 and 2020/688. IGRA is an official diagnostic test for granting/maintaining Officially Tuberculosis-Free (OTF) herds status, and is used also for water buffalo (1). We evaluated the performance of two commercial IGRA kits for TB diagnosis in this species.

Materials and Methods
A total of 216 blood samples, 180 from TST negative buffaloes from 13 OTF herds and 36 from TB-infected buffaloes confirmed by post-mortem tests were used. Samples were collected in heparinized tubes, dispensed into four 1 mL aliquots, and stimulated with Phosphate-buffered saline (PBS, Nil Control Antigen), bovine antigen (PPDB, 20 µg/ mL), avian purified protein derivative (PPDA, 20 µg/mL) and Pokeweed Mitogen (PWM, 2 µg/mL, lymphocyte viability control). After 18-24 hours of incubation, plasma was collected and stored at -80°C until analysis. Samples were tested using IDvet (ID Screen® Ruminant IFN-γ, IDvet) and Bovigam (Bovigam™ TB Kit, Thermo Fisher Scientific,) kits on the same day, according to the manufacturer's instructions. The interpretation of results was done using the EU-RL and manufacturers' cut-offs recommendations: IDvet, Positive: S/P $\geq$ 35%; Bovigam, Positive: OD PPDB - OD PBS $\geq$ 0.1 and OD PPDB - OD PPDA $\geq$ 0.1.

Results
All samples from the TB-infected buffaloes were positive using both kits. Out of the 180 samples from healthy buffaloes, same 4 samples resulted positive in both assays.

Discussion and Conclusion
These results show that the two diagnostic kits provide perfectly overlapping results, considering that the same PPDs were used, as already described for cattle (2). Data should be validated on a large number of animals, considering the age of the animals and the presence of non-tuberculous mycobacterial species to investigate false positive reactions. This research was funded by the Italian Ministry for Health (RC IZSME 014/2022), by ICRAD project, an ERA-NET co- funded under European Union's Horizon 2020 research and innovation programme (https://ec.europa.eu/programmes/ horizon2020/en) under Grant Agreement n°862605, and the EU-RL for Bovine Tuberculosis
.

References
1. Martucciello et al., 2020
2. Gomez-Buendia et al., 2023

P188

Immunology and vaccines

SWINE NEPHROBLASTOMA: A COMPARATIVE MODEL FOR HUMAN WILMS' TUMOR?

F. Fruscione[7], G. Del Zotto[3], C. Cantoni[5], G.M. Spaggiari[6], B. Cafferata[8], E. Brambilla[1], V.G. Vellone[8], L. Gibelli[4], C. Pigoli[4], C.G. De Ciucis[7], L. De Paolis[7], B. Passeri[2], V. Grieco[1], E. Razzuoli[71]Department of Veterinary Medicine and Animal Science, University of Milan, Lodi, Italy[2]Department of Veterinary Science, University of Parma, Parma, Italy

[3]*Integrated Department of Services and Laboratories, IRCCS Istituto Giannina Gaslini, Genova, Italy*
[4]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "Bruno Ubertini", Brescia, Italy*
[5]*Laboratory of Clinical and Experimental Immunology, Integrated Department of Services and Laboratories, IRCCS Giannina Gaslini Institute, Genoa, Italy AND Department of Experimental Medicine, Genoa University, Genova, Italy*
[6]*Laboratory of Clinical and Experimental Immunology, Integrated Department of Services and Laboratories, IRCCS Giannina Gaslini Institute, Genoa, Italy AND Department of Experimental Medicine, Genoa University, Genova, Italy*
[7]*National Reference Center of Veterinary and Comparative Oncology (CEROVEC), Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Genova, Italy*
[8]*Pathological Anatomy, IRCCS Istituto Giannina Gaslini, Genoa, Italy*

Introduction

Nephroblastoma (NB) is a common renal neoplasm of swine, affecting animals < 1 year old, and may resemble the animal counterpart of Wilms' tumour (WT) in children [1]. WT is the most common paediatric renal tumour, with an annual incidence of 1:10.000 [2]. Treatments include pre or post- surgery chemotherapy, but some patients experience recurrence or adverse effects. Understanding NB and WT biology could validate swine as a suitable animal model for WT. Our study, supported by national project 'PRIN 20227FX7KM' and regional funding "REGIONE LIGURIA DD n. 3434/2022", focuses on the histological and molecular characterization of swine NB tissue and human WT primary cell lines, along with their inflammatory microenvironments.

Materials and Methods

Thirty FFPE swine NB samples were histologically evaluated with H&E staining. RNA was extracted from these samples and 10 normal renal FFPE samples using the Maxwell RCS RNA FFPE kit, and from 10 WT primary cell lines (4 epithelial- like, 6 stromal-like). Gene expression related to tumour progression and prognosis (Table 1) was analysed using RT- qPCR.

Results

NB cases were classified as epithelial (2/30), blastemic (2/30), and mixed (26/30) based on the percentage of each component. Only 18 tumour FFPE samples were suitable for molecular analysis and showed downregulation of IL10, IL13, IL12b, IL23, CXCL8, IL17a, IL18, IL1b, IL6, EBI3, IFNß, IFNγ, CDH1, HIF1α, and RANKL, and upregulation of TNFα, TGFß, CDH2, and SNAIL compared to controls. These findings are in line with results obtained in human WT cells, which show low expression of IL10, IL12b, and IFNγ. Stromal-like WT cells exhibited a different expression pattern compared to epithelial-like WT cells, with upregulation of TGF-ß and downregulation of CDH1, IL23, and EBI3.

Discussion and Conclusion

Swine NB tissues show the same morphology as their human counterparts, and gene expression matches the literature for WT tissues [3]. Moreover, human epithelial and stromal-like cells presented different gene expression patterns, resembling WT tissue [3].
These preliminary data indicate that swine NB could be a suitable model for human WT.

References

1. Grieco et al doi: 10.1016/j.jcpa.2005.09.003.
2. Carachi R. The Surgery of Childhood Tumors. Springer; 2016
3. Palmisani F, et al. doi: 10.1016/j.omto.2021.06.016.

GENE	SPECITS	Aooe..;..:ion nunher	PRThU'.RF CE.WARD	PRIMER Rr VERSE
n:.113	human	NII/1 0005 76.3	CAG GGAC.i\:G GATAlG G.il:G CA	ACG C.i\:G GAC AGG TAC AGA TI
Ilh	hum.an	NII/10{1{16{10.5	GAG AGTAGTGAG GAACAAGCC	GGTCAG GGG lGG TIA TIG CA
C'l:CLS	hum.an	NM 000534.4	CE'ICTlGG CAG CCT'ICC T	TIG GGG 1GG AA.4GGTTIG GA
1NF«	hum.an	NM 00ili9·4.4!	CCTCAG CCTCTICECTICC	GGC **'ffi** 1CA*CK:* GGG GTI
HIFIOI	human	NM 001530.4	CCC.4TITIC'Dl.C'ICAGGACAc.ll.GA	GCTITG11.GGACTIGCGCTIT
FrnL1	hum.an	NII/1005438.5	AlGCC.4.CCCll\.GCCAAlG1C	GAAACAGlG GGC AGC TITGG
1GFl31	human	NI\,[0006l50.7	GTIG1G CGG CAG lGG TIG	AGTG 1G 1TAEC C1G C1G 'ICA
IFN'l	hum.an	NI\,[000619.3	.il:GT1CTGC.4.1CG TITlGG **GT**	1CC GCTACA1CTGAA1GACCT
IU3	hum.an	NI\1 016534.3	GAAGCTClG CAC ACTGGC	lGTlGTCCC lGA G'IC CTIGG
Il.12p35	hum.an	NII/1001354!531.1.	ACC ACTCCC AAAACClGC **T**	CCAA**1G G**TA..1\ 4.C AGG CCTCC
n:..np40	hum.an	NII/1 002187.*3*	GCC GTIC.4C A..i\:G C1C A..J!.G TAT	AAG **TIC 11G** GGTGGG 1CA GG
ILI8	human	NMOOI562.4!	A'ITGAC CAJ\.GGAAA1CGG CC	T:.V.. AT:-\.lGG 1CC GGG G1G CA
1\ViST	hum.an	NI\,[0004!74!.4	GGCCGGAGACCIJ!..GA'IG'K:ATI	CCCCACGCCCIGTTICTTIG
IL.II	hum.an	NI\1002190.3	ThC A..4.C CGA1CC *M:;C* 1CA CC	.4.CTTIG CCTCCC AGA'ICACA
MI\11P9	hum.an	NI\;[0041994 .*3*	AA.G GCG C.ll.G AlGGlG GAT	'ICA ACTCAC 1CC GGG AAC1C
n:.13	hum.an	NII/10013549911.	1CAACA "!CACCC AGAACC AGA	GAA1C'C GCTC.il:G C.4.TC-CTCT
ll..lO	hum.an	NI\1 000571..3	GGAGA..4CCTGAAGACCCTC.4	AAC'ICA C1C AlG GCTTIG 1J!.G A
II.27	human	NII/1145659·.3	AGTGAACCTGlACCTCCTGC	lGA.il:GC G'IG G1G GAG A1G AA
R.4NKL	hum.an	NI\,[003 701.4	GCCTIICA..i\:GGAGClG1GCA..4M	GAG CMAAGGC1GAGCTICAAGC
ZEBl	human	NII/!030751	GGC.J\.lACACC'IAC'ICAAC'IACGG	lGGGCGGlG1AGAA1CAG.il:G1C
!:NAJL	hum.an	NII/1 005985	1C1Cll\.GGCCC1GGC1GC'IA.	TIGACA'ICIG AG'IGGG1C1GG
IFN.13	hum.an	NI\1 002.176	**C'ffiG**A11CCDlC.4AAG MGCAGC	'ICCECTICIGG AA.ClGClGCA
CDHl	hum.an	NI\,[004 3,15(1	GCC'ICC1G AAA.J!.G AGAGlGG MG	lGGCAG1G1CTCTCCA..4A'ICCG
CDfil	human	NI!\1 001792	CC'lC'Cll.G11.GTI1AC'IGCC.41GAC	G'Dl.GGA'IC'ICCGCCAC"IGA.TIC
Tp53	hum.an	NM 000546	CC'IC..4.GC.41CTIA1C'CGAG1GG	1GGA1GG1GG1I\.C.AG'ICAGAGC
CNTIB	human	NII/1 001093200	CM::AAGCAGAG**1GCIG**AAGG**1G**	GATIOCIG AGAG'ICCA.AAG ACAG
EBI3 (IL35)	hurnan	NI\1 0-05755.3	C'IGG A'IC-eGTIJ\.CA..4.GC-GlCAG	CACTffi<i ACG'IAG'Il\.CCIGGCT
n:..113	Su, scrofa	NI\,[214055.I	GCCAGETICA.TIG'JICAGGTIT	CCA..4.GG'ICc.4.GGTTilGGGT
IL6,	*Su;* sc,-afa	NI\I 2143399,I	1GGC'II\.C1GCC11CCC'DI.CC	CAGAGATTTIGCCGAGGAlG
CXCLS	Su ,crofa	NII/1 2138·67.I	C'IG'TL\CAACCTK:"IGCACCC.4	11CGA'IGCC.4.G1GC.4TA.llATh
"JNFO.	.S.u; scr-afa	NIvI 214022.I	C'IGGCCC.4..1\:GGACE.4.G A'ICA	CCAGGAGGGC.4TIGGC.4.TAC
HIFI«	Su, scr-afa	NII/1 0-0112'3124.I	EA."i.GA'ICAGCC.il..GC'TI-1.GX:C	C.A..4.G'ICT:.\AAE.4.G1G1CC1GAG'TI.\
FCSL1	Su; scrofa	XII.1 003122519.3	CAG'IAC..4.G'ICCCCC. 4..4CC	CEGGGGCIGA1C1GTIC.4C
1GFl31	S.u; scrofa	XI\•! 00566529-8.3	AGCGCG ATI"!GC.4GG 'D!.TIGA	GCCGG TIGG ACIG**'ffi1 G**C
IFN'f	*S.u;* s,c, afa	NII/1 2139413.I	GC'IC1GGG AAACIGAAIG AC11CG	1GACTIC1CT1CCGC'IJ'ICTDI.GG
II.23	Su, scrofa	NI\,[0-01137-621.1	GCGGGGA'IGGCIG1GA'IC	CA'ICAGGGlG1AGAGA.4.GGCT
Il..l2p35	*Su;* s,c.r afa	NM2I3993,I	AlGCC'ICA..4CCACEcc.4M	1GlGClGGTITD!..'ICTTIGG**1G**A
1Ll2p4(1	.S.u; scrofa	NI\'1 214013.I	CCClGA..4.GMG ACGGCA.'ICA	A'IGG1CAGGG'ITilGCCAG T
n:.IS	*Su;* scr-afa	NII/1 213997.1	GC1GCIG AACCGGAAGAc.4.A.**T**	CGATIC'C.il:GG1C11CAK'G**TIT**
1\VET	Su scr-afa	Xli..,[003130192..5	GCIGAGCA,"i.GA1CCAGACCC	GC1CG'IC.4.ClClG'I:-1.GGACC
Il:..,17	S.u, scr-afa	NI\,[001005729.1	CCC'ICll.G AT:.l..C1CC.AA4CGC	CTier'JECCTIC.4.GC.4TIGA
I\'IMP9•	*Su,* scrofa	NI\'1 001033004.I	GGGCTT-\.G A'ICAClCA.J\.CCG	CCGGGG TIC..4.GG TIIAGGG
n:..,13	*Su;* s,c.r afa	NI\1 213803 .I	CACCc.4GAACe.4.GA..4.GACll.CC	CA'JGC1GG1GG'IC.ll.GG11GA
ILIO	S-u; sc,afa	NII/1 _214}{141.1	AGCC-AGC-ATIA..4.G'IClGAGM	C-C1CE11GG AGCTIGClAA
n:.27	*Su;* s,c.r afa	NI\1 00100752:0.1	ElCCGAGATfIGCA..4.CAGC	GTICTCCICC1CC1CC1G.4.C
RANKL	Su scr-afa	Xli..,[001925694.6	GM::A'ICCCA1CCGG11CCC	CGC.4.'ICG.il:GGTTICC1G.4.A.G
ZEEl	S.u, scrofa	XI\1 0210641%.1	A1CGCOLG11.GAAGAACGAA	GAC"IGCG'ICll.CAlG'ICElGA
SNAIL	.S.u, scrofa	XM 021077%1.1	CACGACICTIC1CCAGAG1ICA	AGAGAG1CCC.i\:G AlGAGCGT
IFN,	.S.u; sc.r-afa	NII/1 214'393.I	AA.GCA'ICIGC.4..J!.GGTICcc.4AT	ECAA...A.G1CCCTIC1G'IGG1C
CDHl	S.u; scr-afa	NI\1 O-OU63060.I	lGG AGA..4.GAGGACe.4.GG AC1TI	GCAGlGGGG1CACIA.'K 4.GC
CDH2	.S.u; scr-afa	XM 0210%205.1	A'ICCCACGThlGGCCTTICC	GGA.CCX:GCCG 'IAGA.J\.lG1C
Tp53	Su; s,c.rafa	NI\1 _213824.3	GGCGAGTA.TIE.AC-CC'ICCA	CTICTDU,M::'IICAGGlGGC'IGG
CNTIB	S.u, scr-afa	NI\I 214367.I	AGACGGAGGA..4.GGECG AG	GC1CCA'IC.4.4..41CAGC'ffiGG
EBB (IL35)	Su ,crafa	NII/1 001315681..1	CAACG1C.4.CAGCCAEC.4.C	GGTI'IC'C.AC'IGC.«:CCA..4.
REFERENCE GENE;				
b2M	S.u; sc.rafa	NI\.,[2.13978.1	A1GG1GA.i\:GG1CGGAG1GAA	AG'IGG.i\:GG'ICAA.lG A..i\:GGGG
GAIDH	S.u, scrofa	NIvI 001206359.1	CCCTICIGG1CCACAC1G A.GT	lGG 1CK'GA'ICCCACTDI. ALT
GAPDH	hum.an	NM 002046.7	**;e:.aaP- t e:.!;t e2:; a t** c;a	**ace .ae:;a ;!!;tt aaa.a .a;;ccc**
RLPO	human	NM 001002.4!	**c:aggga:agacaggge**	**cc:a.eat tgt et ct"c ccac.a**
RIP3JA	human	M-1[000998.5	**..-dg .;ug .a:-g.a. c:_ga ;gct gt:g g;g**	gtc ttc t;g,. tgg cg aet tt
ACTI!	human	NM 0011015	**:ate CJ..!; t ,=;4t!;0ct'a tee et**	**c.at ace cct C!!;t al:!.a te**
HPRT	human	NM 000194.3	**;g;.1J. .a:g gt ttt J.t t** cct c;a,t ·2"-	**gcc tee c.J.t etc ctt c;at c;a**
RSPI4	hurnan	NM 0010250712	**:ate .a.cc gcc eta cac:ate a;a**	ate c-,gc ttg a:tc ttc ;,i.a cc

P189

Immunology and vaccines

DEVELOPMENT AND FIELD VALIDATION OF COMPANION SEROLOGICAL AND MOLECULAR DIVA ASSAYS FOR ASF VACCINES, BASED ON THE DETECTION OF THE P72, EP153R, AND EGFP GENES/CODIFIED PROTEINS

G. González-García[4], J. Fernández-Pinero[1], C. Gallardo[1], C. Galán[4], M. Montón[4], S. Barroso-Arévalo[8], N. Casado[1], J.Á. Barasona[8], Ž. Šteingolde[5], J. Buitkuviene[7], P. Václavek[3], A. Oggiano[2], I. Nurmoja[6], J.M. Sánchez-Vizcaíno[8], Á. Venteo[4], P. Sastre[4], P. Rueda[4]

[1]*Centro de Investigación en Sanidad Animal, CISA, INIA-CSIC, European Union Reference Laboratory for African Swine Fever (EURL), Valdeolmos, Madrid, Spain*
[2]*Department of Animal Health, Istituto Zooprofilattico Sperimentale della Sardegna, Sassari, Italy*
[3]*Department of Virology, State Veterinary Institute Jihlava, Jihlava, Czech Republic*
[4]*Gold Standard Diagnostics Madrid SA (GSD Madrid), Madrid, Spain*
[5]*Institute of Food Safety, Animal Health and Environment "BIOR", Riga, Latvia* [6]*National Centre for Laboratory Research and Risk Assessment (LABRIS), Tartu, Estonia*
[7]*National Food and Veterinary Risk Assessment Institute, Vilnius, Lithuania*
[8]*VISAVET Health Surveillance Center, Complutense University of Madrid, Madrid, Spain*

Introduction

African Swine Fever (ASF) is one of the most significant infectious diseases affecting both domestic pig and wild boar populations. Currently, outbreaks have been reported worldwide, emphasizing the urgent need for an effective and safe vaccine for ASF control. In this context, several promising vaccine candidates based on Lv17/WB/Rie1, a naturally attenuated genotype II ASF virus, have been generated under the VACDIVA project, in which several genes, including the EP153R gene, have been deleted and replaced by the eGFP reporter gene. The objective of the present study was to develop both molecular and serological DIVA (Differentiation between Infected and Vaccinated Animals) companion assays for these vaccine candidates. Additionally, the p72 gene/antigen was included in the assays as a control for infection and to monitor the vaccination.

Materials and Methods

To achieve this objective, we developed a triplex DIVA PCR for the detection of B646L, EP153R and eGFP genes. Similarly, based on the codified proteins, a companion serological assay was developed based on ELISA platform.

Results

Initially, both assays were evaluated with experimental samples. Particularly, the evaluation of the serological assay was performed using 112 samples from 6 domestic pigs (DP) and 87 samples from 8 wild boar (WB), immunized with the parental virus. The results showed that 100% of the animals seroconverted against p72 and pEP153R, although with a delayed onset between both antibody responses, and all resulted negative against eGFP. On the other hand, 207 samples from 16 DP and 96 samples from 8 WB vaccinated with the candidate vaccines were analyzed. All vaccinated animals were negative against pEP153R, and positive against p72 and eGFP, with a similar sero-conversion profile. Subsequently, a field validation of both assays was performed using more than 1,500 serum and tissue samples from European endemic regions, leading to very good diagnostic parameters in the field for both assays.

Discussion and Conclusion

The results demonstrate that both DIVA diagnostic assays could be useful companion tools for surveillance during prospective vaccination campaigns in regions where vaccines, based on modified genotype II ASFV strains in which the EP153R gene has been deleted and/or the eGFP reporter gene has been inserted, could be used.

References

doi: 10.1111/tbed.13132

P190

Immunology and vaccines

DEVELOPMENT OF A *COXIELLA BURNETII* NEUTRALIZATION ASSAY

M. Heijne[2], Y. Corripio-Miyar[1], A. Dinkla[2], M. Van Den Esker[2], T. Mcneilly[1], L. Ravesloot[2], A. Koets[2]

[1]*Moredun Research Institute, the Netherlands*
[2]*Wageningen Bioveterinary Research, the Netherlands*

Introduction
Q fever, caused by the intracellular bacterium *Coxiella burnetii*, is a zoonotic disease having worldwide impact. Ruminants are considered the most important reservoir for *C. burnetii*. In ruminants, one of the main control measures is vaccination, but the current available whole-cell formalin-inactivated vaccine has limitations in terms of safety, ease of manufacture and lack of capacity to differentiate between infected and vaccinated animals (DIVA). Therefore, an improved vaccine that is safer, easier to manufacture and has DIVA capacity would be of added value. To develop such a vaccine, in-vitro tools are needed to evaluate the efficacy of candidate vaccine antigens prior to testing in vaccine efficacy trials.

Materials and Methods
A monoclonal antibody (mAb IBIS-57) was developed to visualize cellular invasion and replication of *C. burnetii*. BGM cells were infected with different MOI (multiplicity of infection) of *C. burnetii* and harvested at different time points. An immune peroxidase monolayer assay (IPMA) with mAb IBIS-57 was used to determine the level of infectivity. Phase 1 immunized goat serum and polyclonal sheep IgG generated against *C. burnetii* vaccine candidates, were tested to investigate a neutralization effect.

Results
C. burnetii infection could be visualized in BGM cells using monoclonal antibody IBIS-57 without background labelling of non-infected cells. Neutralisation was observed 24 hrs post infection with MOI 270, or 7 days post infection with MOI 27 or 2.7 using phase 1 immunized goat serum. Also, in some of the polyclonal sheep IgG a neutralization effect was observed.

Discussion and Conclusion
The monoclonal IBIS-57 could successfully be used in a newly developed neutralization assay for *C. burnetii*. With the assay, neutralization could be observed at different timepoints and MOI. Further optimization of the test lies in an improved quantification of the neutralization effect, for instance by image analysis software. In conclusion, we developed an *in vitro* Coxiella neutralization assay to perform functional analysis of antibodies against *C. burnetii* in sera from vaccinated animals. This tool can be used to select candidate antigens for incorporation into novel Q fever vaccines.

P191

Immunology and vaccines

IN SILICO AND *IN VITRO* CHARACTERISATION OF RECOMBINANT *CHLAMYDIA ABORTUS* ANTIGENS AS POTENTIAL VACCINE CANDIDATES

H. Hill[1], M. Livingstone[1], K. Aitchison[1], C. Cunnea[1], K. Snedden[1], M. Rocchi[1], S. Wattegedera[1], D. Longbottom[1]

[1]*Moredun Research Institute, Edinburgh, UK*

Introduction
Ovine Enzootic Abortion (OEA), caused by *Chlamydia abortus*, is a significant reproductive disease in sheep, leading to late-term abortions, stillbirths, and delivery of weak lambs. Current vaccines face efficacy, stability, and safety issues[1]. The *C. abortus* outer membrane protein complex (COMC) has recently shown promise as a vaccine due to its protective efficacy in pregnant sheep[2]. This study aims to characterise constituent proteins of the COMC and effector proteins of the Type III secretion system (T3SS), which are involved in chlamydial virulence and pathogenesis, as candidate antigens for future vaccine and diagnostic test development.

Materials and Methods
COMC and T3SS effector proteins were identified by mass spectrometry and bioinformatic analysis, respectively. Candidate protein 3D structures and sequence features were predicted using AlphaFold2 and Protean 3D, revealing signal sequences, transmembrane domains, hydropathy, charge, antigenicity, and monoclonal antibody epitopes. Partial or whole genes were cloned, transformed, expressed, and affinity-purified as HIS-tagged fusion proteins. The proteins were then tested by immunoblotting using convalescent sheep sera and *in vitro* lymphocyte re-stimulation assays to evaluate immunogenicity.

Results
In silico modelling led to the design of 29 COMC and T3SS effector proteins. Analysis of 18 constructs resulted in the production of

recombinant proteins that varied in expression levels. The major outer membrane protein (MOMP), Polymorphic membrane protein (Pmp)14G passenger domain, and translocated actin recruiting phosphoprotein (Tarp, T3SS effector) displayed good to moderate expression levels. Additionally, seven other outer membrane proteins exhibited significant expression. MOMP, Tarp, dnaK, Pmp14G, and a putative transmembrane helical protein demonstrated strong immunoreactivity to convalescent sheep sera.

Discussion and Conclusion
Structural analysis of selected antigens facilitated the expression of targeted COMC and T3SS proteins for immunogenicity evaluation, marking an important step towards identifying promising candidate antigens for in vivo subunit vaccine studies against OEA.

References
1. Caspe, S.G., et al. (2021). Pathogens, 10(5), 543
2. Livingstone, M. et al. (2021). Vaccines, 9(8), 898

P192

Immunology and vaccines

POULTRY VECTOR VACCINES: INNOVATIVE SEROLOGICAL ASSAYS FOR VACCINATION MONITORING AND DIVA TESTING FOR H5 AVIAN INFLUENZA A

S. Lesceu[1], M. Gaimard[1], C. Redal[1], J. Drus[1], C. Lefebvre[1], M. Zorbas[1], L. Comtet[1], P. Pourquier[1]

[1]*Innovative Diagnostics, France*

Introduction
Influenza viruses belong to the family *Orthomyxoviridae*. There are four types of influenza viruses: A, B, C and D; which are defined by the nature of their internal nucleocapsid antigen. Type A is the most conserved genus and can be further divided into subtypes based on their Hemagglutinin and Neuraminidase antigens. Some subtypes are associated with highly pathogenic forms of the disease and high rate of mortality. Since 2004, a lineage (H5 HPAI) has been circulating worldwide and has been responsible for important poultry losses. These successive waves of Influenza in Europe pushed the health authorities to review their vaccination strategy. Given the need for rapid and reliable serological tools for monitoring of vaccination, IDvet has developed unique indirect ELISAs: one, based on H5 recombinant protein, for the monitoring of recombinant vaccines, and one, based on NP protein, for DIVA strategy (differentiated Infected from Vaccinated Animals).

Materials and Methods
Flocks vaccinated with different technology of vaccines (H5 RNA, r-HVT-AI(H5) or inactivated sub-unit AIV-H5 vaccines) were tested. Antibody titers for H5 were evaluated using the H5 iELISA. Samples were also tested with the NP iELISA to monitor field challenge. For each tested flock, the following parameters were measured: mean titers, minimum, maximum, and CV%. All samples with titers higher than 732 for H5 iELISA, and higher than 668 for NP iELISA, were considered positive.

Results
All the flocks vaccinated with H5 vaccines were found positive with the H5 iELISA. Some of them were also found positive with the nucleoprotein iELISA. Therefore, the positivity of the H5 iELISA, belonging to negative flocks with the NP iELISA, demonstrated the detection of seroconversion induced by vaccines. The positivity of the NP ELISA in some flocks highlighted the presence of challenge with one HxNy strain.

Discussion and Conclusion
The H5 indirect ELISA presented is the only quantitative test for the specific detection of H5 antibodies which allows for H5 vaccination monitoring. The NP indirect ELISA is an excellent tool for the detection of wild virus in populations vaccinated with recombinant H5 vaccine.

P193

Immunology and vaccines

OPTIMIZATION OF BACTERIAL LIQUID CULTURES FOR AUTOGENOUS VACCINE PRODUCTION TO BE ADMINISTERED TO SPECIES OF AQUACULTURE INTEREST.

D. Prataviera[2], A. Tondo[2], B. Colò[2], M. Picchi[2], L. Cortinovis[1], M. Bottinelli[2], S. Catania[2]

[1]*Centro Specialistico Ittico, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*
[2]*Mycoplasma Unit, WOAH reference laboratory for avian mycoplasmosis (M. gallisepticum, M. synoviae), SCT-1 Verona, Istituto Zooprofilattico Sperimentale delle Venezie, Buttapietra, Italy*

Introduction
Rainbow trout *(Onchorynchus mykiss*) is one of the most farmed fish species in Italy and Europe. *Yersinia ruckeri* biotype 2 (YRB2) and *Lactococcus garvieae* (LG), are two common bacterial pathogens responsible for relevant economic losses causing hemorrhagic symptoms. Therefore, implementation of prevention measures, in particular vaccine development, is fundamental from a perspective of antimicrobial stewardship and its conscious use. Autogenous vaccines consist in inactivated-antigen suspensions obtained through culturing bacterial strain of interest in liquid media. The goal of this project was to maximize viable cells count (VCC)/ml values for vaccine batch production.

Materials and Methods
Batch cultures of each species were prepared inside glass bottles (volume 1000 mL) with screw on top caps, using Heart Infusion Broth medium (volumes 100 ml and 600ml) and incubated for 16-48 hours at 25 °C. Eight experimental groups were defined combining different broth-to-vessel ratios and broth agitation methods (orbital vs magnetic). At different time points, broth turbidity was assessed using a nephelometer and VCC was calculated through Most Probable Number method (1).

Results
YRB2 batch cultures reached Log10 VCC/ml values between 10 and 10.7 and 30 turbidity units, while LG batch cultures reached Log10 VCC/ml values between 8 and 9 and 10 turbidity units. YRB2 experimental groups reached the highest turbidity and VCC values when cultured with 100 ml of broth and 150-180 RPM orbital shaking. On the other hand, the highest turbidity and VCC values for LG experimental groups were reached with 600 ml of broth and magnetic stirring.

Discussion and Conclusion
It can be inferred that the growth of YRB2, a non-motile bacterium, benefits from fast orbital shaking, which likely enhances oxygen dissolution into the broth. On the contrary, in order to achieve high VCC values with LG in broth it is better to culture it with low oxygen concentrations.

References
1. https://www.fda.gov/food/laboratory-methods-food/bacteriological-analytical-manual-bam

P194

Immunology and vaccines

EXPLORING THE ROLE OF INACTIVATED BCG AS AN IMMUNOMODULATOR IN SARS-COV-2 DISEASE

L. Sánchez-Morales[2], N. Porras[5], T. García-Seco[5], M. Pérez-Sancho[2], F. Cruz[5], S. Barroso-Arévalo[2], B. Chinchilla[3], M. Diaz-Frutos[2], A. Buendía[5], I. Moreno[1], A. Rodríguez-Bertos[4], M. Domínguez[1], L. Domínguez[2]

[1]*Unidad de Inmunología Microbiana, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Carretera Pozuelo-Majadahonda, Madrid, Spain*
[2]*VISAVET Health Surveillance Centre, Complutense University of Madrid / Department of Animal Health, Faculty of Veterinary Medicine, Complutense University of Madrid, Spain*
[3]*VISAVET Health Surveillance Centre, Complutense University of Madrid / Department of Animal Production, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain*
[4]*VISAVET Health Surveillance Centre, Complutense University of Madrid / Department of Internal Medicine and Animal Surgery, Faculty of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain*
[5]*VISAVET Health Surveillance Centre, Complutense University of Madrid, Madrid, Spain*

Introduction
SARS-CoV-2 remains a global health problem that requires further studies on effective vaccine strategies to combat new variants. This led

to an exploration of whether the BCG (*Bacillus* Calmette–Guérin) vaccine, used against tuberculosis, could enhance the immune response against SARS-CoV-2. BCG has immunomodulatory properties and offers protection against different heterologous pathogens. However, studies on its efficacy against SARS-CoV-2 have shown controversial results, with both protective and non-protective outcomes

Materials and Methods
A total of 36 K18-hACE2 mice were used to assess the effects of inactivated BCG on SARS-CoV-2 infection. Mice were inoculated with BCG and then infected with SARS-CoV-2, with the control group consisting only of SARS-CoV-2 infected animals. Daily monitoring assessed clinical signs and mortality. Mice were euthanized at 3- or 4-days post-infection or upon reaching endpoint criteria. Necropsies were performed, and tissue samples were collected to quantify viral loads. Blood sera were analyzed to assess cytokine level.

Results
Mortality was 33% in the BCG/SARS-CoV-2 group and 50% in the control group. The BCG/SARS-CoV-2 group presented higher viral loads in the lungs, trachea and nasal turbinates but lower viral load in the brain. IFN-γ and IL-6 levels were elevated in BCG/SARS-CoV-2 mice that became ill. Anti-inflammatory cytokine levels were higher in BCG/SARS-CoV-2 mice that did not show clinical signs.

Discussion and Conclusion
Our findings suggest that inactivated BCG may provide protection against SARS-CoV-2 in K18-hACE2 mice. In particular, we observed a trend toward lower severity in mice inoculated with inactivated BCG, which contrasts with previous findings using live BCG. While no studies have specifically tested inactivated BCG against SARS-CoV-2, research on inactivated mycobacteria against other diseases has shown varying results, including protective effects and lack thereof. Differences in virus variants, BCG strains, and animal models are critical factors to consider across studies.

References
Zhang et al., 2022. Doi: 10.1172/jci.insight.157393 Kaufmann et al., 2022. Doi: 10.1016/j.celrep.2022.110502 Hildebrand et al., 2022. Doi: 10.1128/spectrum.03075-22
Ferreras-Colino et al., 2023. Doi: 10.21203/rs.3.rs-2865092/v1

P195

Laboratory animals

ARTIFICIAL SCAFFOLD IN POLYBUTYLENE SUCCINATE FOR BONE TISSUE REGENERATION: STUDY ON THE *IN VIVO* "RABBIT" MODEL

L. Cicero[3], R. Cirincione[3], M. Licciardi[1], R. Puleio[3], G. Vigni[2], L. D'Itri[2], V. Gargano[3], D. Gambino[3], G. Barbaccia[3], D. Vicari[3], G. Cassata[3]

[1]*Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Palermo, Italy*
[2]*Department of Surgical, Oncological and Stomatological Disciplines, University of Palermo, Palermo, Italy*
[3]*Experimental Zooprophylactic Institute of Sicily, Italy*

Introduction
Polybutylene succinate (PBS) with presence has high potential as a scaffold in bone tissue engineering due to its ability for cell proliferation and differentiation, biocompatibility and bioresorption. The aim of the project was to demonstrate the improvement of the bone regeneration process of the tibial part, following a bone defect, through the use of three- dimensional microfibrillar scaffolds (with or/and without growth factors) based on biocompatible materials capable of stimulating the reconstruction of damaged or removed tissue and generate a positive effect on the repair times created experimentally on the rabbit animal model.

Materials and Methods
A circular defect (8 mm in diameter) was formed on the tibial (frontal) part of the right hindlimb. Six New Zealand white rabbits, followed by implantation of the scaffold (8 mm x 2.5 mm) at the site of a single defect (treated). The control subject's defect underwent spontaneous repair. Bone formation was assessed by in vivo x-ray tomography and histological analysis at 12 (Group 1, n = 3), and 24 (Group 2, n = 3) weeks after implantation.

Results
At 4 and 12 weeks after surgery, we observed new infiltration of bone tissue at the implant site, with a small amount of fibrous tissue forming; in the untreated contralateral defect, however, we detected the presence of tissue fibrosis with small areas of bone growth at the margins of the defect.

Discussion and Conclusion
The preliminary results highlighted that 24 weeks after the implant, after the sacrifice, analyzing the rabbit tibial sections, the histological analysis highlighted that in the tibial defect treated with the scaffold implant, a new infiltration was observed of bone tissue, with greater quantities of mineralized tissue.

References

1. Sen M, Miclau T. Autologous iliac crest bone graft: should it still be the gold standard for treating nonunions? Injury. 2007;38(Suppl 1):S75–80. doi: 10.1016/j.injury.2007.02.012.
2. Kong L, Ao Q, Wang A, Gong K, Wang X, Lu G, Gong Y, Zhao N, Zhang X. Preparation and characterization of a multilayer biomimetic scaffold for bone tissue engineering. J Biomater Appl. 2007 Nov;22(3):223-39. doi: 10.1177/0885328206073706. Epub 2007 Jan 25. PMID: 17255157.

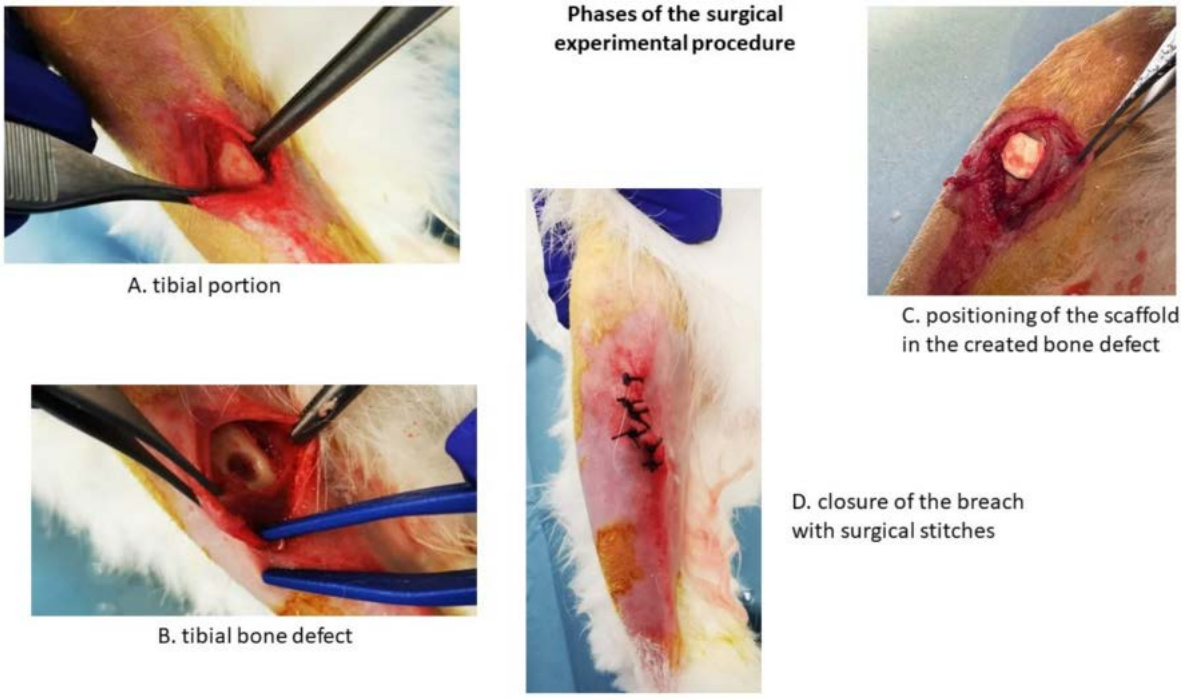


Figure 1. Phases of the surgical experimental procedure.

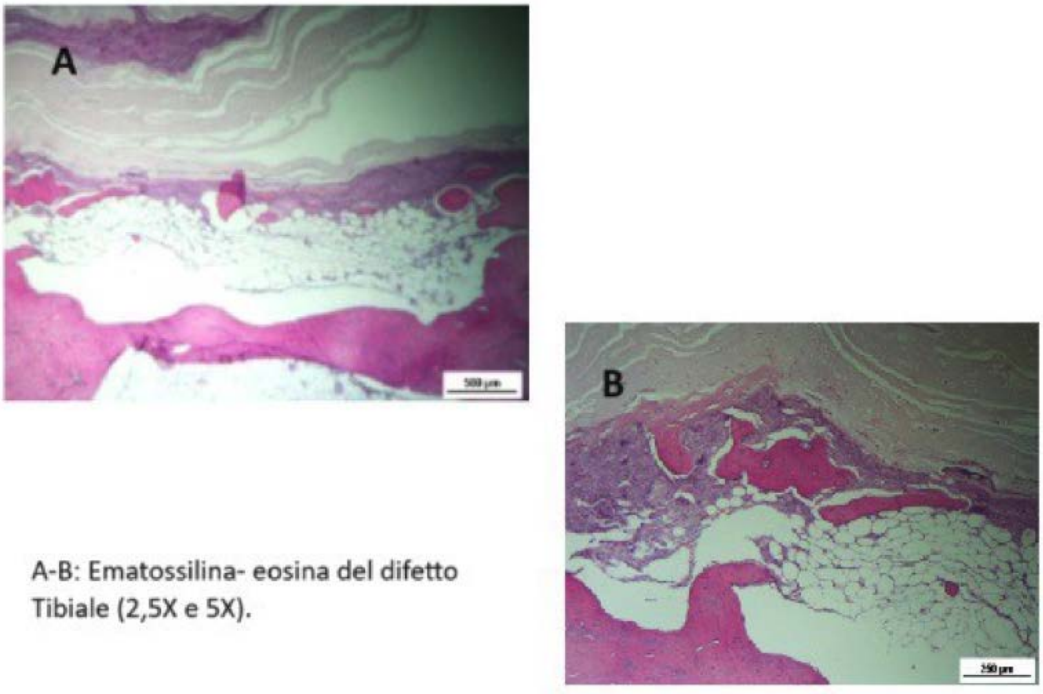


Figure 2. Preliminary histology results.

P196

Laboratory animals

ATP ASSAY FOR TOXICITY EVALUATION OF AUTOGENOUS VACCINES: A REPLACEMENT APPROACH TO RODENT MODEL

De Mattia[1], M.A. Checco[1], R. Villa[1], A. Cacciamali[2], S. Dotti[1]

[1]*Centro di Referenza Nazionale per i Metodi Alternativi, Benessere e Cura degli Animali da Laboratorio, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna-IZSLER, Italy*
[2]*Laboratorio Produzione Vaccini e Reagenti, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna-IZSLER, Italy*

Introduction
Autogenous vaccines represent an important tool in veterinary medicine to improve animal health conditions and prevent antibiotic resistance[1]. These vaccines must be subjected to a series of safety checks, including the evaluation of toxicity performed by the ATT (Abnormal Toxicity Test)[2], an *in vivo* assay which is considered no longer in line with the principles of animal welfare according to the EU Pharmacopoeia[3]. The aim of this study is to propose an *in vitro* test as a replacement for ATT: ATP based Cell Viability Luminescence Assay allows the measurement of cytotoxicity with considerable robustness and accuracy[4].

Materials and Methods
The method is based on the use of a cell culture as a toxicity detection system. The cell line used is the L-929, provided by IZSLER Biobank of Veterinary Resource (www.ibvr.org). The testing principle is the production of adenosine triphosphate (ATP) by viable cells: using the CellTiter-Glo® 2.0 kit (Promega, Italy), ATP present in the sample can be quantified producing a luminescence signal measureble with a luminometer.
Cells were seeded in 96-well plates, vaccines were tested starting from the original material and up to 1:64 dilution and incubated for 24 h before proceeding with the luminescence analysis.

Results
The results showed that the tested vaccines were cytotoxic at the highest concentrations with good linearity: cell viability increases with increasing vaccine dilution.

Discussion and Conclusion
In order to apply the 3Rs principle in pharmaceutical fields, these studies produced a number of data to demonstrate the comparability with the ATT assay and lay the foundation for the possibility of replacing the *in vivo* method with the *in vitro* one.

References
1. Hera, A., Bures, J. Veterinary autogenous vaccines. Dev Biol (Basel) 117, (2004).
2. Grein, K., et al V. Autogenous vaccines: Quality of production and movement in a common market. Biologicals 76, (2022).
3. Lang, C. et al. Replacement, Reduction, Refinement - Animal welfare progress in European Pharmacopoeia monographs: activities of the European Pharmacopoeia Commission from 2007 to 2017. Pharmeur Bio Sci Notes 2018, (2018).
4. Cree, I. A., Andreotti, P. E. Measurement of cytotoxicity by ATP-based luminescence assay in primary cell cultures and cell lines. Toxicology in Vitro 11, (1997).

P197

Laboratory animals

PROTEIC AND GENOMIC CHARACTERIZATION OF NON-PATHOGENIC BUT INVASIVE *BORDETELLA BRONCHISEPTICA* STRAINS IN LABORATORY SPF RABBITS

R. Eliacin[1], C. Pelletier[1], M. Tisserant[1], C. Mathieu[1]

[1]*LDA01, Bourg-en-Bresse, France*

Introduction
During health checks on SPF rabbits, *Bordetella bronchiseptica* is regularly isolated in asymptomatic carriage in two farms (A and B), with different distributions: restricted to the upper respiratory tract for farm A or systemic (sinuses, lungs, spleen, liver) for farm B. As the serum of these rabbits is used in human medicine, it is crucial to characterize the zoonotic health risk by understanding the mechanisms involved in the asymptomatic carriage of these strains.This study had two objectives: -Identify proteic and/or genomic biomarkers to monitor the evolution of strains and control the introduction of new strains -Identify the genetic mechanisms involved in non-pathogenicity of these strains (mutations-deletions in virulence genes or their promoters, in BvgAS regulon involved in expression of Bordetella virulence genes, in pathogenicity islands) and determine their degree of reversibility.

Materials and Methods

350 strains from farms A or B and 2 strains isolated from rabbits in a pathogenic context were analyzed morphologically. 90 representative strains were selected, and their protein expression profiles obtained by MALDI-TOF MS analysis (Biotyper, Bruker) were analyzed using FlexAnalysis software (Figure 1). The complete genome of one strain of each protein profiles was sequenced using MinION™ (Oxford nanopore) and was compared to the reference genome of B. bronchiseptica RB50 using EPI2me.

Results

Only farm B and pathogenic strains are hemolytic. We obtain 1 protein profile for farm A strains, 3 protein profiles for farm B strains and 1 protein profile for pathogenic strains. According to predictions from the UNIPROTKB database of specific protein biomarkers, we determined that only strains from farm B and pathogenic strains express proteins involved in adhesion to host cells and in the colonization of deep organs (fimbriae, pertactin). If we noted an almost perfect homology between genome of the pathogenic and RB50 strains, the genome of farms A and B strains present numerous GAPs (16 and 8 respectively), particularly regarding the regions coding for protein of replication of pathogenicity island (Figure 2).

Discussion and Conclusion

These various data constitute concrete scientific elements supporting the exemption from EU Directive 2019/1833.

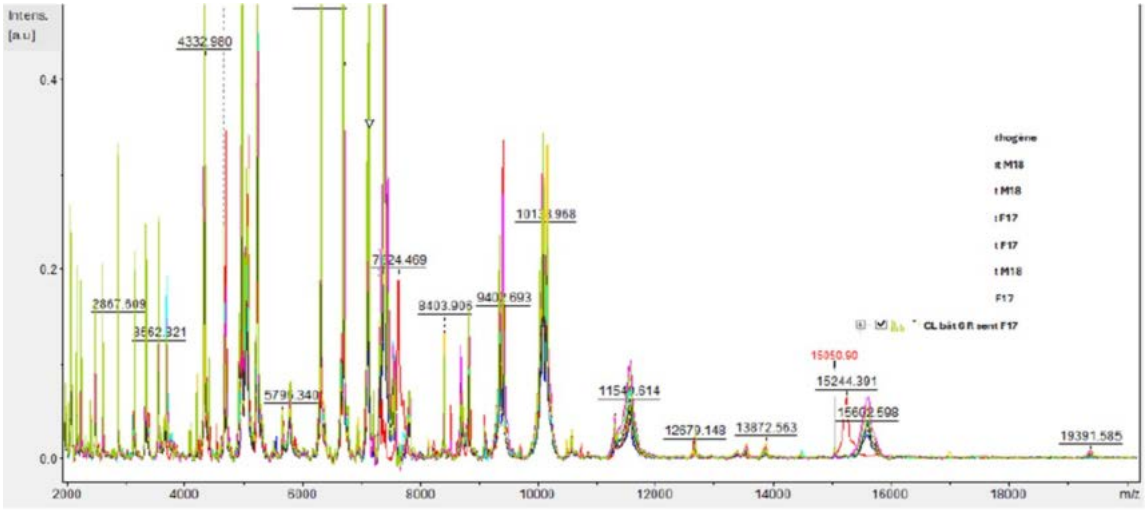


Figure 1: FlexAnalysis spectra obtained for 8 strains of *Bordetella bronchiseptica* in farms A, B or in pathogenic context

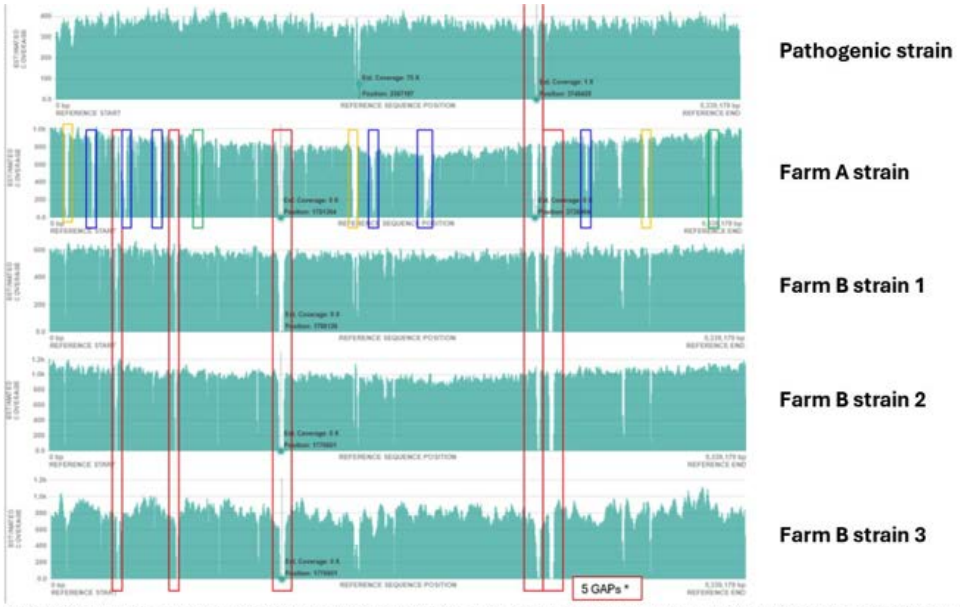


Figure 2: Alignment of the cleaned reads obtained for the 5 sequencing runs on the RB50 reference genome (EPI2me). Red boxes: GAPs common to the 4 non-pathogenic strains, Blue boxes: GAPS only present at farm A, Yellow boxes: regions not covered or weakly common between farm A and B, Green boxes: weakly covered regions present only at farm A.

P198

Miscellaneous

THE DISTRIBUTION OF *CHLAMYDIA ABORTUS* IN LYMPH NODES FROM INFECTED EWE

S.G. Caspe[1], N.D. Sargison[3], E. Milne[3], S.R. Wattegedera[2], D. Longbottom[2]

[1]*Chlamydia Group/Moredun Research Institute, Edinburgh, UK*
[2]*Moredun Research Institute, Edinburgh, UK*
[3]*University of Edinburgh, Edinburgh, UK*

Introduction

Ovine Enzootic Abortion is a disease caused by *Chlamydia abortus* (*C. abortus*) [1]. Infection is oronasal, establishing first in the tonsil and disseminating to the rest of the body by blood or lymph system. Chlamydiae may enter a latent phase, remaining undetectable but reactivating during pregnancy. After abortion, ewes' immunity can protect them from future abortions. Lymph nodes may play an important role in this protective response [2].

Materials and Methods

In this study, pregnant sheep were experimentally infected with *C. abortus* at day 70 of gestation. Samples of lymph nodes from three different regions (lumbo-aortic or LNLA, mediastinal or LNM and prescapular or LNP) and highly vascularised organs (mammary gland, liver, and lung) from infected (n=8) and non-infected control (n=2) ewes were evaluated. The lymph nodes were analysed by immunohistochemistry (IHC) using a monoclonal antibody (mAb) targeting chlamydial-LPS (mAb clone 13/4, Santa Cruz Biotechnology, Heidelberg, Germany) [3] and the remaining tissues by *C. abortus*-specific qPCR [4].

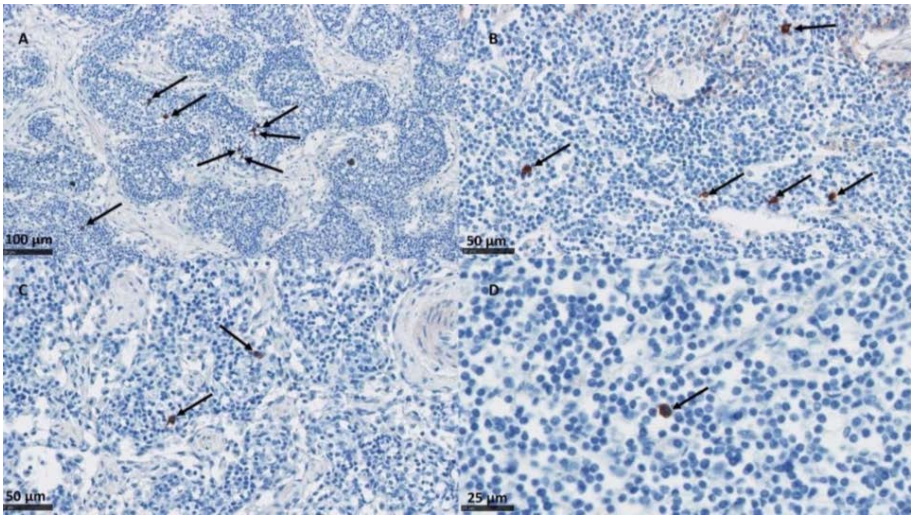


Figure 1. Detection of *C. abortus* in lymph nodes of infected ewes by IHC. The figure shows immunolabelling for *C. abortus* in placenta using mAb targeting *C. abortus* - MOMP. *C. abortus* -MOMP is labelled in red over a blue counterstain. Note the cells showing intracellular inclusion (arrow) in lumbo-artic (A and B), mediastinal (C) and prescapular (D) lymph node IHC using AEC red substrate and counterstained with haematoxylin.

Results

All LNLA (8/8) were positive for *C. abortus* in all infected ewes, but only some LNM (6/8) and LP (5/8) were positive. qPCR analysis was negative for *C. abortus* for all the samples analysed.

Discussion and Conclusion

In this study, the infiltration of chlamydiae in the tissues of the ewes appears to be related to the anatomical positions. The LNLA, which drain the pelvic area, were more frequently positive than the LNM or LNP. This may result from the phagocytic cells migrating from the mucosa to the LN during the recrudescence of infection. Chlamydial antigen in these lymph nodes is likely a major contributor to memory/protective responses to future *C. abortus* infections. However, further studies with larger cohorts are required to confirm the findings.

References

1. Longbottom, D. and L.J., 2003. Coulter. J Comp Pathol, 128(4): p. 217-44.
2. Longbottom, D., et al., 2013. PLoS One, 8(2): p. e57950.
3. Buxton, D., et al., 2002. J Comp Pathol, 127(2-3): p. 133-41.
4. Livingstone, M., et al., , 2009. Vet Microbiol, 135(1): p. 134-141.

P199

Miscellaneous

PREVALENCE OF PARASITIC INFESTATIONS BY CESTODES, NEMATODES AND COCCIDIA IN SHEEP FARMS IN SICILY – ITALY

G. Barbaccia[1], D. Gambino[1], V. Gargano[1], L. Cicero[1], R. Cirincione[1], D. Vicari[1], G. Cassata[1], A. Vella[1]

[1]*Istituto Zooprofilattico Sperimentale della Sicilia A. Mirri, Italy*

Introduction
Sheep farming in the Mediterranean relies on local breeds in extensive systems with feed from various pastures. Grazing improves milk nutrients but exposes sheep to parasites like coccidia, cestodes, and nematodes, causing diseases and reducing productivity. Understanding parasite dynamics in grazing systems is crucial for effective control. This study evaluates parasitic infestations in sheep farms in Palermo, Catania, Messina, and Trapani during 2022-2023, as part of Measure 14 of Sicily's PSR.

Materials and Methods
Fecal samples from sheep were collected and analyzed at the Istituto Zooprofilattico Sperimentale della Sicilia as part of Measure 14 of the Sicilian PSR. Each sample was homogenized, centrifuged, washed, mixed with a sodium nitrate and glucose solution, and parasite eggs were identified under a microscope at 10X and 40X magnification by comparing them with parasitology images.

Results
The analysis revealed a prevalence rate of 54.4% for coccidia, 14.2% for cestodes, and 50.5% for nematodes. These results indicate a relatively high prevalence of coccidia and nematodes in the sheep population, with over half of the samples testing positive for these parasites, while the prevalence of cestodes was significantly lower, affecting about one in seven samples.

Discussion and Conclusion
The study shows a high prevalence of parasitic infections in sheep, notably coccidia (54.4%) and nematodes (50.5%), reflecting Mediterranean climate influences. Effective management requires accurate diagnosis and targeted treatments to prevent anthelmintic resistance. Initiatives like Measure 14 of PSR in Sicily improve sheep health and farming sustainability. Monitoring infestations is crucial for effective control and welfare. Continuous surveillance and strategic interventions are vital to mitigate economic losses and ensure animal welfare in the Mediterranean.

References
1. Sodi, I et al.; doi:10.3390/pathogens12121459.
2. Assessorato Regionale dell'Agricoltura, dello Sviluppo Rurale e della Pesca Mediterranea Dipartimento Regionale dell'Agricoltura; Regione Siciliana Programma Di Sviluppo Rurale Sicilia (PSR) 2014-2022 - Bando Di Attuazione Anno 2022 Misura 14 - Benessere Degli Animali; 2022;
3. Taylor, M.A.; et al.; ISBN 978-1-119-07369-7.
4. Knoll, S.; et al.; doi:10.1186/s13071-021-05013-9.

P200

Miscellaneous

MINIMUM INHIBITORY CONCENTRATION OF ANTIMICROBIALS MAY NOT PREVENT *MYCOPLASMA GALLISEPTICUM* AND *MYCOPLASMA SYNOVIAE* FROM SYNTHESIZING BIOFILM *IN VITRO*

B. Colò[1], M. Bottinelli[1], G. Nai[1], D. Prataviera[1], E. Rinaldi[1], A. Tondo[1], S. Catania[1]

[1]*Mycoplasma Unit, WOAH reference laboratory for avian mycoplasmosis (M. gallisepticum, M. synoviae), SCT-[1] Verona, Istituto Zooprofilattico Sperimentale delle Venezie, Buttapietra, Italy*

Introduction
The biofilm lifestyle is recognized as the predominant mode of bacterial growth in nature, and mycoplasmas are capable of synthesizing biofilms (1). Within biofilms, bacteria are protected from adverse environmental conditions, including immune responses and antimicrobials (2). This study aimed to observe whether mycoplasma biofilm synthesis could be affected by exposure to various concentrations of antimicrobials.

Materials and Methods
Three strains of *Mycoplasma gallisepticum* and three strains of *Mycoplasma synoviae* were used to perform minimum inhibitory concen-

tration (MIC) tests. The strains were propagated using the Avian Mycoplasma Experience broth without inhibitors and placed in MIC plate wells containing different concentrations of 10 antimicrobials. Plates were incubated aerobically at 37°C, with growth checked after 24, 48, and 72 hours and MIC reading was performed once planktonic growth was observed in the positive control well. After MIC reading, the plates were kept at room temperature for 30 days, then emptied, washed, and stained with 5% (v/v) crystal violet for 30 minutes. The wells were subsequently emptied, washed twice with distilled water, and biofilm formation was visually checked after 24 hours.

Results
All strains produced biofilm at drug concentrations that did not inhibit planktonic growth. One strain of each species synthesized biofilm in wells where the drug concentration inhibited visible planktonic growth (MIC wells). No biofilm was observed in wells where drug concentrations exceeded the MIC for each drug.

Discussion and Conclusion
In conclusion, our data showed that common drugs used for mycoplasma treatment do not inhibit biofilm production. On the contrary, we observed that drug concentrations inhibiting planktonic growth did not hinder mycoplasma biofilm synthesis in some cases. Therefore, there is a potential risk of favoring chronicization of mycoplasma infections through biofilm production if proper drug concentrations are not achieved at the site of infection.

References
1. Catania S, et al. (2024). Pathogenic avian mycoplasmas show phenotypic differences in their biofilm forming ability compared to non-pathogenic species in vitro. Biofilm. 6;7:100190.
2. Yin, W, et al. (2019). Biofilms: The Microbial “Protective Clothing” in Extreme Environments. Int. J. Mol. Sci. 20, 3423.

P201

Miscellaneous

QUALITY CONTROL STUDIES ON LARGE BATCHES OF FETAL BOVINE SERUM FOR USE IN DIAGNOSTICS

E. De Haan[1], M. Tacken[1], D. Kalk-Korver[1], M. Span[1], S. Avci-Seker[1], D. Van Zoelen-Bos[1], J. Van Der Goot[1]

[1]*Wageningen Bioveterinary Research, the Netherlands*

Introduction
Fetal Bovine Serum (FBS) is widely used as a supplement to basal growth medium for *in vitro* cell culture applications. It provides a rich culture system known to support a wide range of (animal) cell types. Commercial FBS is derived from the blood of cow fetuses, which is pooled into larger batches. Therefore, the origin of each (large) batch FBS is undefined and its composition can vary between lots. As a result, not every batch of FBS is suitable for use in diagnostics.

Materials and Methods
At the diagnostics department of Wageningen Bioveterinary Research, possible new batches of FBS are subjected to extensive quality controls before being purchased on a large scale. In 2023, we compared the quality and performance of five FBS batches from different suppliers. First, the absence of *Mycoplasma* and sterility of the FBS batches were confirmed. FBS batches were then tested on the absence of Bovine Viral Diarrhea Virus (BVDV) by viral isolation and PCR, after which the absence of antibodies against common pathogens in cows were checked by ELISA, CBR and VNT.

Results
All five FBS batches tested positive against BVDV by PCR, however no live virus was found by viral isolation. In addition, antibodies against either Bovine Herpes Virus type 1 or BVDV were found in two out of five FBS batches resulting in discarding these batches for further quality tests. The three remaining batches were then used in cell culture to compare growth characteristics in our different cell lines used in diagnostics. Major varieties in cell growth were observed in especially IBRS2 and SK6 cells between the FBS batches, highlighting the lack of consistency between lots and therefore the importance of quality controls on new FBS batches.

Discussion and Conclusion
At the end of the quality control, two batches of FBS proved to be suitable for use in our diagnostic tests. We chose the largest batch of FBS since these extensive quality controls are expensive and laboursome.
As our results show, it is difficult to obtain FBS completely free of pathological agents and antibodies to these agents, especially BVDV. New animal-free products are emerging to replace animal-derived FBS. However, based on the current state of the literature, there seems to be no clear replacement for FBS for all aspects of its current use (yet).

P202

Miscellaneous

CHICKEN IN LAMB'S CLOTHING: SPECIES SUBSTITUTION IN SINGLE PROTEIN DOG FOOD

V. Filipello[2], C. Wehbe[1], G. Magagna[2], M. Tilola[2], E. Butovskaya[2], E. Faggionato[2]

[1]*Department of Comparative Biomedicine and Nutrition, University of Padova, Legnaro (PD), Italy*
[2]*Department of Food Safety, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Brescia, Italy*

Introduction

Single protein canine diets are commonly used in the diagnosis and management of adverse food reaction (AFR). However, the possibility of undeclared ingredients jeopardizes the efficacy of such approach. Moreover, such diets are often more expensive than regular extruded diets. The objective of this study was to verify the consistency of the animal origin ingredients declared in the label through barcode sequencing.

Materials and Methods

Thirty-three single protein source adult canine diets were purchased either at specialized shop or at supermarket. The foods belonged to 9 different brands, ranging in price from 3.83 to 14.99 €/kg. DNA was extracted using the Small Fragment protocol of the DNeasy mericon Food Kit (Qiagen). The animal species was determined targeting either COI or cytb (cytochrome oxidase subint I and cytochrome b) using mini- PCR protocols. Sequencing was carried out on a SeqStudio Genetic Analyzer (Thermo Fisher Scientific) and species identification was determined using BoLD or GenBank databases.

ID	Brand	Priice (€/kg)	Dedared proteimsomce	Indentifiied speciies	Reselle:r
1	a	5156,	turrkey	*Me!agris gallopavo*	Supermarkeit
2	b	3,83	pig	*Susscrofa*	Supermarkeit
3	b	4,1	beef	*Bo:sta urus*	Supermarkeit
4	b	4,1	.salmon	*Salmo salar*	Supermarkeit
5	b	8,8	raibbit	*Meleagris gollopavo*	Pet store
6	b	8,8	pig	*Susscrofa*	Pet store
7	b	8,8	dudk	*Anse.raruer x cygrmides*	Pet store
8	b	8,8	trout	*Salmo salar*	Pet store
9	b	9136,	.salmon	*Salmo salar*	Pet store
10	b	9136,	lamb	*Galius ga!lus*	Pet store
11	e	9166,	hiorse	Fish	Pet store
12	e	9166,	deer	*Rarigifer tarom:lus*	Pet store
13	d	12,71	.sea bass	*Salmo salar*	Pet store
14	d	14,99	deer	*Susscrofa*	Pet store
15	e	8186,	hiorse	*Equ us cabaUus*	Pet store
16	e	8186,	fish	*Salmo tr.utta*	Pet store
17	e	9125	pig	*Susscrofa*	Pet store
18	e	9,25	turna fish	*Susscrofa*	Pet store
19	f	14,49	pig	*Susscrofa*	Pet store
.20	g	7175	.salmon	*Galius ga!lus*	Pet store
.21	g	8149	rabbit	*O.ryctola_g.us cu:nicul.us*	Pet store
.22	g	7,75	lamb	*Ovis aries*	Pet store
.23	g	7164	dudk	*Anas platyrhynchos*	Pet store
24		1175	Pi.El!:	*Susscrofa*	Pet store
.25	h	10	beef	*Bo:sta urus*	Pet store
.26	h	10	pig	*Susscrofa*	Pet store
27	h	10	.sole	*Ga lh.iSqallus*	Pet store
28	h	10	.salmon	*Susscrofa*	Pet store
.29	h	10	pig	*Susscrofa*	Pet store
30	h	10	quail	*Cotumix spp.*	Pet store
31	i	5104	pig	*Susscrofa*	Pet store
32	i	5104	lamb	*Ovis aries*	Pet store
33	i	5104	.salmon	*Salmo salar*	Pet store

Table 1. Results of the species identification of the analysed samples.

Results
All 33 samples were successfully sequenced, in 33% of the them (n=11) the species identified did not correspond to that declared as single protein source on the package. The more frequent substitution were chicken and pig, often in place of fish proteins. Salmon was used twice in place of fishes with higher economical value. In one case turkey was identified instead of rabbit and in another fish instead of horse. In one sample reindeer was identified instead of deer, though in this case a problem of translation to Italian language may also be the cause.

Discussion and Conclusion
The species substitution was identified only in products placed in the more expensive price range (premium diet) and pertained 7 out of 9 brands. These situations may arise due to economic reasons, usually involving meats cheaper than those originally foreseen. Sanger sequencing does not allow identification of multiple species within the same sample, further investigation supported by metabarcoding analysis may prove useful to have a more complete overview of the problem.

References
1. L. A. Fossati, et al, 2018, doi: 10.1002/vms3.125.
2. R. Ricci et al., 2018, doi: 10.1186/s12917-018-1528-7

P203

Miscellaneous

COMPARISON OF PROTOCOLS USEFUL TO ISOLATE AND COUNT OF MICRO PLASTICS IN *CARETTA CARETTA* STOOL SAMPLES

C. Lomonaco[1], E. Morici[2], G. Schirò[1], R. Disclafani[1], L. Tranchina[2], F.P. Bonomo[2], V. Monteverde[1], M. Licciardi[2], S. Dara[1], P. Galluzzo[1]

[1]*Centro di Referenza Nazionale sul Benessere, Monitoraggio e Diagnostica delle Malattie delle Tartarughe Marine (C.Re.Ta.M), Sicily (Italy) - Istituto Zooprofilattico Sperimentale della Sicilia "A.Mirri, Palermo, Italy*
[2]*Advanced Technologies Network Center (ATeN Center), University of Palermo, Palermo, Italy*

Introduction
Microplastics (MPs) have been found in the whole globe and can persist in the environment for long periods (1). Microplastics have heterogeneous physical and chemical properties and differ in size, shape, color, specific density and composition (2). Marine organisms are impacted by plastic debris by entanglement and ingestion. The sea turtle *Caretta caretta* is one of the most important bioindicator organism for monitoring the marine plastic pollution and is classified as endangered species. Therefore, it is important to monitor the impacts of marine plastic pollution for the conservation of this species (3). Few studies on MPs extraction methods from sea turtles' stools were reported. This work aims to compare different methods used for the MPs extraction from sea turtle stools.

Materials and Methods
Twelve stool samples recovered by two subjects of *Caretta caretta* hospitalized at the C.Re.Ta.M, Sicily (Italy) were collected. For each sample, 1gr of stools freeze-dried was used to apply five different methods (A-E) (Table 1).

Methods	Steps	Reagents	Reference
A	2	1. H_2O_2 30% 2. Flotation	(1)
B	2	1. H_2O_2 30% with Fenton's reactive 2. HNO_3 69,5%.	(5)
C	3	1. H_2O_2 30% 2. C_2H_6O 99,9% 3. HNO_3 69,5%.	(6)
D	3	1. H_2O_2 30% 2. NaOH 10M 3. HNO_3 69,5%.	(7)
E	5	1. H_2O_2 30% 2. NaOH 10M 3. HNO_3 69,5% 4. C_2H_6O 99,9%.	-

Table 1. Outline of the methods used. The number of steps and types of reagents required to carry out the method are shown. Each step is followed by a filtration step. Method E has not yet been published and is the subject of this study.

Results
Method A detected only 10% of the microplastics. The Fenton's reagent used in method B can lead to the adhesion of the surface of MPs

by hydroxyl radicals causing the degradation of smaller MPs (4). Using C and D methods, filters rich in fatty matrix were obtained and microplastic counts were hard. The method E allowed a drastic reduction in the fat matrix of the sample.

Discussion and Conclusion

While it is possible to detect threads using C and D methods since the thickness of filaments are usually greater than the those of the fat layer on filters, some MPs fragments and films are detectable only using the E method (Figure 2). Therefore, although method E requires several steps, the filters are cleaner, allowing easier, faster and higher resolution reading.

References

1. Aranda D. A. et al., 2024, https://doi.org/10.1016/j.marpolbul.2024.116092
2. Hidalgo-Ruz V. et al., 2012, doi.org/10.1021/es2031505
3. Dong-Min H. et al., 2022, https://dx.doi.org/10.17582/journal.pjz/20210820080823
4. Ortiz D. et al, 2022, https://doi.org/10.1016/j.chemosphere.2022.136809
5. Zehua Y. et al., 2020, https://doi.org/10.1016/j.jhazmat.2019.121489
6. Djouina M. et al.,2022, https://doi.org/10.1016/j.envres.2022.113230
7. Perez-Guevara F. et al., 2021, https://doi.org/10.1016/j.scitotenv.2021.146395

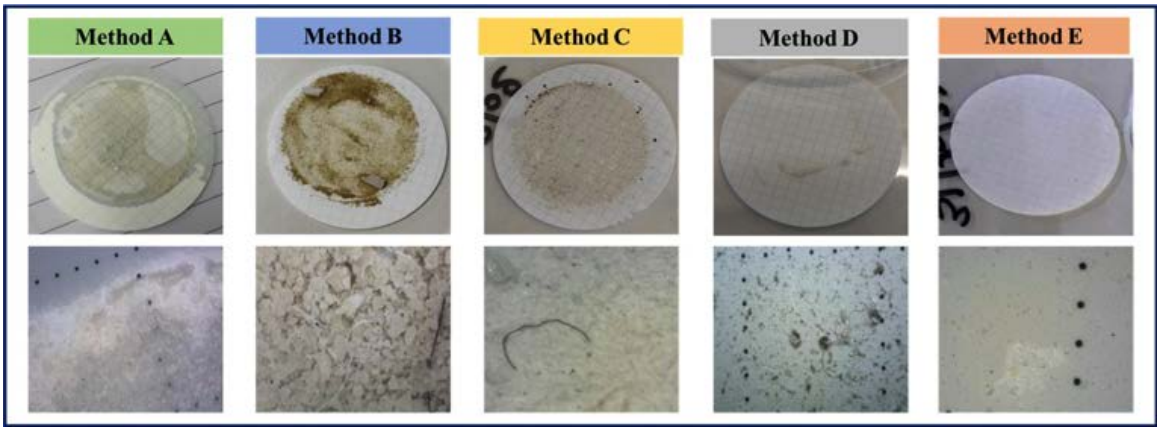


Figure 1. Macroscopic image and enlargement (92X) of filters obtained by the different 5 methods used for the isolation and count of microplastics (A-E).

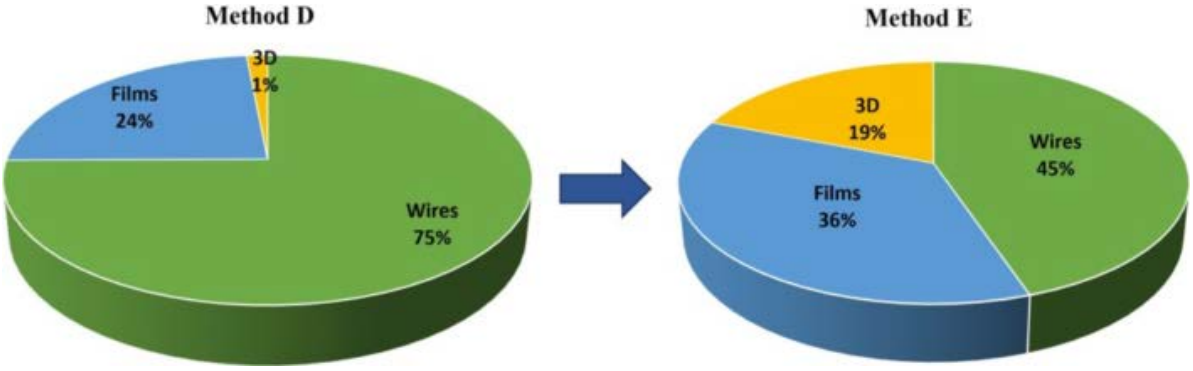


Figure 2. Pie chart comparing methods D and E. The graph of the E method underlines a more homogeneous characterization of MPs shape (3D = MPs fragments).

P204

Miscellaneous

DETECTION OF *NEOSPORA CANINUM* ANTIBODIES IN SOWS AND THEIR OFFSPRING

L.V. Athanasiou[1], E.G. Katsogiannou[1], C.N. Tsokana[2], D. Gougoulis[1], S.M. Papadakis[1], V.G. Papatsiros[1]

[1]*Clinic of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece*
[2]*Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece*

Introduction
Neospora caninum, a protozoan parasite, significantly affects livestock, particularly cattle and swine, causing reproductive issues like abortion and neonatal mortality1. This study assessed the prevalence of IgG and IgM antibodies against *Neospora caninum* in sows and the umbilical cords (UCB) of stillborn piglets using a previously employed indirect immunofluorescent assay (IFA)2.

Materials and Methods
The study included 39 pig farms on the Greek mainland, encompassing around 10,800 sows. Blood samples were obtained from 247 sows via jugular venipuncture, and UCB samples were collected from 247 stillborn piglets within 12 hours post-farrowing or directly from the umbilical cord immediately after birth, before colostrum intake. Convenience sampling targeted litters from sows farrowing during the visits. Statistical analyses were conducted using MedCalc Statistical Software version 14.8.1 and the online "Fisher's Test for Exact Count Data".

Results
Out of 247 serum and UCB samples tested, 22 serum samples (8.90%) and 8 UCB samples (3.24%) tested positive for anti-IgG. For anti-IgM, 9 serum samples (3.64%) and 2 UCB samples (0.81%) tested positive. Additionally, 2 UCB samples (0.81%) and 1 UCB sample (0.40%) tested positive for both antibodies. Higher seroprevalence was observed in smaller farms compared to larger ones.

Discussion and Conclusion
The presence of antibodies in UCB suggests transplacental transfer of the parasite from infected sows to fetuses. The increased prevalence of antibodies in sows and umbilical cords underscores the significance of this pathogen in swine herds. Regular monitoring and serological surveys are essential to manage and control *Neospora caninum* in livestock populations.

References
1. Snak A. et al 2019. Does *Neospora caninum* cause reproductive problems in pigs? Vet Parasitol 275,108934
2. Athanasiou L.V. et al .2021 *Neospora caninum* and/or Toxoplasma gondii Seroprevalence: Vaccination against PCV2 and Muscle Enzyme Activity in Seropositive and Seronegative Pigs. Microorganisms. 20;9(5):1097.

P205

Miscellaneous

PRELIMINARY RESULTS ON THE ISOLATION OF SPECIFIC *BACILLUS ANTHRACIS* BACTERIOPHAGES FROM NATURALLY CONTAMINATED SITES AND THEIR POTENTIAL USE AS ENVIRONMENTAL DECONTAMINANTS

V. Manzulli[1], D. Farina[1], A. Bianco[1], S. Castellana[1], A. Fasanella[1], V. Rondinone[1], L. Serrecchia[1], L. Pace[1], E. Poppa[1], M. Iatarola[1], D. Galante[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*

Introduction
Anthrax is a historically known and feared disease caused by *Bacillus* anthracis and its spores can survive in soil for centuries. Existing chemical decontaminants, though effective, are environmentally harmful. This study aimed to isolate specific *B. anthracis* bacteriophages from environmental samples collected in geographical areas where anthrax outbreaks have been recorded in the past.

Materials and Methods
Soil samples were collected from burial sites in Pollino National Park, Italy (2012), Gargano Promontory, Italy (2019), different area of Bangladesh (2011, 2015) and Albania (2012, 2013) over the last decade. The detection of *B. anthracis* from soil samples was carried out by the Ground Anthrax *Bacillus* Refined Isolation (G.A.B.R.I.) method (1) and confirmed by Real Time PCR. The isolation of phages from positive environmental samples was performed according to the method of Walter M.H. et al. (2) (Figure1). DNA from phage lysates was extracted and sequenced using the Illumina MiSeq platform. The sequences obtained were subjected to Quality Control using fastp v0.23.2 and assembly with SPAdes 3.15. Identification and annotation of phage genomes was performed with https://phastest.ca (July – August 2023).

Results

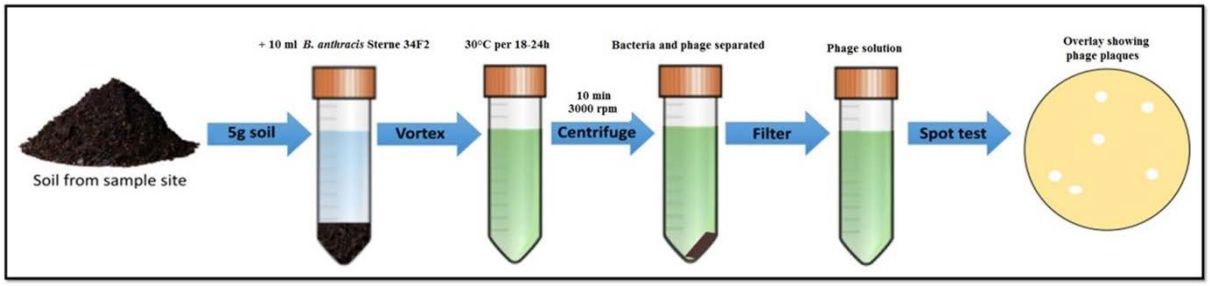
Out of 182 soil samples, 16 tested positives for the presence of *B. anthracis* spores: Albania (2/32), Bangladesh (11/71), Gargano (2/12) and Pollino National Park (1/67). Phage isolation was successful in 8 samples. Only for two phages, FAGO-8 (Gargano) and FAGO-C (Bangladesh), we could obtain complete genomes, identified respectively as phage vB_BtS_BMBtp3 (Gargano) and TsarBomba (Bangladesh), both matching to phages specific for *Bacillus* cereus group bacteria (Figure 2).

Discussion and Conclusion

The results obtained, although preliminary, should be considered as the beginning of the development phage cocktails as eco-friendly environmental decontamination in order to reduce the number of spores in contaminated sites, thus carrying out an effective and targeted preventive action.

References

1. Fasanella A, et al. Ground Anthrax *Bacillus* Refined Isolation (GABRI) method for analyzing environmental samples with low levels of *Bacillus* anthracis contamination. BMC Microbiol. 2013; 13:167.
2. Walter MH, Baker DD. Three *Bacillus* anthracis bacteriophages from topsoil. Current Microbiology. 2003;47(1):55–58.



1. Fasanella A, et al. Ground Anthrax Bacillus Refined Isolation (GABRI) method for analyzing environmental samples with low levels of Bacillus anthracis contamination. BMC Microbiol. 2013; 13:167.
2. Walter MH, Baker DD. Three Bacillus anthracis bacteriophages from topsoil. Current Microbiology. 2003;47(1):55–58.

Figure 1. Technical procedure for phage isolation from soils sampled in environmental sites in which carcasses of animals that died from anthrax had been buried.

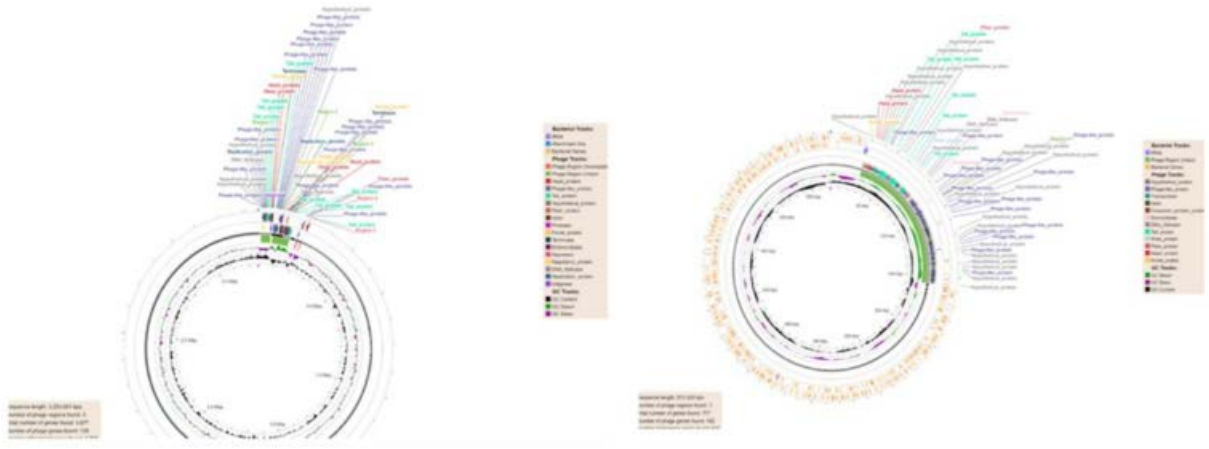


Figure 2. Circular map of the assemblies resulting from the genetic sequences of the FAGO-8 (right) and FAGO-C (left).

P206

Miscellaneous

MICROPLASTICS BIODEGRADATION POTENTIAL OF BACTERIA ISOLATED FROM AQUATIC ENVIRONMENT OF THE ADRIATIC SEA

S. Pieralisi[2], S. Di Lullo[2], D. Maiolatesi[2], G. Angelico[2], G. Talevi[2], S. Nardi[2], F. Leoni[3], F. Barchiesi[1], E. Rocchegiani[2], D. Ottaviani[2]

[1]*Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", Centro di Referenza Na-zionale per il controllo microbiologico e chimico dei molluschi bivalvi vivi, Ancona, Italy*
[2]*Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", Laboratorio Controllo Alimenti, Ancona, Italy* [3]*Istituto Zooprofilattico Sperimentale Umbria e Marche "Togo Rosati", LNR per il Controllo Batteriologico dei Molluschi Bivalvi, Ancona, Italy*

Introduction
Microplastics (Mps) smaller than 5 mm are increasingly documented in marine organisms and their trophic transfer along food chain is a growing concern for the health of marine environment and animals. MPs provide a substrate for the adhesion of microorganisms that activate their degradation, particularly *Pseudomonas* spp (1). However, their MPs biodegradation potential needs to be further investigated. The aim of our study is to evaluate the biodegradation potential of bacteria isolated from aquatic environments through the development of microbiological screening methods easily reproducible in the laboratory.

Materials and Methods
P. aeruginosa and *P. putida* ATCC, which previously demonstrated biodegrading power towards LPDE (low-density polyethylene) (1), were used to standardize the screening protocol which was then used to test 3 *P. putida* and 3 *P. aeruginosa* strains isolated from marine organisms. This Clear Zone Method is based on the inoculation of the strain into a medium containing plastic as the only carbon source. A lysis halo around the inoculum is an indicator of the use of plastic by bacteria. Two different medium formulations were tested: one containing PEG (polyethylene glycol) and one containing LPDE particles, both tested with and without yeast extract (0.1%). After incubation, staining with Comassie blue was carried out and the bacteria that produced a blue area on the dark background were considered positive.

Results
The best medium for displaying the biodegradation halo was the one containing PEG and yeast extract. Of the 6 strains tested, 2 strains of *P. putida* and one strain of *P. aeruginosa*, originating from Adriatic sea, gave positive results.

Discussion and Conclusion
A first appreciable result was the standardization of an easily reproducible method to select bacteria with biodegradative potential against MPs. Furthermore, we have identified 3 Pseudomonas strains originating from the Adriatic Sea potentially capable of biodegrading Mps. The next step will be to confirm their biodegradation activity on plastic strips in an aquatic environment on laboratory scale.

This work was funded by Ministry of Health (RF201912370587, RC0042019 and RC0072023)

References
1. Kiav et al. (2012). doi 10.1007/s12088-012-0250-6

P207

Miscellaneous

PRELIMINARY STUDY ON THE HAEMOLYSIS INFLUENCE ON PROTEIN ELECTROPHORESIS IN CALVES SERA

M. Soncin[2], M. Bertola[2], C. Falcaro[2], E. Passabì[2], D. Peruffo[2], V. Bertazzo[2], A. Stefani[2], R. Tessari[1], F. Peraro[1], F. Mutinelli[2]

[1]*Freelance veterinarian, Veneto, Italy*
[2]*Istituto Zooprofilattico Sperimentale delle Venezie, Padua, Italy*

Introduction
Haemolysis, due to in vivo process or *in vitro* management, is the leading cause of sample rejection in clinico-chemical laboratories and it is widely recognized to bias the results of most routine analyses such as capillary electrophoresis, used to evaluate the transfer of colostral passive immunity in calves. This study aims to: i) evaluate the influence of haemolysis on the proteins determination in calves' serum samples, and ii) establish the threshold of haemolysis able to produce significant bias on the studied parameters.

Materials and Methods

Blood samples without anticoagulant were obtained by jugular venepuncture in 18 healthy Holstein-Friesian calves of 1-4 days of life. From these blood samples, different degrees of haemolysis (HD) were created using 25G needle and thermal shock. Based on the amount of serum available, it was possible to obtain 89 aliquots, distributed among seven groups of HD, according to Larrán et al. (2024) (Table 1). Protein fractions (albumin, α, β and γ globulins) were obtained through capillary electrophoresis while serum total proteins (TP) were determined with Biureto's method. The effect of artificially induced haemolysis on the sera, according with their group assignment, was evaluated by two different statistical models and, if significant, by Tukey post hoc comparison test.

	HAEMOLYSIS GROUPS						
	1	2	3	4	5	6	7
N aliquots	16	12	15	27	10	7	2
HD interval	0 - 0.19	0.20 - 0.49	0.50 - 0.99	1.00 - 2.49	2.50 - 4.99	5.00 - 9.99	> 10
HD (g/L)	0.11 ± 0.05	0.36 ± 0.09	0.70 ± 0.13	1.65 ± 0.45	3.71 ± 0.86	7.34 ± 1.30	11.66 ± 0.78
Albumins (g/L)	20.71 ± 1.81	21.41 ± 2.32	21.16 ± 2.44	21.04 ± 1.76	20.57 ± 2.17	19.20 ± 2.49	19.45 ± 1.34
α globulins (g/L)	14.76 ± 1.35	14.34 ± 1.88	15.19 ± 2.16	14.70 ± 1.42	14.76 ± 1.30	13.75 ± 1.06	14.85 ± 0.64
β globulins (g/L)	9.48 ± 1.06	9.38 ± 0.97	10.25 ± 0.98*	11.00 ± 1.17**	12.76 ± 1.32**	15.30 ± 0.74**	20.05 ± 2.05**
γ globulins (g/L)	18.21 ± 6.20	16.60 ± 5.09	17.47 ± 5.99	17.03 ± 5.60	15.38 ± 7.21	15.55 ± 6.48*	18.65 ± 1.48
TP (g/L)	63.19 ± 8.07	61.58 ± 8.94	64.07 ± 7.29	63.74 ± 7.56	63.45 ± 8.8.4	63.83 ± 8.86	73.00 ± 4.24

* p<0.05 e **p<0.001.

Table 1. Nnumber of aliquots analysed, haemoglobin intervals and proteins (mean and standard deviations) values according to assigned haemolysis group.

Results

The study findings indicate that albumins, α globulins and TP levels did not appear to be significantly affected by HD of samples while β globulins appear to be significantly sensitive to haemolysis with each successive haemolysis group (table 1). In addition, high levels of haemolysis (HD >7.34 g/L) can compromise the determination of γ globulins.

Discussion and Conclusion

This is an important aspect because a calf is considered to be adequately drained when more than 10 g/L of γ globulins are present in the serum (Bragg et al., 2020). Increasing the number and type of samples analysed (i.e., add calf sera with failure of passive transfer) and standardizing haemolysis groups numerousness will consolidate the results obtained.

References

Larrán et al. (2024) - DOI: 10.1016/j.rvsc.2024.105203 Bragg et al. (2020) - DOI: 10.1016/j.prevetmed.2020.105059

P208

New Diagnostic Testing Technologies

DEVELOPMENT AND OPTIMIZATION OF A MULTIPLEX BEAD-BASED ASSAY FOR THE DETECTION OF ANTIBODIES AGAINST BOHV1, BVDV1 AND BVDV2 IN SERUM AND MILK SAMPLES.

B. Colitti[1], E. Locatelli[1], A.R. Vlad[1], D. Giaccone[3], C. Nogarol[2], L. Bertolotti[1], S. Rosati[1]

[1]*Department of Veterinary Sciences, University of Turin, Italy*
[2]*In[3]diagnostic s.r.l., Grugliasco, Italy*
[3]*Regional Association of Farmers of Piedmont, Turin, Italy*

Introduction

Infectious Bovine Rhinotracheitis (IBR) and Bovine Viral Diarrhea (BVD) are two viral diseases endemic in bovine population all over the world. They are responsible for considerable economic and health damage in farms; thus, control programs have been implemented in many Countries. The detection of antibodies in both serum and milk samples is a cost-effective tool for the monitoring of diseases [1]. In this context, serology tests have been one of the most widely used diagnostic techniques for the diagnosis of IBR and BVD.
The aim of the study was to develop and validate a bead-based tetraplex serology assay for the simultaneous detection of antibodies directed against BoHV-1 whole-virus, BoHV-1 glycoprotein E, BVDV-1 and BVDV-2 E2 antigens in bovine serum and milk samples using the Bioplex-200 platform (Biorad) and Luminex xMAP technology.

Materials and Methods
The correct antigen-magnetic-beads coupling, reagents and serum and milk concentrations, monoplex or tetraplex interference and protocol parameters were determined using 24 well-characterized samples. Assay cut-off values, sensitivity and specificity of the method were assessed using 356 samples (198 sera and 158 milk) belonging to farms with known IBR and BVDV status.

Results
The best sample dilution was assessed at 1/20 for sera and undiluted for milk. The sensitivity of the method on sera was defined as 99%, 100%, 99% and 99%, while the specificity was 97%, 96%, 82%, and 93% for BoHV1 gE, BoHV-1 whole-virus, BVDV-1, and BVDV-2 respectively. Meanwhile, the sensitivity and specificity of the test applied on milk samples were demonstrated to be slightly lower. The test showed a good agreement with Cohen's kappa statistic of 0,86, 1, 0,61 and 0,94 when 94 paired sera and milk samples were tested.

Discussion and Conclusion
The developed assay was demonstrated suitable for the simultaneous detection of antibodies against BoHV-1, with discrimination among IBR infected and vaccinated animals, BVDV-1, and BVDV-2 antigens in both serum and milk samples, making it a valid alternative to the classic ELISA test for the large-scale monitoring and control of these infectious diseases.

References
1. Conrady et al. Editorial: Global Control and Eradication Programmes for Cattle Diseases. Front Vet Sci. 2021

P209

New Diagnostic Testing Technologies

AN IMPROVED COMPETITIVE ELISA FOR A RELIABLE DETECTION OF CONTAGIOUS BOVINE PLEUROPNEUMONIA (CBPP)

L. Comtet[1], J. Gaborit[1], K. Martin[1], C. Derendinger[1], A. Greatrex[1], A. Limozin[1], P. Pourquier[1]

[1]*Innovative Diagnostics, France*

Introduction
Contagious Bovine PleuroPneumonia (CBPP) is a severe and insidious pneumonic disease for cattle, responsible for major financial losses. The causative agent of CBPP is *Mycoplasma mycoides s*ubsp. mycoides (Mmm), member of the *Mycoplasma* mycoides cluster. CBPP is very similar to other respiratory disease in cattle, especially in subacute infection. In the absence efficient vaccines, a reliable and easy-to-use diagnostic assay for recurrent testing, combined with isolation and treatment of positive animals, represents the best option for CBPP control. This study presents the performances of the ID Screen® CBPP Competition, an ELISA designed to detect antibodies against Mmm. in bovine serum and plasma, developed from CIRAD's biologicals (Montpellier, France).

Materials and Methods
Diagnostic specificity and sensitivity were assessed respectively on 336 bovine negative samples from free areas and 20 positive samples (6 sera from immunized herds and 14 from hyperimmunized goats with an adjuvanted killed Mmm whole-cell extract) provided by a european NRL. The ability to discriminate between negative and positive population was compared to formerly available blocking ELISA (kit A). Detection of seroconversion was evaluated on 2 goats vaccinated with an adjuvanted, killed whole cell extract of Mmm. day 0 and day 60. Exclusivity with respect to other Mycoplasmas was assessed.

Results
Measured diagnostic specificity and sensitivity were respectively 100% [98.9,100] and 100% [83.9,100]. The ID Screen® kit shows a better discrimination between negative and positive samples than kit A does. Seroconversion was detected as of 38-40 days after vaccination. The ID Screen® has a perfect exclusivity with respect to *Mycoplasma mycoides* subsp. capri, *M. capricolum* subsp. capripneumoniae, *Mycoplasma bovis*, *Mycoplasma agalactia*, and *Mycoplasma hyopneumoniae.*

Discussion and Conclusion
The ID Screen® kit demonstrate excellent specificity and high sensitivity. This blocking ELISA has a perfect exclusivity with respect to other Mycoplasmas, is user-friendly as all reagents are ready-to-use. Being the only commercially available ELISA for CBPP, ID Screen® CBPP Competition is a reliable solution contributing for both CBPP monitoring and eradication programs.

P210

New Diagnostic Testing Technologies

A NEW TRIPLEX RT-QPCR TO DETECT AND DIFFERENTIATE BLUETONGUE AND EPIZOOTIC HEMORRHAGIC DISEASE IN A SINGLE WELL

L. Comtet[1], L. Despois[1], E. Bianchini[1], A. Limozin[1], P. Pourquier[1]

[1]*Innovative Diagnostics, France*

Introduction
BlueTongue Virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) are Orbiviruses respectively responsible for bluetongue (BT) and Epizootic Hemorrhagic Disease (EHD), which were reported in 2023 across Europe : BTV-3 (NL, BE, UK), new variant of BTV-8 (FR, IT) and EHDV (ES, FR). There huge economic impacts and similar clinical symptoms makes laboratory testing essential for diagnosis. Innovative Diagnostics offers a new kit, the ID Gene™ BTV&EHDV Triplex. It allows, in a single well, for the detection and differentiation of both BTV&EHDV alongside with a sample endogenous control. Results can be obtained in 50 min with a rapid amplification program, compatible with all IDGeneTM kits, enabling to test on the same run different Orbiviruses RT-qPCRs, therefore offering maximum flexibility & testing capacity by optimizing lab equipments ressources.

Materials and Methods
Blood RNA purifications were performed with the ID GeneTM Mag Fast magnetic beads (21 min). Diagnostic specificities for BTV&EHDV were both assessed on 327 negative samples. Sensititivies were tested on 70 BTV positive samples and 143 EHDV positive samples. Inclusivity was assessed on 3 reference panels: 13 EHDV RNAs and 36 BTV RNAs (French NRL for BTV&EHDV, Anses), 7 EHDV RNAs (The Pirbright Institute, UK) and 10 BTV RNAs (FLI, Germany).

Results
The ID GeneTM kit measured 100% [99.1-100] specificity for both BTV and EHDV (n=327). Measured diagnostic sensitivity for BTV and EHDV targets was respectively 100%, [99.1-100], n=70 and 98.9%, [98.2-100], n=143. BTV+/ EHDV+ were constituted by mixing vol/vol positive samples from each virus. Even for low Cq values, no competition was observed on the qPCR, indicating the ability to detect possibly co-infected animals. All strains tested, including the BTV and EHDV strains detected in Europe in 2023, were efficiently detected by the new ID GeneTM kit, indicating a perfect inclusivity.

Discussion and Conclusion
The new ID GeneTM BTV&EHDV Triplex enables to efficiently detect and differentiate BT and/from EHD in only one reaction. In regions where both viruses can co-circulate, this RT-qPCR is, in complement to the existing IDGeneTM qPCRs, the ideal tool for differential diagnosis testing, for disease surveillance and testing before animal movements.

P211

New Diagnostic Testing Technologies

EVALUATION OF AN INDIRECT ELISA BASED ON E2 RECOMBINANT PROTEIN FOR THE SEROLOGICAL DETECTION OF OVINE PESTIVIRUS IN PORCINE SERA.

M. Corsa[1], G. Pezzoni[1], A. Castelli[1], R. Benevenia[1], M. Giammarioli[2], D. Meyer[3], P. Becher[3], E. Sozzi[1]

[1]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna "Bruno Ubertini" (IZSLER) Brescia, Italy*
[2]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati" (IZSUM) Perugia, Italy*
[3]*University of Veterinary Medicine Hannover, Foundation Institute of Virology, Hannover, Germany*

Introduction
In 2019, a novel Ovine *Pestivirus* (OVPV) was isolated in Italy [1]. This atypical pestivirus can infect pigs [2]. The available ELISAs for serological screening of Classical Swine Fever Virus (CSFV) cannot discriminate between the two pestiviruses due to the high antigenic identity causing cross-reactivity. Our study aimed to express the glycoprotein E2 of OVPV and CSFV (Diepholz), considered to be a major antigenic target of pestiviruses, and to develop two indirect ELISAs capable of discriminating between sera from pigs infected with OVPV and CSFV, respectively.

Materials and Methods
The E2-OVPV and E2-CSFV sequences obtained respectively from a field OVPV strain and from the Diepholz reference strain (AFJ79215.1), were optimised for mammalian cell expression and cloned into pcDNA3.4. The protein expression was carried out in the Expi293F system. At 3 and 6 days after plasmid transfection, the culture supernatants were collected and the proteins were purified and evaluated in Western Blotting (WB) thanks to the presence of an his-tag. A total of 11 sera from pigs infected with OVPV and 50 sera from

pigs infected with CSFV, were tested in dilution by indirect ELISA (iELISA) with purified antigens adsorbed on microtiter plates. The sera antibody titre was calculated. A total of 342 non-infected pig sera were used to evaluate the test specificity.

Results
Both recombinant proteins were expressed at the expected molecular weight as evaluated in WB. A total of $9*10^7$ suspension cells yielded 2.36 mg of purified E2-Diepholz and 1.4 mg of purified E2-OVPV. In iELISA, the titre of OVPV or CSFV infected sera was significantly higher when tested against the homologous antigen than against the heterologous antigen.

Discussion and Conclusion
The developed iELISAs represent a valid strategy to differentiate CSFV infections from other cross-reactive atypical pestiviruses. The recombinant proteins were expressed in good yields and their use makes the test robust, specific and reproducible.

References
1. Sozzi E. et al. Isolation and Full-Length Sequence Analysis of a *Pestivirus* from Aborted Lamb Fetuses in Italy. Viruses. 2019 Aug 13;11(8):744.
2. Bohórquez J.A. et al. The new emerging ovine pestivirus can infect pigs and confers strong protection against classical swine fever virus. Emerg Infect Dis. 2022 May;69(3):1539-1555.

P212

New Diagnostic Testing Technologies

DEVELOPMENT OF A SENSITIVE AND RAPID HOMOGENEOUS BIOLUMINESCENT DIAGNOSTIC ASSAY PLATFORM FOR ANIMAL INFECTIOUS DISEASES

M. Dart[1]

[1]*Promega Corporation, USA*

Introduction
Rapid and accurate diagnosis of animal infectious diseases is essential for effective management and control. Current methods, limited by slow turnaround times, low sensitivity, and complex equipment requirements, often fall short in diverse settings.

Materials and Methods
This study introduces a novel bioluminescent analyte detection assay platform using ternary split NanoLuc® luciferase complementation as the reporter system in a homogeneous immunoassay (Lumit Flex®). The system employs three non-functional fragments of NanoLuc® luciferase that reassemble into an active enzyme only in the presence of a target molecule, producing a bioluminescent signal. The enzyme components were enhanced through directed evolution for improved chemical and thermal stability and reduced background noise.

Results
To showcase the utility of this platform, we developed Lumit Flex® immunoassays for the rapid detection of Leptospirosis and SARS-CoV-2 antigens, and for serology recognition of these agents, demonstrating superior performance in LOD and ULOQ compared to conventional ELISAs. Additionally, we proved the feasibility of lyophilizing the assay components to create a shelf-stable, aqueous buffer soluble, point-of-care test that utilizes a handheld luminometer.

Discussion and Conclusion
The study highlights the system's adaptability to diverse environmental conditions, operational simplicity, and the ability to rapidly deliver diagnostic results. The development of the ternary split NanoLuc® system, tailored for increased stability and usability, offers a robust tool for analyte detection in varied diagnostic environments. This advanced system provides transformative solutions for the rapid and accurate diagnosis of animal infectious diseases, significantly enhancing disease surveillance and management strategies. It simplifies the diagnostic process across various settings, thereby revolutionizing veterinary medicine.+

References
https://pubs.acs.org/doi/10.1021/acs.analchem.0c05074
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9643700/

P213

New Diagnostic Testing Technologies

VALIDATION OF AIV AND RABV MOLECULAR METHODS EMPLOYING COMMERCIAL FREEZE-DRIED REAGENTS AND DEPLOYMENT IN SUB-SAHARAN DIAGNOSTIC LABORATORIES

P. Drzewnioková[1], I. Brian[2], A. Fortin[2], M. Marcin[2], M. Gourlaouen[7], A. Angot[7], M. Niang[8], I. Dah[6], K. Berete[3], A. Diakite[4], F. Tall Lo[5], V. D'Amico[2], V. Valastro[2], P. De Benedictis[2], I. Monne[2], V. Panzarin[2]

[1]Department of Public Health, University of Pavia, Pavia, Italy; at the time of the study: Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy
[2]Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Padua, Italy [3]Laboratoire Central de Diagnostic Vétérinaire (LCDV) de Conakry, Conakry, Guinea [4]Laboratoire Central Vétérinaire (LCV) de Bamako, Bamako, Mali
[5]Laboratoire National d'Elevage et de Recherches Vétérinaires (LNERV) de Dakar, Dakar, Senegal
[6]Laboratoire National Vétérinaire (LANAVET) de Yaoundé, Yaoundé, Cameroon
[7]One Health and Disease Control (NSAH-CJW) – Animal production and Health division (NSA) Animal Health Services (NSAH-CJW), Food and Agriculture Organization of the United Nations (FAO-UN), Roma, Italy
8Regional Office for Africa (RAF), Emergency Centre for Transboundary Animal Diseases (ECTAD), Food and Agriculture Organization of the United Nations (FAO-UN), Accra, Ghana

Introduction

Molecular methods are widely accepted as gold standard techniques for the laboratory diagnosis of several animal pathogens. However, these methods commonly employ wet reagents that require freezing for transportation and storage, and thus their use can be challenging in settings where the cold-chain maintenance cannot be guaranteed. Over the years, alternative lyophilized molecular products have been developed to overcome such issues. We evaluated their applicability for the molecular diagnosis of avian influenza (AIV) and rabies (RABV) viruses at laboratory level and under realistic Sub-Saharan scenarios.

Materials and Methods

We performed an inventory of commercial lyophilized one-step RT-PCR kits. One reagent was selected and compared to standard wet master mixes on two RT-PCR and eight qRT-PCR assays for AIV and RABV detection and typing. Thermostability tests were also carried out. Finally, an inter-laboratory exercise involving four African Central Veterinary Laboratories (CVLs) was organized for the implementation of the validated assays using freeze-dried reagent.

Results

The Qscript lyo 1-step kit (Quantabio) was selected for its single-reaction format that allows a simplified reaction assembly and reduces cross-contamination risk. The lyophilized reagent showed the same detection limit as wet reagents for all the qRT-PCR assays with Cq CV ≤ 0.04, and a slightly lower performance for RT-PCR tests. Overall diagnostic sensitivity was $\geq$ 96.55%. Storage of the lyophilized kit for 9 months at RT and for 10 days at 30°C did not affect its performance. Finally, reproducibility study through multi-site evaluation at Africans CVLs confirmed an almost perfect agreement with the expected results (Cohen's-Kappa 0.921–1). Data from individual participants further confirmed a perfect or almost perfect agreement with the results obtained with wet reagents.

Discussion and Conclusion

The Qscript lyo 1-step kit proved to be interchangeable with wet reagents for the molecular diagnosis of AIV and RABV, coupling stability over a range of environmental conditions while retaining its performance. More in general, lyophilized reagents have the potential for broader applications in remote areas also to other relevant infectious diseases upon proper validation.

References

doi:10.1016/j.jviromet.2023.114686 doi:10.3390/v15030680
doi:10.4060/cc3956en

P214

New Diagnostic Testing Technologies

DEVELOPING A RECOMBINANT PROTEIN ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DIAGNOSIS AND PREVALENCE OF *BABESIA DIVERGENS*

P. Formisano[1]

[1]*Institute of Biodiversity Animal Health and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK; Moredun Research Institute, Pentlands Science Park, Penicuik, Scotland, UK*

Introduction

Bovine babesiosis is a major tick-borne disease caused by a number of parasite species which results in significant economic loss across the world. In Northern Europe, *Babesia divergens* affects cattle and poses a zoonotic risk to humans. It causes disease of varying severity in naïve adult cattle and a rapidly progressing, life-threatening haemolytic anaemia in immunocompromised or splenectomised humans. Recently, diagnosed cases of human babesiosis have been rising and this has been attributed (in part) to the increased availability of serological testing. An immunofluorescent antibody test for *B. divergens* is available, but it is time-consuming and challenging to interpret. A rapid, simple test is needed to diagnose babesiosis in Europe. Therefore, this study aims to develop a recombinant protein ELISA for *B. divergens*.

Materials and Methods

Candidate *B. divergens* antigens were identified, cloned into bacterial expression vectors and recombinant proteins expressed. Immunoreactivity was tested using Babesia-positive bovine serum samples and promising antigens were selected for ELISA validation.

Results

Four *B. divergens* antigens were identified as promising ELISA candidates and based on these, representative recombinant proteins were expressed and purified. Immunogenicity data will be presented and discussed, along with preliminary ELISA performance results.

Discussion and Conclusion

It is anticipated that this recombinant protein ELISA will be able to identify animals with low antibody titres, thus facilitating prevalence studies on *B. divergens*, and enabling pre-movement testing of cattle into endemic areas, reducing the negative impact of bovine babesiosis. It should increase laboratory capacity for sample testing and allow better estimates of parasite prevalence in both bovines and humans.

References

Zintl, Annetta, Grace Mulcahy, Helen E. Skerrett, Stuart M. Taylor, and Jeremy S. Gray. 2003. 'Babesia divergens, a Bovine Blood Parasite of Veterinary and Zoonotic Importance', Clinical Microbiology Reviews, 16: 622-36.

McFadzean, H., N. Johnson, L. P. Phipps, V. Swinson, and L. A. Boden. 2023. 'Surveillance and Risk Analysis for Bovine Babesiosis in England and Wales to Inform Disease Distribution', Animals (Basel), 13.

P215

New Diagnostic Testing Technologies

DESIGN AND VALIDATION OF A QRT-PCR ARRAY TO DISCRIMINATE VIRULENT AND AVIRULENT APMV-1

A. Fortin[4], A. Laconi[2], I. Monne[4], S. Zohari[3], K. Andersson[3], C. Grund[5], M. Cecchinato[1], M. Crimaudo[4], V. Valastro[4], V. D'Amico[4], A. Bortolami[4], M. Gastaldelli[4], M. Varotto[4], A. Abdelrahman[6], N. Amarin[6], M.B. Abubakar[6], R. Belayneh[6], Y.B. Cyprien[6], V. Christodoulou[6], I. Chvala[6], A. Dodovski[6], S.A. Ghafouri[6], M. Giasuddin[6], M. Hassan[6], A. Kammon[6], I. Shittu[6], C.J. Snoeck[6], M. Steensels[6], D. Suarez[6], M.K. Torchetti[6], S.M. Tshipambe[6], L.H. Ouermi Zerbo[6], C. Terregino[4], V. Panzarin[4]

[1]*Department of Animal Medicine, Production and Health, University of Padua (Unipd), Legnaro (Padua), Italy*
[2]*Department of Comparative Biomedicine and Food Science, University of Padua (Unipd),Legnaro (Padua), Italy*
[3]*Department of Microbiology, Swedish National Veterinary Institute (SVA), SE751 89 Uppsala, Sweden*
[4]*EU/WOAH/National Reference Laboratory for Avian Influenza and Newcastle Disease, Division of Comparative Biomedical Sciences, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro (Padua), Italy*
[5]*Institute of Diagnostic Virology, Federal Research Institute for Animal Health, Friedrich-Loeffler-Institut (FLI), Greifswald-Insel Riems, Germany*
[6]*Newcastle Disease Collaborating Diagnostic Group (established before February 2022)*

Introduction
Newcastle disease (ND) caused by virulent strains of avian paramyxovirus type 1 (APMV-1) is an infectious disease of poultry with severe direct and indirect costs consequent to high mortality rates and control measures. Current diagnostic methods capable to discriminate virulent and avirulent strains are lengthy, do not identify co-infections or are developed to detect only certain genotypes resulting in suboptimal diagnosis and delayed interventions. To overcome these limitations, we developed a new array of RT-qPCR for the sensitive discrimination of virulent and avirulent APMV-1 in less than 2 hours.

Materials and Methods
The array consists of three simultaneous RT-qPCR assays designed to cover any genetic variability of APMV-1. Pathotyping can be accomplished in dual mode, i.e. by pathotype specific probes and by downstream Sanger sequencing to obtain the fusion protein cleavage site (CS) directly from the amplification product. The validation process was compliant with the WOAH guidelines and assessed analytical specificity and sensitivity (ASp, ASe), repeatability, diagnostic specificity and sensitivity (DSp, DSe) and reproducibility.

Results
The RT-qPCR array showed 100% ASp and correct discrimination between virulent and avirulent APMV-1. In all cases but one (i.e. APMV-1 genotype V.1) the limit of detection was $\leq 10^{2.83}$ EID_{50}/100 µl. The overall %CV of intra- and inter- assay Cq was ≤ 4.9. DSe assessed on a variety of samples representing different genotypes and matrices from poultry and wild birds from Europe, Asia and Africa resulted in 100% DSp and DSe and correct virulent/avirulent discrimination by pathotype-specific probes. For 81% APMV-1 positive samples we obtained the CS sequence. In a domestic pigeon, the array was able to detect virulent APMV-1 and the vaccine strain. Reproducibility as assessed by ring trial and an inter-laboratory exercise resulted in perfect (Cohen K = 1) or almost perfect (Fleiss K = 0.88) agreement.

Discussion and Conclusion
The RT-qPCR array provides a rapid and accurate method for the discrimination of virulent and avirulent APMV-1. Additional validation tests are required to assess its performance on genotypes not included in the study and on vaccinated poultry populations as potential DIVA strategy.

References
doi:10.1016/j.jviromet.2023.114813

P216

New Diagnostic Testing Technologies

COMPLETE DIAGNOSTIC SOLUTION FOR EPIZOOTIC HAEMORRHAGIC DISEASE MONITORING

C. Aira[2], S. Hernández-Antón[2], L. López[2], C. Galán[2], M.Á. Risalde[1], P. Rueda[2], A. Fresco-Taboada[2]

[1]*Departamento de Anatomía y Anatomía Patológica Comparadas y Toxicología, Grupo de Investigación GISAZ, UIC Zoonosis y Enfermedades Emergentes ENZOEM, Universidad de Córdoba, Córdoba, Spain.*
[2]*Gold Standard Diagnostics Madrid S.A. (GSD Madrid), Madrid, Spain*

Introduction
Epizootic haemorrhagic disease (EHD) is a non-contagious disease of wild and domestic ruminants transmitted by midges of the genus Culicoides. In deer, it often results in non-negligible levels of mortality, while in cattle, less severe infections are usually observed. The causative agent is the EHD virus, a virus belonging to the *Orbivirus* family. First cases in Europe were reported in autumn 2022, and outbreaks increased recently showing an important impact to cattle. Its evolution is unclear although experts suggest it might show a similar evolution to BTV in Europe. To respond to these outbreaks, we developed a complete diagnosis for the surveillance and monitoring of EHD.

Materials and Methods
In this work, we show the development of an ELISA for the detection of specific antibodies to EHDV and two lateral flow assays for the detection of antigen and antibodies. The ELISA was developed in a competitive format using VP7- coated plates and a specific monoclonal antibody which was shown to be EHDV-specific (no cross-reactions with BTV or AHSV). The new ELISA was evaluated with a total of 626 serum samples, and it was validated to be used with filter- paper blood samples. For LFAs, an analytical evaluation has been performed.

Results
The competitive ELISA showed a sensitivity of 99.7 % out of the 304 positive samples tested. Additionally, we assayed a total of 81 cattle samples collected in parallel as fresh blood and in filter paper. This study showed the same diagnostic parameters regardless of the sampling method used. To evaluate ELISA's specificity, 98 negative field samples from cattle, deer, sheep, and goat, and 24 cattle samples collected from BTV-infected animals have been evaluated. The ELISA exhibited a 100% specificity. Analytical evaluation of LFAs showed a limit of detection of 5 ng/strip for the antigen-detection LFA and the antibody- detection LFA detected all the analysed serotypes (1, 2, 4-6 and 8).

Discussion and Conclusion
These results indicate that the ELISA is a good tool for monitoring disease evolution, and, thanks to the filter paper blood collection, it can be easily applied to wildlife monitoring. Sample panel will be extended in the following months. Moreover, LFAs can help in field investigation of outbreaks.

P217

New Diagnostic Testing Technologies

IMPROVED SEROLOGICAL ASSAYS FOR THE DIAGNOSIS OF CANINE LEISHMANIOSIS

R. Mahdavi[2], C. Aira[1], H. Redondo[1], L. López[1], C. Galán[1], P. Rueda[1], A. Fresco-Taboada[1], U. Steinhoff[2]

[1]*Gold Standard Diagnostics Madrid S.A. (GSD Madrid), Madrid, Spain*
[2]*Institute of Medical Microbiology and Hospital Hygiene, Phillips University of Marburg, Marburg, Germany*

Introduction
Leishmaniosis is a zoonotic disease caused by parasites of the genus *Leishmania*. It is a disease of slow and progressive evolution which can be treated if detected on time, otherwise it can be fatal. Among the multiple affected species, dogs are of great importance since they act as a parasite reservoir, and they can play a role as intermediates to human transmission. To properly control this disease, rapid and reliable diagnostic tests are needed. Serology is the preferred method for diagnosis because it better correlates with disease stage. Commercially available serology tests are mainly based on whole parasite or on the recombinant kinesin rK39, which have several limitations in terms of specificity. In this work, we developed two novel multi-species tests for improving serological detection of leishmaniasis.

Materials and Methods
For this purpose, a new recombinant kinesin antigen from *Leishmania* was bioinformatically developed based on the most conserved regions among different *Leishmania* species. These highly conserved regions were selected and, among the different designed proteins, the rKLi8.3 was chosen as the most immunogenic form. This rKLi8.3 antigen was used to develop an enzyme-linked immunosorbent assay (ELISA) and a lateral flow assay (LFA) in an indirect multispecies format.

Results
The new ELISA and LFA tests were evaluated with a panel of samples collected from symptomatic and asymptomatic dogs, from animals infected with other parasites, or from healthy dogs. The ELISA and the LFA showed a sensitivity (Sn) of 94% and 93%, and a specificity of 99% and 98% respectively; what improved parameters obtained with a commercial ELISA based on the whole parasite (Sn 84% and 88%). The new serological assays improved the detection of infected asymptomatic animals, which are challenging to detect, and showed no cross-reactivity with samples collected from dogs with other parasite infections.

Discussion and Conclusion
In conclusion, the new tests based on the rKLi8.3 antigens were shown to have better diagnostic parameters than conventional diagnostic assays based on whole parasite/rK39 antigen, improving the identification of infected animals.

P218

New Diagnostic Testing Technologies

APPLICATION OF A RUMINANT ABORTION MULTIPLEX QPCR-HRM TECHNIQUE TO INVESTIGATE ABORTIONS IN SMALL RUMINANTS, GREECE.

I. Gouvias[1], M. Lysitsas[1], G. Valiakos[1]

[1]*Faculty of Veterinary Science, University of Thessaly, Karditsa, Greece*

Introduction
Abortifacient pathogens of small ruminants are extremely challenging to deal with because of their regular co- implication in abortion cases and diversity in both their diagnosis and subsequent treatment [1]. They cause significant economic losses and animal welfare issues, and their zoonotic potential has emerged as a concern for human health [2]. The objective of this study was to preliminarily evaluate the efficacy of using a novel multiplex qPCR-high resolution melting (HRM) technique for the simultaneous detection of various abortogenic agents in small ruminant samples obtained throughout Greece.

Materials and Methods
Ovine and caprine vaginal swab samples were obtained from animals that aborted during the preceding week. Nucleic acid extraction was performed using a commercial spin column kit (INDICAL Bioscience) and an IDEAL™ 32 extraction robot (Innovative Diagnostics). Molecular investigation was performed using a novel commercial kit (ID Gene™ Ruminant Abortion Multiplex HRM, Innovative Diagnostics) according to the manufacturer's instructions on an MIC qPCR Cycler (Bio Molecular Systems).

Results
The results indicated a high prevalence of *C. burnetii* and *Chlamydophila* spp. in the investigated group, detected in approximately 49% and 42% of the animals, respectively. *Toxoplasma gondii*, *A. phagocytophilum, Salmonella* spp., *Brucella* spp., *C. fetus* and *N. caninum* were also identified, in lower rates. Mixed infections occurred in approximately one-third of the animals examined.

Discussion and Conclusion
This multiplex qPCR-HRM technique allows for the simultaneous detection of many abortogenic pathogens in a cost- effective and accurate assay. Pathogens not commonly investigated in small ruminants, such as *C. fetus* and *N. caninum*, are included; their detection in variable cases indicates that their role in ovine and caprine abortions may be underestimated. Furthermore, the detection of T. gondii and N. caninum can be accomplished in many cases through vaginal swabs, even though they are not the recommended type of sample.

References
1. Menzies, P.I. Control of Important Causes of Infectious Abortion in Sheep and Goats. Veterinary Clinics of North America: Food Animal Practice 2011, 27, 81–93.
2. Roest, H.I.J. et al. The Q Fever Epidemic in The Netherlands: History, Onset, Response and Reflection. Epidemiol. Infect. 2011, 139, 1–12.

P219

New Diagnostic Testing Technologies

COMPARATIVE EVALUATION OF MASTITIS BACTERIOLOGICAL IDENTIFICATION METHODS IN DAIRY COWS DURING THE PRE-DRYING OFF PERIOD

F. Laforêt[3], T. Glouden[3], M. Vandermolen[3], M. Beaudor[1], M. Liégeois[2], V. Dave[4]

[1]Industrialization Leader, Molecular Biology Business Unit, Unisensor, Liège, Belgium [2]Product Manager, Molecular Biology Business Unit, Unisensor, Liège, Belgium
[3]R&D Expert, Molecular Biology Business Unit, Unisensor, Liège, Belgium
[4]R&D Leader, Molecular Biology Business Unit, Unisensor, Liège, Belgium

Introduction
Mastitis, an udder inflammation primarily caused by bacterial invasion via the teat canal, poses significant challenges for dairy farmers. It reduces milk quality and quantity, resulting in substantial economic losses from treatment costs and disposal of antibiotic-treated milk. Bovine mastitis is the most frequent reason for antibiotic use in dairy production. Accurate identification of mastitis-causing bacteria is crucial for effective treatment and prevention strategies. Various methods exist, including culture-based, MALDI-TOF mass spectrometry and molecular biology. Culture methods on agar plates are slow and have limited sensitivity. MALDI-TOF is expensive and requires specialized equipment and expertise. PCR, the reference method, needs a thermocycler and is predominantly conducted in an accredited laboratory. An interesting alternative is the isothermal DNA amplification utilized in the MastiSensor kit. This kit detects 8 targets commonly responsible for mastitis, including Gram-positive bacteria, *Streptococcus uberis* and *S. dysgalactiae, Escherichia coli*, *Klebsiella* spp., *Staphylococcus* aureus, *Enterococcus* spp., and the blaZ gene. This test provides results in less than an hour, is easy to use for all veterinarians, and shows a high sensitivity.

Materials and Methods
This study aims to compare the bacteriological identification methods used by the veterinarian themselves (culture- based vs MastiSensor). The sample are cow's milk from approximately a hundred cows, specifically before drying off— a critical period for mastitis onset. This study, conducted in summer 2024 in Liège (Belgium) focuses on researching bacteria in weekly milk samples collected from 18 cows. Data collected will include, among others, parity, clinical signs, and mastitis history. All positive cultures will undergo MALDI-TOF analysis for colony identification confirmation.

Results
Expected outcomes include results consistency across isothermal amplification, culture, and MALDI-TOF.

Discussion and Conclusion
If the results are consistent, it will demonstrate the significant value of MastiSensor as a rapid, reliable, and user- friendly identification method for veterinarians which enable quick decision-making, tailored and effective treatment, and daily animal monitoring.

P220

New Diagnostic Testing Technologies

RECOMBINANT ANTIBODY GENERATION AND ADAPTATION TO ANIMAL HEALTH DIAGNOSTIC TESTS

J. Martínez-Cano[1], E. Soria[1], A. Camuñas[1], M. Terrasa[1], J. Sarraseca[1], Á. Venteo[1], S. Collado[1], P. Rueda[1], M. García-Durán[1]

[1]Gold Standard Diagnostics Madrid SA (GSD Madrid), Spain

Introduction
Antibodies are widely used as recognition reagents in immunodiagnostic based tests. Compared to the polyclonal and monoclonal antibodies traditionally used for these purposes, recombinant monoclonal antibodies (rMab) offer many advantages because they are based on a known and defined DNA sequence. Their functionality is therefore secured, their industrial production becomes highly reproducible, and they can be further engineered to improve their downstream applications. Here we show the adaptation of two monoclonal antibodies, 1DB12 anti-TGEV and 2BD1 anti- gE ADV, into their recombinant analogs as each hybridoma line initially presented production problems. Our results show that the new antibodies perform as well as their hybridoma-derived analogs in their actual ELISA competition kits for animal diagnostic.

Materials and Methods
RNA was extracted from samples of hybridoma lines, and their antibody variable chains were amplified by RT-PCR using a specific mix

of degenerate primers. Sequences were cloned in a scFv-Fc format, transfected into HEK293 cell suspension cultures and rMab purified by protein A columns. rMab were labelled with horseradish peroxidase and diluted according to the requirements of the diagnostic test. INgezim CORONA DIFERENCIAL 2.0 and INgezim ADV gE PLUS ELISA tests were performed with rMab and Mab in parallel, using panels of positive and negative sera.

Results

The results are summarised in Table 1 and Table 2 (attached).

	Mab 1DB12	rMab 1DB12
Corona +	103	103
PRCV +	27	27
TGEV +	74	75
Doubtful	2	1
TOTAL sera	206	

Table 1. Classification of sera from animals with porcine corona pathologies by INgezim CORONA DIFERENCIAL 2.0 using rMab and compared to reference (Mab).

	Mab 2BD1	rMab 2BD1
ADV +	13	12
ADV -	156	161
Doubtful	7	3
TOTAL sera	176	

Table 2. Detection of anti gE antibodies in porcine sera by INgezim ADV gE PLUS using rMab and compared to reference (Mab).

Discussion and Conclusion

Both rMab showed high concordance with the reference test in OD and classification (see Table 1 and 2). Differences were related to small changes in percentage of competition which can be easily resolved by fine-tuning of ELISA controls. The results obtained in the final test demonstrate their applicability to the final commercial kit, incorporating the advantages of increased batch-to-batch reproducibility.

P221

New Diagnostic Testing Technologies

LAMP : NEW DNA AMPLIFICATION TECHNOLOGY AS A POINT OF CARE TOOL TO HELP DIAGNOSTIC OF PATHOGENS IN ANIMALS

L. Melo[1], T. Thibault[1], L. Valot[1], A. Zocevic[1], M. Simonnet[1], W. Yakoub[1], D. Schieb[1], M. Gene[1], E. Schaeffer[1], C. Bettin[1], L. Thiery[1]

[1]*Enalees, France*

Introduction

Currently, the direct detection of pathogens contaminating animals can be performed by 1) PCR, giving highly sensitive results but this requires to send the samples to a specialized laboratory which can give the results only few days later. 2) Antigenic tests which can give a result quickly but with a lower sensibility than PCR. LAMP technology (Loop mediated isothermal AMPlification) (Notomi T) is a NAAT technology (Nucleic Acid Amplification test) which can occur at constant temperature, unlike PCR which need thermal cycles. This allows the use of this technology as a POC (point of care) test and allow the obtention of the result in only 30 min without the necessity to use a thermal cycler. This technology can be useful to detect highly contagious pathogens causing diseases in animals and the possibility to administrate the appropriate medicine or isolate the animal immediately.

Materials and Methods

LAMP Primers and LAMP mastermix were chosen for their capabilities to amplify nucleic acids for pathogens infecting animals. Quick nucleic acid extraction and purification from complex samples (blood, urine, faeces, nasopharyngeal swabs, …) were developed to be compatible with LAMP reactions without loss of performance (Valot L). LAMP reader was chosen to be efficient, robust and portative.

Results

LAMP assays, containing 6 LAMP primers allowing a good specificity (instead of 2 for PCR), strand displacement polymerase and a quick nucleic acid extraction, were developed to diagnose pathogens infecting horses, dogs or cats. LAMP has shown performances similar than conventional PCR performed in a laboratory. Kits were developed to have a portative format and to be used by a non-specialized technician quickly (less than 30 min between the sampling and the result). Differences between PCR and LAMP will be explained. Development of a LAMP test will be detailed, and results obtained during the development of a LAMP test will be shown.

Discussion and Conclusion
LAMP tests are a new generation of DNA/RNA amplification test, specific and sensitive, which can be performed near a sick animal and can give a result in only 30 min. These parameters allow the consideration to use this technology to analyse quickly potential pathogens holder and treat or isolate the animal immediately.

References
Notomi, T. et al. (2000)
Valot, L. et al. WO2021160849

P222

New Diagnostic Testing Technologies

VALIDATION OF A DROPLET DIGITAL PCR ASSAY USING THE LAMBDA PROPHAGE TYPE 3 TARGET TO DETECT *B. ANTHRACIS*

L. Pace[1], M.G. Basanisi[1], V. Rondinone[1], G. La Salandra[1], V. Manzulli[1], A. Aceti[1], L. Serrecchia[1], F. Tolve[1], A. Scarinci[1], V. Vetritto[1], G. La Bella[1], G. Nobili[1], D. Galante[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Foggia, Italy*

Introduction
Bacillus anthracis, the causative agent of anthrax, is a zoonotic pathogen that is relatively common throughout the world and may cause life threatening diseases in animals and humans. Most of the developed PCR-based assays for the detection of *B. anthracis* rely on unique markers present on virulence plasmids pXO1 and pXO2, but relatively few assays incorporate chromosomal DNA markers due to the close relatedness of *B. anthracis* to the *B. cereus* group strains. We propose a method for B.anthracis detection using a highly conserved chromosomal marker for *B. anthracis*, the lambda prophage type 3 (PL3), present in single copy, using the digital PCR (dPCR) to identify *B. anthracis*.

Materials and Methods
The validation of this test method was carried out according to the guidelines described in part 1 – chapter 1.1.6 of the "WOAH Terrestrial Manual-12th edition". The protocol involved the determination of analytical accuracy as well as the calculation of the limit of detection (LOD). Briefly, we tested 20 DNA of *B. anthracis* strains used as positive controls and 20 strains belonging of *B.cereus* group and other bacteria species used as negative controls (Table 1). Digital PCR reactions were performed in QX200™ Droplet Digital™ PCR System, using customize dPCR Probe Assays (Biorad).

Results
Our results suggest that the PL3 marker is a good marker for the identification and the quantification of *B. anthracis* spores, since it is highly specific and shows no cross-reaction with a large panel of genetically closely related bacteria belonging to *B.cereus* group and other non-*Bacillus* bacteria (Figure 1). Furthermore, we obtained a good LOD of 20000 spores/ml (Table 2).

Positive Controls	
1	Bacillus anthracis
2	Bacillus anthracis
3	Bacillus anthracis
4	Bacillus anthracis
5	Bacillus anthracis
6	Bacillus anthracis
7	Bacillus anthracis
8	Bacillus anthracis
9	Bacillus anthracis
10	Bacillus anthracis
11	Bacillus anthracis
12	Bacillus anthracis
13	Bacillus anthracis
14	Bacillus anthracis
15	Bacillus anthracis
16	Bacillus anthracis
17	Bacillus anthracis
18	Bacillus anthracis
19	Bacillus anthracis
20	Bacillus anthracis

Negative Controls	
1	Bacillus thuringensis
2	Bacillus thuringensis
3	Bacillus cereus group
4	Bacillus cereus group
5	Bacillus cereus group
6	Bacillus cereus group
7	Bacillus cereus group
8	Bacillus cereus group
9	Bacillus cereus group
10	Bacillus cereus group
11	Bacillus cereus group
12	Bacillus cereus group
13	Bacillus cereus group
14	Bacillus cereus group
15	Bacillus cereus group
16	Bacillus cereus group
17	Escherichia coli
18	Clostridium perfringens
19	Clostridium perfringens
20	Clostridium perfringens

Table 1. Samples used for the Validation of Droplet digital PCR assay.

Spores/ml	*n. copies/µl (I)*	*n. copies/µl (II)*	*n. copies/µl (III)*	*n. copies/µl (IV)*	*n. copies/µl (V)*
20 x 10^6	157	165	164	163	160
20 x 10^5	17,8	19,1	18,3	18,4	19,1
20 x 10^4	0,88	1,2	1,7	1,5	1,6
20 x 10^3	0,32	0,20	0,19	0,19	0,18
20 x 10^2	0	0	0	0	0

Table 2. Five replicates of DNA serial dilutions were analysed to determine the limit of detection (LOD) for *B.anthracis.*

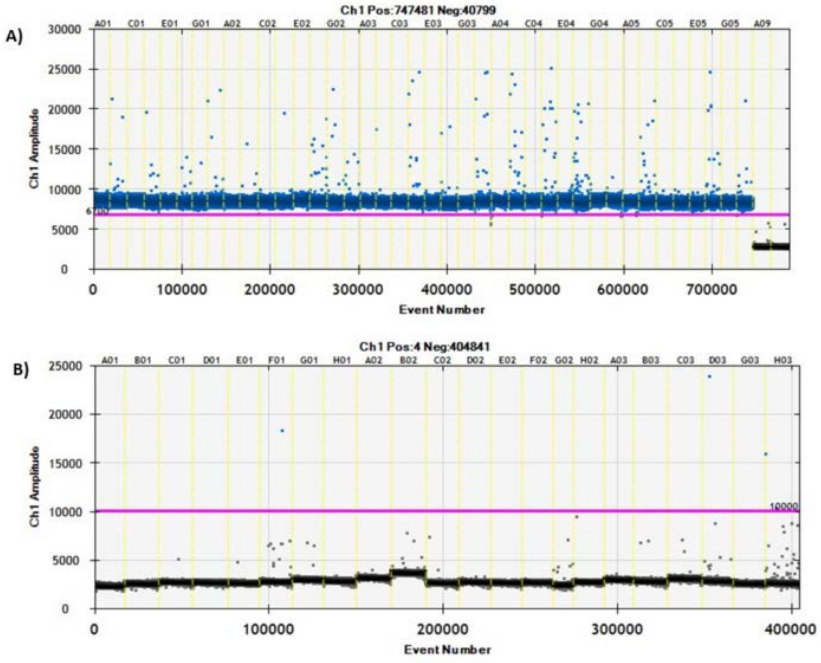


Figure 1. Droplet digital PCR assay for PL3. A) Positive samples droplet populations are shown in blue droplets blue. B) Negative samples in grey droplets. Threshold was set manually (pink).

Discussion and Conclusion

In the last years dPCR has rapidly gained importance. Therefore, this approach represents a robust and powerful novel molecular biology technique for the detection of *B. anthracis* that it can provide higher sensitivity and specificity, as well as, the rapid quantification of genomic copies of the samples. In conclusion, these results are important for a standardization of an alternative molecular assay for B.anthracis detection.

References

Straub T, et al. Estimated copy number of *Bacillus* anthracis plasmids pXO1 and pXO2 using digital PCR. J Microbiol Methods. 2013 Jan;92(1):9-10. doi: 10.1016/j.mimet.2012.10.013.

P223

New Diagnostic Testing Technologies

VALIDATION OF PASSIVE SAMPLERS FOR THE DETECTION OF AVIAN INFLUENZA VIRUS IN WETLANDS

M. Crimaudo[1], S. Marciano[1], P. Berto[2], S. Bofill Mas[3], M. Rusiñol[3], A. Bortolami[1], F. Pascoli[2], F. Bonfante[1], C. Terregino[1], V. Panzarin[1]

[1]*EU/WOAH/National Reference Laboratory for Avian Influenza and Newcastle Disease, Division of Comparative Biomedical Sciences, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro (Padua), Italy*
[2]*Fish Virology Laboratory, Division of Comparative Biomedical Sciences, Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), Legnaro (Padua), Italy*
[3]*Laboratory of Viruses Contaminants of Water and Food, Secció de Microbiologia, Departament de Genètica, Microbiologia i Estadística, Facultat de Biologia, Universitat de Barcelona (UB), Barcelona, Spain*

Introduction

Avian influenza viruses (AIVs) pose continuous threats to the poultry industry, wildlife conservation and public health. Surveillance in wild birds is crucial to be prepared against new viral incursions and monitoring water bodies where thousands of animals gather can further improve its effectiveness. Passive samplers (PS) are cost-effective solutions successfully used for pathogens monitoring in wastewater, but their application to AIV is yet to be evaluated. The study aims to address this issue and optimize protocols for PS use in wetlands.

Materials and Methods

Nine different materials (cotton gauze - CG, sponge, nitrocellulose - NC, nylon, polycarbonate - PC, polyethersulfone - PES, ZetaPlus VR filters) were immersed for 24h in brackish water spiked with AIV. Virus was eluted with a PBS- based buffer and concentrated for subsequent qRT-PCR. For protocol optimization, two additional elution buffers (glycine- and guanidine-based) were also evaluated on five adsorbent materials. The experiment was repeated with AIV contaminated freshwater using the best elution protocol. To establish optimal deployment time, five adsorbent materials were inserted in torpedo shells and immersed for 3h, 24h and 7d in 2,5L brackish and freshwater spiked with AIV. The effect of complete water change after 24-hour deployment was also evaluated to assess possible virus detachment from PS.

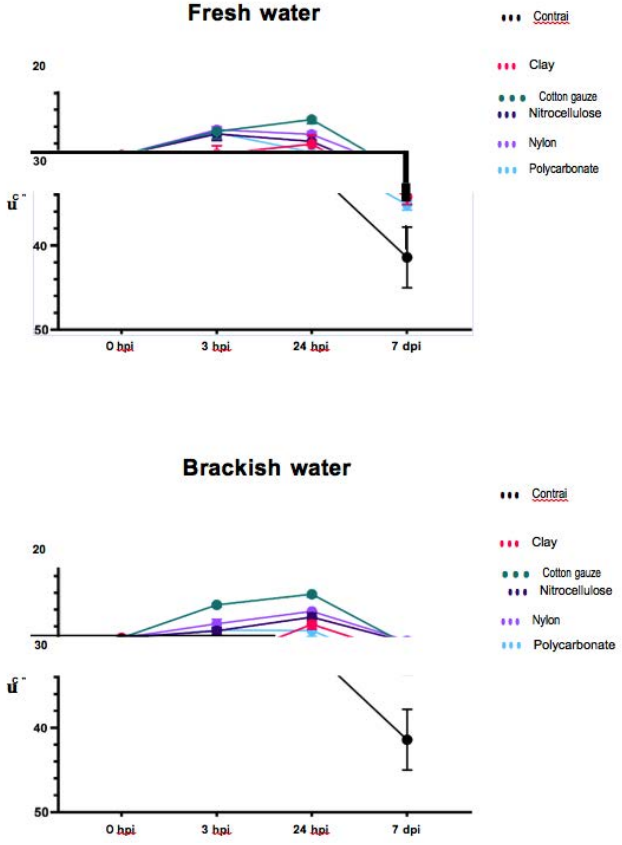


Figure 1. Comparison of different adsorbent materials immersed in brackish and freshwater contaminated with AIV.

Results
Adsorption with clay, CG, NC, nylon and PC followed by elution with the guanidine-based buffer showed the best performance and were employed for subsequent trials. After 3h, all the materials but clay adsorbed the virus with significant improvement of viral detection. Adsorption increased after 24h, except for PC that suffered from virus detachment over time. After 7d, all the materials retained whole viral particles. Finally, water replacement simulating watering down events did not significantly impair virus recovery.

Discussion and Conclusion
PS allow efficient AIV detection in water samples and can be used for surveillance in wetlands. Assessment of different deployment times allows end-users to select the most convenient option based on the sampling regimen. Further testing to determine PS detection limit and performance in real field scenarios are ongoing and will provide additional data for recommendations of use.

References
doi:10.1021/acs.est.1c01530

P224

New Diagnostic Testing Technologies

VETERINARY RAPID POINT-OF-CARE TESTING USING THE PORTABLE ISOTHERMAL LOOP MEDIATED NUCLEIC ACID AMPLIFICATION PLUSLIFE PLATFORM

V. Sarchese[2], F. Di Profio[2], E. Prato[1], C. Commisso[1], F. Marsilio[2], V. Martella[3], B. Di Martino[2]

[1]BioMed S.r.l., Via A. Vespucci [12], [16156], Genova, Italy.
[2]Department of Veterinary Medicine, Università degli Studi di Teramo, Teramo, Italy
[3]Department of Veterinary Medicine, University of Bari Aldo Moro, Valenzano, Italy

Introduction
Feline panleukopenia virus (FPV), canine parvovirus (CPV), and pathogens causing feline upper respiratory infection (FURI) are common infectious agents in companion animals. Recently, point-of-care (POC) tests using nucleic acid amplification methods have been developed, showing comparable sensitivity and specificity to traditional molecular techniques. This study aims to evaluate the performance of the RHAM-technology-based Pluslife Mini Dock (PMD) compared to PCR and qPCR.

Materials and Methods
Ninety-eight rectal swabs of 47 cats and 51 dogs either with or without enteritis, and 90 feline respiratory samples of cats with respiratory signs, were tested using PCR and qPCR assays, specific for FPV, CPV, and for URI pathogens, including Feline Calicivirus, Feline Herpesvirus-1, Chlamydia felis and *Mycoplasma* felis (1-7). POC testing was performed according to manufacturer's instructions. Positive percent agreement (PPA), negative percent agreement (NPA) and accuracy of POC tests were calculated in comparison to the reference molecular methods.

Results
As for FPV and CPV, PMD tests showed PPA values ranging between 83.0% to 94.1%, NPA values ranging 77.8%-100%, with accuracy values ranging from 70.9% to 90.7%, in comparison to PCR and qPCR assays. As for the POC tests specific for FURI, the PPA values ranged from 93.3% to 100%, the NPA values from 88.2% to 100%, and the accuracy values ranged from 91.5% to 97.7% compared to methods used as gold standards.

Discussion and Conclusion
The PMD POC technology demonstrated good analytical PPA, NPA, and accuracy values for the identification of the infectious agents investigated, compared to the PCR and qPCR assays used as gold standards. PMD technology represents a rapid and reliable method for pathogens detection in a POC setting, speeding up the adoption of measures useful in preventing and controlling infectious diseases in companion animals.

References
1. Buonavoglia et al., 2001. J Gen Virol. 82:3021-5.
2. Streck et al., 2013. J Virol Methods. 93:6-8.
3. Chalker et al., 2004. Vet Microbiol. 100:77-82.
4. Marsilio et al., 2004. Vet Res Commun. 1:295-8.
5. Söderlund et al., 2011. J Vet Diagn Invest. 23:890-3.
6. Helps et al., 2003. J Clin Microbiol. 41:2734-6.
7. Helps et al., 2002. J Virol Methods 106:241–4.

P226

New Diagnostic Testing Technologies

DEVELOPMENT OF A ROUTINE ANALYSIS METHOD FOR CREATININE IN MILK

J. Schaep[1], I. Jacobs[2], V. Huys[1], L. Allais[1], N. Botteldoorn[1], E. Van Driessche[1], T. Vanblaere[1]

[1]*Animal Healthcare Flanders, Torhout, Belgium*
[2]*Milk Control Centre, Lier, Belgium*

Introduction

Creatinine is commonly analysed in animal serum and urine. It is a non-ureum nitrogen compound also present in the milk. For cattle an analysis in milk is relevant as it could be a tool to monitor the protein intake in practice. Little information is available for routine analysis of creatinine in milk.

Materials and Methods

Milk samples were taken from three bulk milk containers. Acetic acid was added to the milk for the sample preparation, after centrifugation resulting in a three-layer sample with the clear middle liquid-layer being the solution of interest. Two methods were compared to determine creatinine: an HPLC-UV method and a discrete analyser method with photometric analysis (Table 1).

Sample preparation	
Make-up	4 ml milk + 2,5 ml of 3% (v/v) acetic acid, diluted to 10 ml with water
Centrifugation	10 min at 1500 rpm
HPLC-UV	
Equipment	Thermo Vanquish Core
Column	Spherisorb S5NH2 COL 25CMX4.6MM
Injection parameters	injection volume 5 µl , flow 1,2 ml/min
Detection parameters	255 nm
Discrete analyser	
Equipment	Thermo GalleryPlus
Settings	Enzymatic reaction (Thermo 981845), analysis at 540 nm

Table 1. Method information.

Results

Starting from published information we observed that the milk matrix impacted the baseline to such an extent that integration was not straightforward. Changing parameters such as injection volume and wavelength allowed to set up a reproducible method. A chromatogram is shown in Figure 1. Creatinine concentrations in the tested bulk milk samples were found to be around 8-10 mg/l with the method being repeatable and reproducible. The recovery of creatinine for additions of 15 mg/l was very good, with all recoveries between 85-105 %. As creatinine is commonly analysed photometrically in serum, it was studied if that technique could be used also for milk matrices. Using the same sample preparation the liquid as injected in the HPLC was analysed by a discrete analyser. The milk creatinine concentrations found were comparable. Results are summarized in Table 2.

Characteristic	HPLC	Discrete analyser
Concentration range	8,0 – 9,5 mg/l	8,2 – 9,5 mg/l
Repeatability	0,6 %	1,3 %
Reproducibility	8,7 %	6,8 %
Mean recovery	94,9 %	98,6 %

Table 2. Results for two methods (r, R, %recovery)

Discussion and Conclusion

Creatinine can be analysed by both the HPLC-UV method as the discrete analyser method in a reproducible way with an excellent recovery. The milk creatinine concentrations showed no significant difference for the two methods. As the photometric determination is more straightforward to carry out and cheaper it is the method to be preferred for routine analysis. The relationship between the milk creatinine concentration and the protein intake of the cow will be studied next.

References
Zuo et al. (2015), Food Chemistry, vol 182, p 242-245
Indyk et al. (2004), Journal of AOAC International, vol 87(1), p 116-122 Cavazzini et al. (2023), Liquid Chromatography, Chapter 10, p 227-249

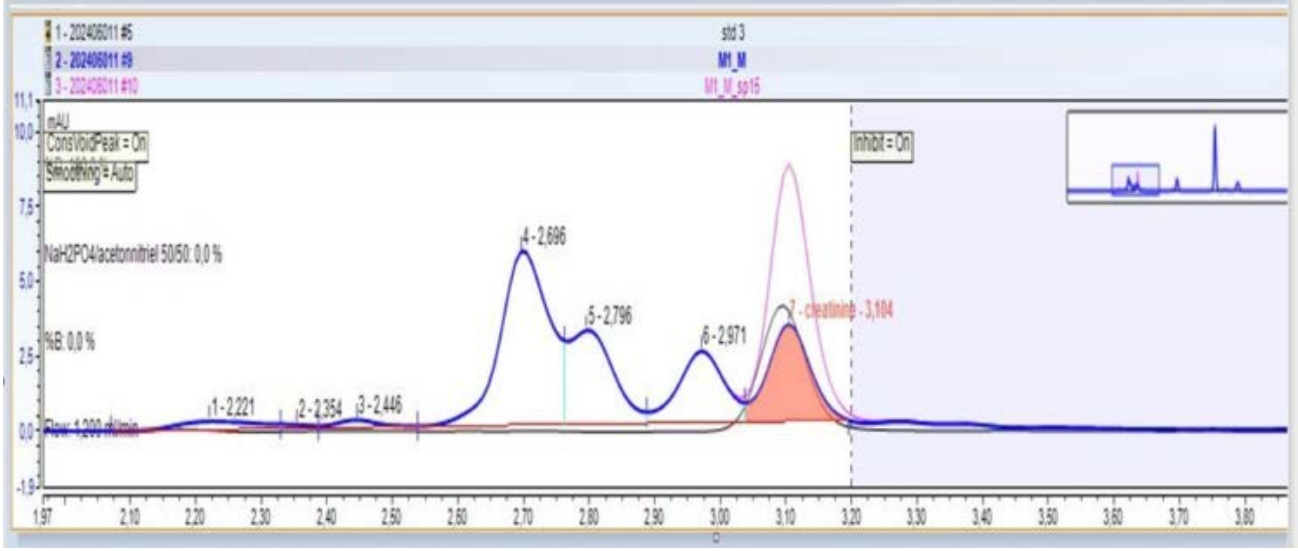


Figure 1. HPLC chromatogram, overlay std 5 ppm – bulk milk – spiket bulk milk (automatically integrated)

P227

New Diagnostic Testing Technologies

PCR-BASED METHODS FOR THE DETECTION OF BOTULINUM PRODUCING-CLOSTRIDIA TYPE C, D, AND THEIR MOSAIC VARIANTS

S. Taglieri[1], C. Scalfaro[1], F. Anniballi[1]

[1]*Istituto Superiore di Sanità, Department of Food Safety, Nutrition and Veterinary Public Health, National Reference Centre for Botulism, Rome, Italy*

Introduction
PCR-based techniques play a crucial role in botulism laboratory diagnosis and epidemiological monitoring. They are widely adopted as *in vitro* approaches for detecting botulinum neurotoxin-producing-clostridia, aiding in risk assessment. In the present study, we report the development and in-house validation of a new primers-probes set capable of working in probe-based multiplex real-time PCR, SYBR Green real-time PCR and multiplex digital PCR.

Materials and Methods
The primers-probes set was designed using the conserved regions of publicly available sequences encoding for botulinum neurotoxins type C, D, CD, and DC. Selectivity, specificity, sensitivity, accuracy, repeatability, and the Limit of Detection were established by testing DNA extracted from purified strains, spiked samples and naturally contaminated specimens.

Results
The results showed 100% selectivity, specificity, sensitivity, accuracy, and repeatability. The different protocols developed showed at least seven logarithms of linearity and can recover less than ten *Clostridium botulinum* cells in 25g of samples.

Discussion and Conclusion
Historically, types C, D, CD, and DC *C. botulinum* (also known as group III *C. botulinum*) are mainly responsible for animal botulism; however, the recent recovery of a new variant of type C toxin in a domestic botulism outbreak in Japan and a pig farm in France poses

new concerns for public health and the need for an in-depth assessment of this new emerging risk. Although probe-based multiplex PCR is a suitable approach for laboratory diagnosis of botulism, probes, especially those containing Locked Nucleic Acid bases, can be too expensive to address the isolation process of these organisms. Indeed, isolating *C. botulinum* group III could be challenging because of the ease with which these organisms can lose genes encoding for botulinum neurotoxins during laboratory manipulations. We also developed and validated a SYBR Green approach to make the method cheaper. Finally, the digital PCR approach can be particularly suitable for risk assessment because it can provide quantitative results without the calibration curve.

References

Anniballi F, et al. Vet Microbiol. 2012;154(3-4),332-338. Woudstra C, et al. Anaerobe. 2013;22,31-47.
Gao P, et al. Front Microbiol. 2022;13:860992.

P228

New Diagnostic Testing Technologies

MIKROWIN 2020 – AN AUTOMATED ELISA INTERPRETER

M. Van Den Brandt-Willekes[1], P. Van Kalsbeek[1], I. Heite[1]

[1]*Department of Diagnostics and Crisis Organization, Wageningen Bioveterinary Research, Wageningen University and Research, Lelystad, The Netherlands*

Introduction

The ELISA diagnostics laboratory has been using Mikrowin 2010 and Mikrowin 2013 for an extended period. Issues with test validation and software performance prompted inquiries about support for both versions of Mikrowin. Mikrowin 2013 was discontinued and replaced by Mikrowin 2010, which was developed until 2020 and supported until 2025. The objective was to find a new software solution that aligned with the laboratory's needs.

Materials and Methods

Key requirements included the ability to connect to hardware (ELISA reader) and software (Laboratory Information Management System), protocol selection based on measurement names, execution of calculations based on measurements, and interpretation of results for ELISA validation. However, the market was predominantly focused on research, leaving a gap in software offerings for diagnostic laboratories. Ultimately, the laboratory reverted to Mikrowin, opting for Mikrowin 2020 this time.

Results

All 18 of our existing protocols needed to be adjusted to the new software. Due to the differences between the two versions, it was decided to create each protocol from scratch. During the various validation processes, it was noted that the rules of rounding were not consistent. The problem was mostly resolved with the latest version (V 6.15), which also made the use of rounding definitions unnecessary.

Discussion and Conclusion

The 18 protocols have been established. Upon initiating a measurement, the software will identify the relevant protocol based on the four digits in the measurement name using Measurement-ID Protocol Linking. The measurement name can be scanned from the work protocol barcode, so no manual input is needed. Mikrowin will initiate the ELISA reader to conduct a reading using the specified wavelength in the protocol. Calculations will be automatically executed using the obtained optical density value and test controls. The test validation will be conducted by comparing the calculated values against predefined criteria. If the test is deemed valid, an export file will automatically be generated, and the results will be exported to the LIMS software.

P229

Proficiency Testing – participant and organizer perspective

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELLE VENEZIE - PROFICIENCY TESTING (PT) AQUA ON FOOD MICROBIOLOGY: 20 YEARS OF EXPERIENCE

A. Bevilacqua[1], R. Trevisan[1], F. Loriggiola[1], M. Favretti[1], L. Barco[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

Introduction

The PT AQUA is aimed at determining the performance of the participating laboratories and identifying any problems in the implementation of the procedures by laboratories. In 1999 the Food Microbiology laboratory of IZSVe organized the first PT by sending test samples to the 10 peripheral laboratories of the IZSVe. In 2003 the PT was created for the quality assurance of results in the Food Microbiology

field and was destined to the10 national laboratories involved in this analytical field. In 2012 IZSVe AQUA MA (received the Accreditation by the Italian Accreditation Body (ACCREDIA).

Materials and Methods

The planned determinations aim to identify those microorganisms most implicated in food safety and covered by current legislation. The materials supplied to the registered laboratories consists of: i) food matrices, ii) the inoculum of the target microorganisms to be used for contamination and iii) documentation for operational management. Both food matrices and inoculum are freeze-dried, thus ensuring greater stability and ease of transport (Figure 1). Annually, the participants can view the test calendar on the IZSVe website (Figure 2).

Results

Currently, there are approximately 250 customers registered in AQUA MA with an average of 40 laboratories for each PT; annually 7 PTs are provided. Furthermore, unlike the first samples consisting only of freeze-dried microorganism, today test sample more closely simulates a real sample as it is also composed of the food matrix.

Discussion and Conclusion

The customer is provided with a Report containing the assigned values and the statistical processing. For quantitative tests the z-score is provided, while for qualitative tests the agreement/discordance is assessed. For non-compliant results, the Organizer provides the laboratories with comments and/or suggestions in the conclusions of the report and the customer can request a repetition of the test free of charge. PT AQUA MA is the first experience at a national level in which a public laboratory involved in food safety analysis has developed a PT scheme to meet all the main quality requirements of laboratories involved in this sector of activity. After more than 20 years of activity it is a consolidated and successful reality.

References

ISO IEC 17043:2023 Conformity assessment - General requirements for the competence of proficiency testing providers.

Figure 1. Food matrices and inoculum.

Sample cod	Date	Matrix	Analytes
AQUA MA 1	January	Meat, cheese	Enumeration of coagulase positive staphylococci, detection of staphylococcal enterotoxin
AQUA MA 2	March	Meat, molluscs	Enumeration of Bacillus cereus and Escherichia coli, detection of Salmonella spp
AQUA MA 3	May	Meat, milk	Detection and enumeration of Campylobacter spp
AQUA MA 4	June	Meat	Enumeration of Escherichia coli, enterobacteriaceae, total aerobic mesophilic count
AQUA MA 5	September	Meat, milk	Enumeration of Listeria monocytogenes, detection of Escherichia coli O157
AQUA MA 6	October	Meat, milk, vegetables	Detection of Listeria monocytogenes and Yersinia enterocolitica
AQUA MA 7	November	Meat, milk	Enumeration of Clostridium species and Clostridium perfringens, detection of Salmonella spp

Figure 2. Calendar.

P230

Proficiency Testing – participant and organizer perspective

HOW A PHARMACEUTICAL INDUSTRY ENSURES RELIABLE TESTING FOR POST-VACCINATION MONITORING: CEVA ANIMAL HEALTH INITIATIVES

E. Dupas[1], G. Dauphin[1]

[1]*Ceva Santé Animale, France*

Introduction
Ceva offers to its customers a poultry and swine post-vaccination monitoring service of high quality. The launch of each Ceva key vaccine is supported by a panel of diagnostic tools used to validate vaccine application and efficiency.

Materials and Methods
Following vaccination and through the animal production cycle Ceva Veterinary Services teams perform frequent and multiple animal and environmental sampling. Serological and molecular results are recorded into a Ceva database along with other vaccination and animal production parameters such as vaccination program, type and age of birds, production type, etc. This large dataset of more than 100,000 samples per year can then be compiled, analyzed, traced, and used for interactions with customers. Given the power for analysis of the data platform, it is a must for Ceva to rely on high quality data, most of which are laboratory results. These samples for vaccine monitoring are either tested by Ceva's internal laboratories (SSIU – Scientific Support and Investigation Units) or by selected external local laboratories.

Results
The 14 laboratories running PCR vaccine take testing for Ceva have been selected based on their strong competences in diagnostic, and/or molecular biology. They are trained and certified to each Ceva vaccine's new vaccine take test. Depending on Ceva Veterinary Services teams requirements, laboratories have been certified for up to 11 assays. Ceva requests them to participate to annual proficiency tests to maintain their certification. Besides, over 100 laboratories around the world run poultry serology at Ceva's request. Ceva has invited the main laboratories to participate to a quality recognition program that includes a common positive serum control against five pathogens and a proficiency testing panel

Discussion and Conclusion
These two laboratory programs have proven their added value in several ways, including harmonization, standardization, comparison of diagnostic kits, etc.

P231

Zoonoses / vector borne diseases

CIRCULATION AND PREVALENCE OF ENTERIC VIRUSES IN COASTAL WATERS OF THE CAMPANIA REGION

F. Serra[2], M. Levante[2], L. Marati[2], A. Pucciarelli[2], I. Di Bartolo 1, M. Monini[1], G. Ianiro 1, D. Iaccarino[2], E. Esposito[2], E. De Carlo[2], G. Fusco[2], M.G. Amoroso[2]

[1]*Istituto Superiore di Sanità Department of Food Safety, Nutrition and Veterinary Public Health, Rome, Italy*
[2]*Zooprofilactic Institute of Southern Italy- Department of Animal Health, Naples, Italy*

Introduction
Enteric viruses are among the leading causative agents of acute gastroenteritis (AGE). Most of them can survive for long periods in marine environments polluted by human sewage and can be spread over long distances (1). In this study, 485 seawater samples were analyzed by molecular methods to investigate the presence of 11 different enteric viruses: 5 well known pathogens (KP) (rotavirus, adenovirus, hepatitis A virus, norovirus GI and GII) and 6 emerging pathogens (EP) (astrovirus, enterovirus, sapovirus, parechovirus, aichivirus, salivirus). Samples were collected from 2020 to 2022 along the Campania Region coast

Materials and Methods
Seawater samples underwent a filtration pre-treatment with the aim to concentrate viruses (from 10L to 50mL) in the environmental water. Nucleic acids extraction was carried out using QIAsymphony automated extraction system with the DSP Virus/Pathogen Midi kit (Qiagen). Viruses of interest were investigated by Real-Time PCR using virus-specific protocols already published (2) or following the UNI EN ISO 15216-2:2019 for NoV.

Results
Results showed that 210 out of 485 samples (43.3%) were positive to least at one virus. Among these positive samples 85/210 (40.5%) were contaminated by two o more viruses. In detail, salivirus was the most prevalent virus (95/485, 19.6%) (Figure 1). Among the KP, the

most prevalent virus was adenovirus (12.4%) while among the EP beyond salivirus, an accountable prevalence was recorded also for aichivirus (13.4%). Regarding seasonality, positive samples were mainly found during the cold season (autumn-winter) (120/226, 53.0%) with respect to hot season (spring-summer) 90/259, 35%.

Discussion and Conclusion

Results obtained showed a significant circulation of enteric viruses in the sea-water samples. All searched viruses were found making exception for hepatitis A virus. Very interestingly, the most prevalent virus was salivirus, an emerging enteric pathogen not previously investigated in environmental samples in Campania Region, but recently found at low prevalence (1.3%) in faeces of children hospitalized for AGE (2). Monitoring seawater viral circulation could represent a good tool to evaluate viral circulation also among people living in the same area.

The Research was financed by the Italian Ministry of Health (Project: IZSME 07/19).

References

1. Fong TT et al. Microbiol Mol Biol Rev. Vol.69. 2005
2. Amoroso MG et al. Journal of Medical Virology. Vol.96. 2024

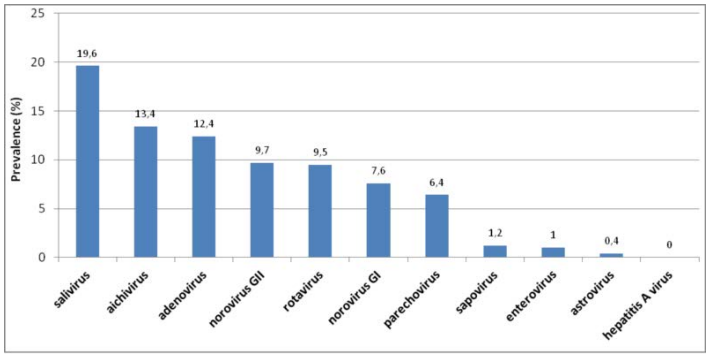


Figure 1. Prevalence of the viruses investigated

P232

Zoonoses / vector borne diseases

GENETIC AND ANTIMICROBIAL RESISTANCE CHARACTERIZATION OF LACTOCOCCOSIS ISOLATES FROM MEDITERRANEAN FISH OUTBREAKS

J.L. Arnal Bernal[1], C. Sanz Tejero[1], E. Planas Callao[2], S. Del Caso Yagüe[1], G. Chacón Pérez[1], D. González Martín[1]

[1]*Exopol, Spain*
[2]*Veterinaria Biomar Iberia, Spain*

Introduction

Lactococcosis is an emerging global disease with significant zoonotic potential, particularly associated with the consumption of raw or undercooked fish. In Europe, the disease has traditionally impacted rainbow trout farming. However, sea bass Mediterranean farms have also been recently affected. The disease is primarily caused by two species: *L. garvieae* and *L. petauri*. Both species can exhibit significant antibiotic resistance. This study aimed to develop a comprehensive diagnostic including species identification, assessment of AMR, and genetic characterization.

Materials and Methods

A collection of 18 clinical isolates of *Lactococcus* spp. was obtained from fish farms placed in Mediterranean countries from 2021 to 2024 (Table 1). All the isolates were analyzed by qPCR for specific identification, confirmed through ITS sequencing[1] and characterized by MLST[2]. AMR studies included MIC testing for drugs allowed in fish farming detailed in Table 3.

Results

All the isolates were successfully identified (Table 2). All the continental isolates resulted *L. petauri* ST14 (CC14). In contrast, all the marine isolates were identified as *L. garvieae*. Specifically, the gilthead bream and sea bass isolates resulted ST95, while the tuna strains were newly described as ST139 (CC17). *L. garvieae* demonstrated a broader range of drugs within sensitivity CMI90 compared to *L. petauri*. Significant oxytetracycline behaviour differences were observed between the two species. Most isolates exhibited substantial resistance to flumequine and trimethoprim-sulfamethoxazole; however, they showed broad sensitivity to florfenicol.

Discussion and Conclusion

L. petauri isolates (ST14) showed homology with those from previous outbreaks reported in Europe and the US. *L. garvieae* ST95 had been found Italy3 and Japan. The clonal complex CC17 detected in tuna has been previously documented in cases of human infection in China and Spain, highlighting the zoonotic potential of this pathogen. The qPCR methodology enables laboratories to quickly diagnose infections directly from animal tissues. Furthermore, subsequent genetic and AMR studies are needed to implement accurate treatments and effective control measures.

References

1.- https://doi.org/10.3390/microorganisms11051320
2.- https://doi.org/10.1371/journal.pone.0084796

Table 1: Isolates under study sorted by host fish and origin.

Host	Origin	*n*
Sea bass (*Dicentrarchus labrax*)	marine	7
Gilthead Bream (*Sparus aurata*)	marine	1
Atlantic bluefin tuna (*Thunnus thynnus*)	marine	2
Rainbow trout (*Oncorhynchus mykiss*)	continental	4
Unknown	unknown	4

Results for two methods (r, R, %recovery)

Table 2: MLST results

Lactococcus sp.	origin	Host	ST	CC	*n*
L. petauri	continental	Oncorhynchus mykiss	14	14	4
		unknown	14	14	3
L. garvieae	marine	Dicentrarchus labrax	95	95	7
		Thunnus thynnus	139	17	2
		Sparus aurata	95	95	1
		unknown	95	95	1

Table 3: MIC results

	Percentage of sensitive isolates	
	L. garvieae (n = 12)	*L. petauri (n = 7)*
Amoxicillin	100%	100%
Oxytetracycline	92%	14%
Trimethoprim-sulfamethoxazole	17%	14%
Florfenicol	92%	86%
Flumequine	0%	0%

P233

Zoonoses / vector borne diseases

FIRST REPORT OF *LEISHMANIA INFANTUM* IN CAPTIVE NON-HUMAN PRIMATES IN ITALY

G. Barlozzari[3], V. Galietta[2], L. Salvato[3], F. Romiti[4], C. De Liberato[4], G. Marafini[3], V. Russini[5], K.G. Friedrich[1], P. Di Cerbo[1], M. Scarpulla[3], C. Eleni[2]

[1]*Fondazione Bioparco, Rome, Italy*
[2]*Laboratorio Anatomia Patologica ed Istopatologia, UOC Diagnostica Generale, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Rome, Italy*
[3]*Laboratorio Malattie Infettive, UOSD Sierologia, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Rome, Italy*
[4]*Laboratorio Parassitologia ed Entomologia, UOC Diagnostica Generale, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Rome, Italy*
[5]*UOC Microbiologia degli Alimenti, Istituto Zooprofilattico Sperimentale del Lazio e della Toscana M. Aleandri, Rome, Italy*

Introduction
The leishmaniases are a group of diseases transmitted by phlebotomine sand flies. *Leishmania* infects many mammalian hosts, including non-human primates (NHPs). Natural infections in NHPs are rare, especially in Old World NHPs (OWNHPs), which generally develop milder forms and often recover spontaneously, unlike New World NHPs (NWNHPs) which experience potentially lethal forms (1).

Materials and Methods
In the period 2021-2023, a total of 28 NHPs (23 OW, 1 NW, 4 prosimians) residing at Bioparco Zoological Garden of Rome, were tested for the presence of leishmania DNA through ITS1 nPCR on blood and spleen samples (2). Positive samples were subjected to Restriction Fragment Length Polymorphism (RFLP) and sequencing. Phlebotomine sand fly catches were carried out in the zoo every 15 days using CDC traps.

Results
Five on a total of 28 NHPs (17.9%) and 5/23 OWNHPs (21.7%) were positive for *L. infantum* DNA on blood. The positive samples were confirmed through RFLP and sequencing. Positive animals were 4 Japanese macaques (*Macaca fuscata*) and 1 sooty mangabey (*Cercocebus atys lunulatus*). A total of 92 phlebotomine sand flies, all belonging to Phlebotomus perniciosus species, were collected in the zoo.

Discussion and Conclusion
Several NHP species were screened but *L. infantum* DNA was detected only in 5 OWNHPs with a prevalence of 21.7%. Positive NHPs did not show clinical signs in line with previous reports. Natural infections in OWNHPs are rare, with few studies assessing prevalence. Surprisingly, we found a prevalence of 21.7% by PCR. To our knowledge, this is the first case of natural infection by *L. infantum* in NHPs in Italy, where the disease is endemic and the first report in Japanese macaques and sooty mangabey worldwide. Further analyses will be needed to clarify if the positivity detected is consistent with a recent or transient infection. The identification of competent vectors in the zoo suggests the need to implement proper surveillance and preventive measures.

References
1. Santos RL, de Oliveira AR. Leishmaniasis in non-human primates: Clinical and pathological manifestations and potential as reservoirs. J Med Primatol. 2020;49(1):34-39.
2. Schönian G et al. PCR diagnosis and characterization of Leishmania in local and imported clinical samples. Diagn Microbiol Infect Dis. 2003 Sep;47(1):349-58.

P234

Zoonoses / vector borne diseases

INVESTIGATION ON Q FEVER AGENT IN DONKEY MILK

L. Bellinati[3], L. Ceglie[3], T.O. Kevenk[1], L. Lucchese[3], A. Natale[3], F. Giacometti[2], L. Alberghini[2]

[1]*Aksaray University, Faculty of Veterinary Medicine Department of Food Hygiene and Technology, Aksaray, Türkiye*
[2]*Department of Animal Medicine, Productions and Health, University of Padova, Legnaro, Italy*
[3]*Istituto Zooprofilattico delle Venezie, Padova, Italy*

Introduction
Donkey milk closely resembles human milk in composition and could potentially serve as a substitute in patients with allergies and intolerance to cow milk proteins, particularly in the neonatal period. Consequently, interest in it is rising, despite limited research on its micro-

biological risk. We aimed to demonstrate the applicability of analytical methods to donkey milk to investigate the presence of *Coxiella burnetii* (Cb), the zoonotic agent responsible for Q fever.

Materials and Methods

Preliminary testing on two bulk tank samples of raw donkey milk were performed using a commercial real-time PCR (qPCR) kit to detect Cb's DNA and an i-ELISA to detect anti-Cb antibodies, both validated for ruminant milk (1). To test qPCR applicability to donkey milk, a Cb plasmid was added to four serial dilutions (from 1:3 to 1:3000) of one milk sample, and pre- and post-contaminated samples were tested (Table 1). The i-ELISA kit contains a multispecies conjugate already validated for equids.
As a preliminary screening, we applied these methods to 90 individual milk samples collected in 13 donkey farms (Table 2).

Results

Of the 92 samples collected, no Cb DNA or anti-Cb antibodies were detected (Table 2). Table 1 summarizes the results obtained in pre- and post-contamination milk dilutions, showing no interference of donkey milk on qPCR analysis.

Discussion and Conclusion

Cb in donkey milk is rarely tested due to lack of regulatory requirements and low oral infection risk. Few studies are present on the risk of Cb in donkey milk and in Italy only one previous study reported a 10.5% prevalence by qPCR in milk samples collected from 3 Italian donkey herds (2). In our study, the methods used showed to be suitable for the investigation of Cb in donkey milk and detected no positivity in commercial milk samples. The low prevalence recorded in Italy is encouraging, but strategic surveillance and risk assessment for operators and consumers are crucial considering the low infectious dose of Cb and its zoonotic potential.

References

1. WOAH (2018) Manual for Terrestrial Animals Cap 3.1.17 par B.1.3 – Q fever
2. Auriemma C., et al. (2012) Preliminary survey on the presence of Coxiella burnetii in milk samples from donkeys raised in Campania. XIV Congresso Nazionale SIDiLV

Sample dilution	qPCR results sample N	qPCR results sample O	Plasmid dilution	qPCR results on sample N post-contamination
1:3	Negative	Negative	-	-
1:30	Negative	Negative	$5*10^4$ EG/mL	Positive Ct 30.17
1:300	Negative	Negative	$5*10^3$ EG/mL	Positive Ct 32.53
1:3000	Negative	Negative	$5*10^2$ EG/mL	Positive Ct 36.01

Table 1. Two bulk-tank raw milk (farm N and O) were tested in 4 serial dilutions in an inactivating molecular transport media. The diluted samples of farm N were also tested after contamination with a Cb plasmid quantified in 5*106 Equivalent genome/mL (EG/mL). This plasmid was used at the serial dilution 5*104 (expected Ct 29.32), 5*103 (expected Ct 33.22) and 5*102 (expected Ct 35.08).

Farm	Region	Herd breed	Feeding	Aim	Sample type	Milking practice	i-ELISA positives/total	qPCR positives/total
A	Veneto	Crossbreed	Grazing/Hay	Foal	I	H	0/2	0/2
B	Veneto	Crossbreed	Grazing/Hay	Foal / Pet therapy	I	H	0/2	0/2
C	Veneto	Martina Franca	Grazing/Hay	Foal	I	H	0/1	0/1
D	Veneto	Crossbreed	Grazing/Hay	Foal	I	H	0/1	0/1
E	Veneto	Crossbreed	Grazing/Hay	Foal	I	H	0/1	0/1
F	Friuli-Venezia Giulia	Crossbreed	Grazing/Hay	Foal	I	H	0/5	0/5
G	Basilicata	Crossbreed	Grazing/Hay PS	Commercial	I	A	0/12	0/12
H	Lazio	Crossbreed	Grazing/Hay PS	Commercial	I	PM	0/14	0/14
I	Lazio	Crossbreed	Grazing/Hay PS	Commercial	I	A	0/29	0/29
L	Abruzzo	Crossbreed	Grazing/Hay	Commercial	I	A	0/19	0/19
M	Emilia-Romagna	Ragusana	Grazing/Hay	Foal / Pet therapy	I	H	0/4	0/4
N	Veneto	Crossbreed	Grazing/Hay	Commercial	BTM	A	0/1	0/1
O	Emilia-Romagna	Crossbreed	Grazing/Hay	Commercial	BTM	A	0/1	0/1
Total							0/92	0/92

Legend: PS: protein supplement; I: milk collected from an individual jenny; BTM: bulk tank milk; H: by hand; A: automatic in a milking room; PM: portable automatic milker.

Table 2. Serological and qPCR results of sampled farms. For each farm, the table 2 lists their location in Italy, breed, feeding type and milk usage (foal nutrition or commercial use in the food, pharmaceutical, medical, or dermocosmetic sectors) and if the farm kept animals for pet therapy. It also details the sample type and the milking method.

P235

Zoonoses / vector borne diseases

WEST NILE VIRUS SURVEILLANCE IN THE CAMPANIA REGION, YEAR 2023

M. Buonanno[1], S. Smeraldo[1], F. Gargano[1], M. Ottaiano[1], R. Brunetti[1], L. Baldi[1], E. De Carlo[1]

[1]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy*

Introduction

The abstract describes the surveillance measures and epidemiology of the West Nile Virus (WNV) and Usutu Virus (USUV) in the Campania region, Italy, for 2023, as part of the National Plan for Prevention, Surveillance, and Response to Arboviruses 2020-2025. This plan integrates national surveillance measures for autochthonous and imported arboviruses, promoting a multidisciplinary approach in managing surveillance and control activities.

Materials and Methods

Passive surveillance (PS) was conducted on wild birds found dead throughout the year. Active surveillance (AS) targeted culled wild birds of specific species during the high-risk season. In Campania, sera from rural free-range poultry less than six months old were examined. Entomological surveillance (ES) involved using traps in WNV-risk areas, while clinical surveillance (CS) focused on equids. For each sampled bird, the central nervous system, heart, spleen, and kidney were analyzed using real-time RT-PCR to detect WNV Lineage 1 and 2 and USUV. Sera were tested with ELISA for WNV antibodies, and all positive samples were sent to the WOAH National Reference Centre for confirmation.

Results

From January to December 2023, 623 samples from wild birds (PS) were analyzed by real-time RT-PCR, and 202 sera from rural free-range poultry (AS) were tested. Four positive cases were confirmed in September-October: one Lineage 1 in a stork, two Lineage 2 in a peregrine falcon and a buzzard, and one in a horse with an untraced lineage. None tested positive for USUV. Viral circulation was unconfirmed at all trap placement sites. Figure 1 shows a map of the Campania region with confirmed WNV cases and active traps for entomological surveillance in 2023.

Discussion and Conclusion

A significant epidemiological finding in recent years is the spread of Lineage 2 across Europe and the Mediterranean Basin, including areas like Campania where the circulation of both lineages coexists. The emergence and spread of WNV in the Campania region highlight the need for ongoing vigilance and proactive public health strategies. Strengthening surveillance, enhancing vector control, and promoting public awareness are essential to mitigate the impact of this disease.

References

Piano Nazionale di prevenzione, sorveglianza e risposta alle Arbovirosi (PNA) 2020-2025.

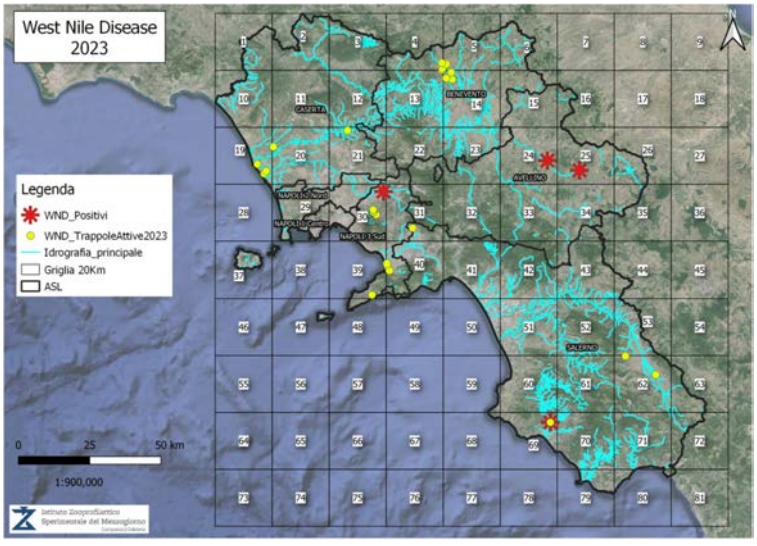


Figure 1. The map of the Campania region with confirmed WNV cases and active traps for entomological surveillance in the year 2023

P236

Zoonoses / vector borne diseases

ENTOMOLOGICAL SURVEILLANCE FOR WEST NILE AND USUTU VIRUS IN APULIA REGION (SOUTHERN ITALY): FIRST EVIDENCE OF WN VIRUS IN MOSQUITOES (2023 YEAR)

M.A. Cafiero[1], D.A. Raele[1], L. Marino[1], N. Cavaliere[1], D. Pugliese[1], M. Beverelli[1], M.G. Cariglia[1], F. Speranzoso[1], S.P. Grimaldi[1], M.C.F. Nardella La Porta[1], O. Mongelli[2]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*
[2]*Regione Puglia, Sezione Promozione della Salute e del Benessere, Servizio di Sicurezza Alimentare e Sanità Veterinaria, Italy*

Introduction

The National Plane of Prevention, Surveillance and Response to Arboviruses (PNA, 2020-2025) aims to prevent risk of pathogen transmission by arthropods, including the mosquitoes. Since several years, mosquitoes monitoring is conducted in the Apulia region (Southern Italy) as part of the integrated Surveillance Plan for Usutu and West Nile (WN) infection, and funded by Regional Grants. We report the results obtained in 2023 year.

Materials and Methods

In April-November 2023, CDC-light traps were positioned by Veterinary Services in selected sites (animal farms) in each Apulian province. The collected mosquitoes were morphologically identified1 and pooled according to species, sex, date. Females were molecularly tested for WNV and USUV RNA by real-time RT-PCR in use.

Results

A total of 5971 (5589 F, 382 M) adult mosquitoes from five genera and 12 species (*Ae. albopictus* (1.92%), *Ae. vexans* (0.24%), *Aedes* (Ae) spp (0.30%), *Anopheles* (An) *algeriensis* (3.72%), *An. maculipennis* s.l. (0.21%) *An.* spp (0.36%), *Coquillettidia richardii* (1.80%), *Culex* (Cx) *laticinctus* (0.15%), *Cx. pipiens* s.l. (24.27%), *Cx.* spp (2.70%), *Cx. theileri* (0.78%), *Culiseta (Cul) annulata* (3%), *Cul. longiareolata* (0.36%), *Cul*. spp (0.21%), *Ochlerotatus (Ochl) caspius* (38.46%), *Ochl. detritus* (21.15%), *Ochl.* spp(0.36%), were collected at the 36 selected sites (6 in FG/ 2 in BA/5 in BAT/5 in BR/ 10 in LE/8 in TA province) for a total of 773 caughts and 3333 pools. The prevalent species resulted both *Ochl caspius* (3202/5971, 53.60%) and *Cx. pipiens* (1212/5971, 20.30%). Out of all examined pools, one pool (size =2F) of *Cx.pipiens* specimens from BAT province resulted positive to WNV-lineage 2.

Discussion and Conclusion

Subsequently to the positivity of the collected mosquitoes (collection July, 21), a total of six human authocthonous cases of WN neuroinvasive disease and six WN-infection cases in equines were diagnosed during the summer season (Figure 1); all of them were attributed to WNV lineage 2. *Cx pipiens* s.l.,is the most important vector of WNV in Europe and Italy; the constant entomological monitoring results a fundamental activity to early detect the mosquito-borne virus and its strengthening and maintaining is strongly recommended.

References

1. Severini F. et al. Fragm Entomol. 2009; 41:213.

Figure 1. WNV lineage 2 infection cases in animals (mosquitoes, equines) and humans in Apulia region during 2023 year.

P237

Zoonoses / vector borne diseases

DETECTION OF TOXOPLASMA GONDII DNA IN RAW MILK OF WATER BUFFALOES (*BUBALUS BUBALIS*) FROM SOUTHERN ITALY

G. Cappelli[1], A. Mancusi[1], D. Vecchio[1], G. Di Vuolo[1], C. Grassi[1], A. Bosco[2], A. Martucciello[1], Y. Proroga[1], M. Serrapica[1], C.A. Denise[1], O. Di Maro[1], L. Rinaldi[2], E. De Carlo[1]

[1]*CReNBuf National Reference Centre for Hygiene and Technologies of Water Buffalo Farming and Productions, Istituto Zooprofiltattico Sperimentale del Mezzogiorno, Portici, Italy*
[2]*Unità di Parassitologia e Malattie Parassitarie, Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II, Napoli, Itlay*

Introduction
Toxoplasma gondii is the aetiological agent of one of the world's most important zoonoses and its distribution is cosmopolitan. Consumption of raw milk from infected animals is considered a risk factor for infection in humans. *T. gondii* DNA and anti-*T. gondii* antibodies have already been detected in the milk of some animal species. Current diagnosis of *T. gondii* is based on serological detection, cell culture and molecular methods. Several studies have demonstrated the higher accuracy, sensitivity and specificity of the PCR technique compared to traditional diagnostic methods. This study was conducted to detect the presence of *T. gondii* in raw milk samples from buffaloes bred in Campania.

Materials and Methods
The study was conducted from November 2023 to May 2024 on a total of 43 dairy buffaloes serologically positive for specific anti-*T. gondii* IgG from two farms, one in the province of Salerno and the other in the province of Caserta. 50 ml of individual milk and a bulk sample were taken during evening milking. 5 ml of milk samples were centrifuged for 1 hour at 10000g at 4 °C. After centrifugation, the fat layer and supernatant were carefully removed. Then total DNA was extracted, using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The ddPCR reaction was performed in a QX200 system (Bio-Rad, Hercules, CA, USA), using the protocol described by Mancusi et al. (2022) to amplify the 529 bp repeat element.

Results
In this study, 63% (27/43) 95% Confidence Interval [CI] = 47.8-75.69 of the milk samples and 35% (15/43) 95% Confidence Interval [CI] = 22.4-49.8 of the serum samples were positive for *T.gondii,* respectively. Of these, nine negative milk samples tested positive in the corresponding serum samples. The highest prevalence of *T. gondii* in the milk and serum samples studied was found in buffaloes aged (> 90 months).

Discussion and Conclusion
This is the first preliminary study to find a 63% seroprevalence of *T.gondii* in buffalo milk in Campania. The results cannot be used as representative for the entire study area, and the IgG antibodies of the samples do not indicate the viability of the strains, however the data found are valuable for public health and food safety. Further studies will be necessary to further investigate the results obtained.

References
Mancusi A. et al. (2022).

P238

Zoonoses / vector borne diseases

SKIN LESIONS CAUSED BY *MYCOBACTERIUM AFRICANUM* IN A DOMESTIC CAT

A. Caprioli[2], P. Alba[2], C. Cocumelli[2], C. Eleni[2], V. Galietta[2], L. Sorbara[2], F. Stravino[2], F. Feltrin[2], R. Amoruso[2], C. Buccella[2], F. Ceccaroni[3], M. Frega[1], A. Franco[2], B. Antonio[2]

[1]*Azienda Sanirataria Roma [1], Servizi Veterinari, Rome, Italy*
[2]*Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Rome, Italy*
[3]*Veterinary practitioner, Rome, Italy*

Introduction
Tuberculosis in humans is mainly caused by 2 highly related bacteria within the *Mycobacterium tuberculosis* complex (MTBC), *M. tuberculosis* and *M. africanum. M. tuberculosis* is widely spread, while M. africanum is generally restricted to Africa (1). To the authors' knowledge, infections due to *M. africanum* have never been described in animals.

Materials and Methods
In 2023 we examined a skin biopsy from a 3-year-old male domestic cat with multifocal nodular cutaneous lesions. The animal was an

indoor cat kept in Rome, reportedly taken as a stray kitten at a camping site in the south of Italy. The biopsy was examined by histology and Ziehl–Neelsen staining. Bacterial cultures using both non-selective media and media for the isolation of *Mycobacterium* spp. were performed (1). Suspected *Mycobacterium* spp. colonies were tested by a multiplex end-point PCR for identification at the genus level and to assess their belonging to MTBC (2). The obtained MTBC isolates were also investigated by whole-genome sequencing (WGS) analysis.

Results

Skin histopathological lesions were characterized by ulceration and multifocal to coalescing pyogranulomatous dermatitis, occasionally with a necrotic centre and variably distributes lymphocytic infiltrates (Figure 1). The Ziehl- Neelsen stain highlighted intramacrophagic acid fast bacilli. After 10 weeks post inoculation Mycobacterium spp. colonies were isolated from the specific media and tested PCR positive for MTBC, but negative for *M. bovis*, *M. caprae* and *M. tuberculosis*. WGS analysis indicated M. africanum lineage 6 as the aetiological agent.

Discussion and Conclusion

We report, for the first time, an infection due to *M. africanum* in an animal. The infection caused skin lesions resembling those of other cutaneous mycobacteriosis in cats. No further sample/information on clinical presentation, beside the skin nodules, was available for the laboratory. Regarding the origin of the infection, it might be speculated that the cat was exposed to infected humans/fomites coming from Africa. Follow up of the case and further analyses on the isolate are still ongoing.

References

1. World Organisation for Animal Health (WOAH) (2022). Chapter 3.1.13.
2. Kulski et al., 1995. Use of multiplex PCR to detect and identify *M. avium* end M. intracellulare in blood culture fluids of AIDS patients. J. Clin. Microbiol. 33, 668–674.

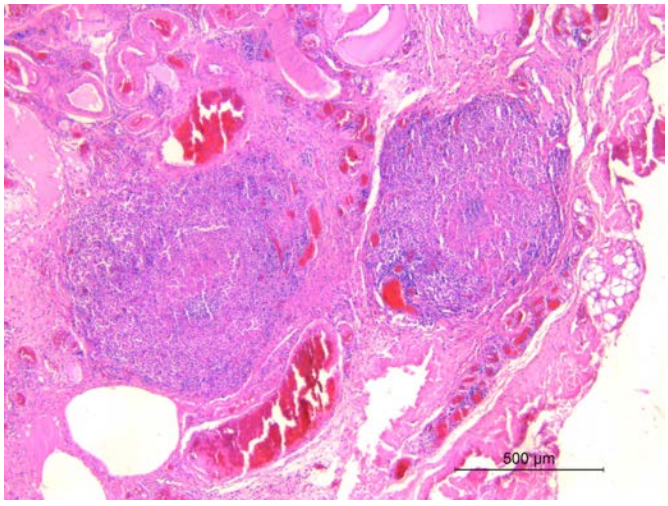


Figure 1. Cat, Skin. Multifocal pyogranulomatous dermatitis, severe, chronic. Hematoxylin-eosin stain.

P239

Zoonoses / vector borne diseases

MOLECULAR DETECTION OF *BABESIA/THEILERIA* SPP. IN TICKS COLLECTED FROM MIGRATORY BIRDS CAPTURED AT FAUNISTIC OBSERVATORY OF THE ASINARA ISLAND, SARDINIA, ITALY.

V. Chisu[1], P. Bianco[1], L. Giua[1], G. Masu[1], C. Foxi[1], G. Masala[1], I. Piredda[1]

[1]*Istituto Zooprofilattico Sperimantale della Sardegna, Italy*

Introduction

The role of migratory birds as hosts of vectors and human pathogenic microorganisms has been increasingly recognized. Birds can cross geographical barriers and contribute to the dispersal of bacteria, viruses, and protozoa. The aim of this study was to increase the knowledge on the potential role of migratory birds as disseminators of hemoprotozoan parasites of the genera *Babesia* and *Theileria* and to determine their prevalence in ticks infesting birds during migration flows.

Materials and Methods

Ticks were collected from birds captured at the Asinara Island in north-west of Sardinia. Ticks were morphologically classified to stage

of development and engorgement status and then identified to genus and species level by PCR and sequencing targeting the 16S rRNA gene. Specimens were tested for Babesia/Theileria infection by PCR standard and sequenced using primers targeting the18S rRNA gene.

Results

A total of 188 birds were tick-infested during this study. Ticks collected were 332 identified as *I. frontalis, I. inopinatus, I. ricinus,* and *I. ventalloi* species. A total of 14 ticks tested positive for Babesia/Theileria spp. after molecular analysis. Results of sequencing showed that two larvae of *I. ricinus* tested positive for *B. venatorum*, one *I. ricinus* nymph for *B. capreoli*, while 4 larvae of *I. ricinus* and one of *I. ventalloi* were positive for Theileria ovis. Moreover, three larvae of I. frontalis showed 100% of identity with *Th. equi* while *Th. orientalis* was detected in 2 larvae of *I. ricinus* and 2 of *I. frontalis* ticks.

Discussion and Conclusion

We report the identification of tick species belonging to the *Ixodes* genera as well as the presence of the emerging zoonotic species *B. venatorum* and the *B. capreoli* whose presence had not yet been reported in the island. The presence of *T. ovis, T. equi* and *T. orientalis* was also confirmed in this study. DNAs of these *Theileria* species was previously detected in ticks and domestic and wild mammals collected in Sardinia. These results highlight the importance of migratory birds in the spread of infected ticks in a geographic context.

References

Toma L, Mancuso E, d'Alessio SG, Menegon M, Spina F, Pascucci I, Monaco F, Goffredo M, Di Luca M. Tick species from Africa by migratory birds: a 3-year study in Italy. Exp Appl Acarol. 2021 Jan;83(1):147-164. doi: 10.1007/ s10493-020-00573-4.

P240

Zoonoses / vector borne diseases

FIRST REPORT OF *LISTERIA MONOCYTOGENES* IN A SEPTICAEMIC PET CHINCHILLA (*CHINCHILLA LANIGERA* MOLINA, 1782) IN ITALY.

L. Cirasella[1], E. Massella[2], S. Pongolini[3], C. Bracchi[3], S. Russo[2], G. Tosi[1], C. Siclari[1], L. Fiorentini[1], S. Perulli[1]

[1]*Istituto Zooprofilattico Sperimentale Della Lombardia e dell'Emilia Romagna (IZSLER), Forlì, Italy*
[2]*Istituto Zooprofilattico Sperimentale Della Lombardia e dell'Emilia Romagna (IZSLER), Piacenza, Italy*
[3]*Risk Analysis and Genomic Epidemiology Unit, Istituto Zooprofilattico Sperimentale Della Lombardia e dell'Emilia- Romagna (IZSLER), Parma, Italy*

Introduction

The long-tailed chinchilla (*Chinchilla lanigera*) (LTC) is a wild small-sized rodent of the family Chinchillidae, native to South America. Because of the increasing trend to keep exotic animals as pets, chinchillas have been progressively domesticated in the last decade. They are currently found worldwide as companion animals, becoming popular pets also in Italy. However, little is known about infectious diseases these exotic animals could harbour, representing a potential risk to their owners. Considering bacterial infection, one of the most common disease reported in chinchilla is listerioris, which is transferable to human. Here we present, to the best of our knowledge, the first case study of a pet LTC with a systemic infection caused by *Listeria monocytogenes* (Lm) in Italy.

Drug class	Antimicrobial	MIC breakpoint and interpretation		
		Susceptible	Intermediate	Resistant
β-Lactams	Amoxicillin + Oavulanic Acid	0.12		
	Ampicillin	<= 0.12		
	Oxacillin + 2% NaCl	2		
	Penicillin	0.12		
Lincosamides	Clindamycin			4
Tetracyclines	Doxycycline	1		
	Tetracycline	1		
Fluoroquinolones	Enrofloxacin	1		
Macrolides	Erythromycin	0.25		
	Tilmicosin		16	
Aminoglycosides	Gentamicin	<=2		
	Kanamycin	<=8		
Rifamycin	Rifampicin	<=0.06		
Sulphonamides	Sulfisoxazole	<=128		
Potentiated sulphonamides	Trimethoprim/Sulfamethoxazole	<=0.12		

Table 1. Phenotypic antimicrobial resistance profile of *Listeria monocytogenes* ST451.

	Genes
Antimicrobial resistance	*norB, FosX, mprF, lin*
Virulence	*inlJ, clpC, vip, inlF, pdgA, inlA, inlB, lntA, iap/cwhA, hpt, lplA1, aut, clpE, inlK, oatA, lap, lapB, fbpA, lspA, lpeA, bsh, clpP, gtcA, ami, prsA2, pfrA, plcA, hly, mpl, actA, plcB, incC*

Table 2. Antimicrobial resistance and virulence genes carried by *Listeria monocytogenes* ST451.

Materials and Methods
In February 2024, a LTC carcass was committed to the IZSLER Laboratory of Forlì. According to the owner's declaration, the only clinical symptom was ataxia, occurred shortly before his death. Necropsy was performed according to standard protocols. Samples of liver, spleen and lungs were processed for routine microbiological analysis. The antimicrobial profile of 15 molecules was determined with broth microdilution method. Minimal Inhibitory Concentration (MIC) results were interpreted according to CLSI. Whole genome sequencing (WGS) was implemented to identify Sequence type (ST), antimicrobial resistance (ARG) and virulence (VAG) genes.

Results
Necroscopy revealed plurivisceral congestion, multifocal miliary necrosis of liver, edema and hemorrhages of lungs. Lm was isolated from all the organs. MIC results are reported in Table1. Lm ST, ARGs and VAGs are described in Table 2.

Discussion and Conclusion
This case-report described Lm in a pet chinchilla as the likely cause of death. Lm was resistant to clindamycin, confirmed by the presence of lin gene. The genetic virulence profile was typical of Lm species, with genes (e.g. inl) implicated in cellular invasion and replication. Notably, Lm ST451 has already been reported in human patients in Europe (1). The present case report underlines the potential health risks associated with exotic pet ownership, in particular the possible exposure to zoonoses.

References
1. Cabal, A., et al. (2019). Isolate based surveillance of *Listeria monocytogenes* by whole genome sequencing in Austria. Front. Microbiol. 10:2282.

P241

Zoonoses / vector borne diseases

PRELIMINARY STUDY OF ANTI-LEISHMANIA ACTIVITY OF TWO NEW SEMI-SYNTHETIC CHALCONES

F. Facciponte[1], E. Anselmo[1], L. Siragusa[1], S. Giardina[1], E. Oliveri[1], F. Bruno[1], G. Castelli[1], F. Vitale[1]

[1]*WOAH Leishmania Reference Laboratory, Istituto Zooprofilattico Sperimentale della Sicilia, Centro di Referenza Nazionale per le Leishmaniosi (C.Re.Na.L.), Palermo, Italy*

Introduction
*Leishmania*sis is a parasitosis, transmitted by the bite of the phlebotomist with clinical manifestations such as cutaneous, muco-cutaneous and visceral leishmaniasis. Although there are some drugs commercially available but they show high costs, side effects, resistance. This study analyzes the anti-leishmania activity and the mechanism of cell death of two new semi-synthetic compounds, chalcones, synthesized from a botanic molecule, icaridin.

Materials and Methods
L. infantum (MCAN/IT/1265), *L. tropica (*MHOM/SU/74/K27), *L. major* (MHOM/SU/73/5ASKH) cultures were treated with serial concentrations of two chalcones, called 3033 and 3130. After 48h was evaluated cell viability by Burker chamber. The cytotoxic action was evaluated by MTT assay on U937 (Human Caucasian Histiocyte Lymphoma). All data were obtained from three indipentend experiments. To evaluate cell cycle distribution by propidium iodide procedure at cytofluorimeter, IC90 of each chalcone was analyzed after 48h in MCAN coltures.

Results
Figure 1(A) and 1(B) show decreased in *Leishmania* viability at chalcone doses from 5uM to 50uM compared with control (100% viability); instead MTT assay shows no cytotoxicity in U937(data not shown). According to cytofluorometer analysis, Pre-G1 and S phases present increased cell density (Fig 2). All data obtained are statistically significant.

Discussion and Conclusion

Chalcones analyzed cause a significant reduction in *Leishmania*s viability as the concentration increases. The chalcones analyzed cause a significant reduction in viability of all *Leishmania* species as concentration increases, by acting in the preG1 and S phases of the cell cycle. Future objectives of our study will evaluate the mode of parasite killing by annexin assay5, and evaluation of cytokines expressed by U937 infected and treated with the compounds.At the end of the clinical trial, it could be a safe and low cost alternative to traditional drugs.

References

Ortalli M, Ilari A, Colotti G, De Ionna I, Battista T, Bisi A, Gobbi S, Rampa A, Di Martino RMC, Gentilomi GA, Varani S, Belluti F. Identification of chalcone-based antileishmanial agents targeting trypanothione reductase. Eur J Med Chem. 2018 May 25;152:527-541. doi: 10.1016/j.ejmech.2018.04.057.

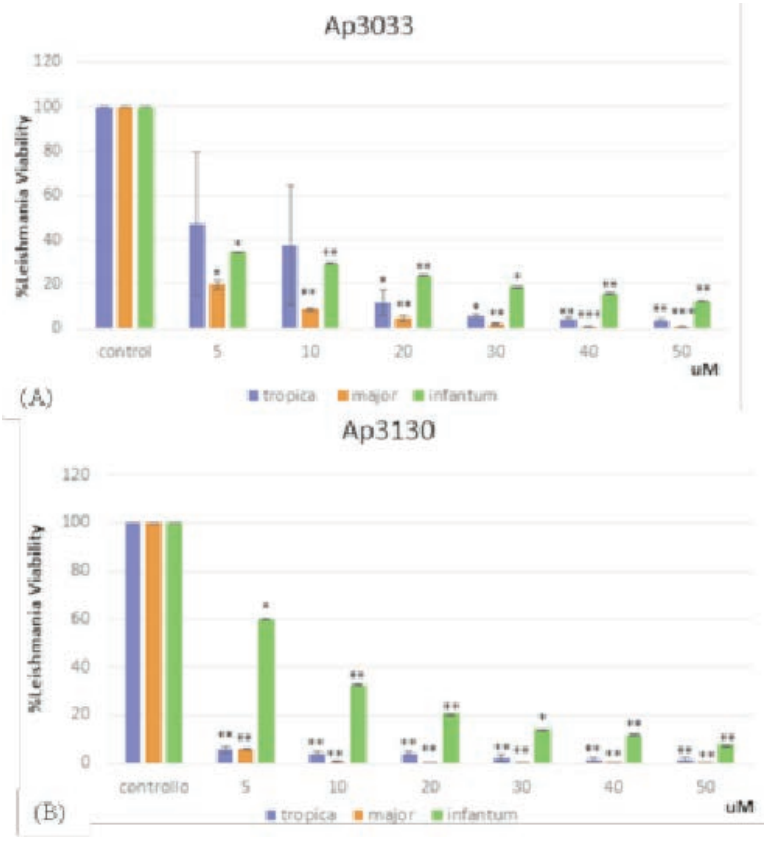
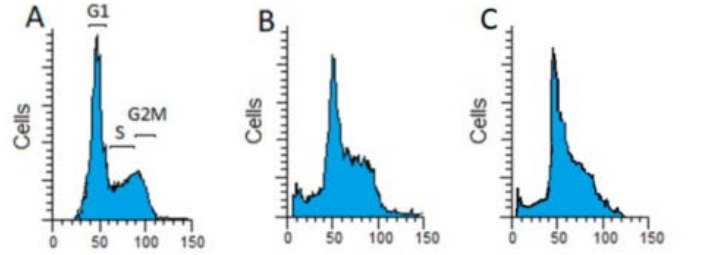


Figure 1. Promastigotes viability after 48h colture with serial concentrations of 3033 (A) and 3130 (B)



	Control (A)	3130 (B)	3033 (C)
Pre-G1	2%	11.1%	7.6
G1	60.1%	44.6%	52.3
S	29.2%	40.3%	34.8
G2M	8.7%	4%	5.3

Figure 2. Cell cycle distribution by propidium iodide procedure at cytofluorimeter, IC90 of 3130(B), 3033 (C) and control (A) after 48h in MCAN coltures.

P242

Zoonoses / vector borne diseases

DEVELOPMENT OF A MOLECULAR ASSAY FOR THE DETECTION OF *BORRELIA* SPP. IN ANIMAL SAMPLES

V. Facile[1], M. Magliocca[1], L. Gallina[1], L. Urbani[1], A. Balboni[1], M. Battilani[1]

[1]*Department of Veterinary Medical Science, University of Bologna, Ozzano dell'Emilia (BO), Italy*

Introduction
Borrelia is a genus of spirochete bacteria which includes two main groups: *Borrelia burgdorferi* sensu lato, a genospecies complex causing Lyme Disease (LD), and the Relapsing fever group which includes several species causing flu-like symptoms. *Borrelia* is one of several tick-borne pathogens that can affect both animal and human health [1]. Infections of dog have been reported worldwide but few molecular prevalence studies have been carried out. The diagnosis of this disease is mainly serological, while molecular assays, often less sensitive, allow to genetic characterise the identified bacteria. Our study aimed to develop a molecular assay for the detection of *Borrelia* spp. DNA in animal samples and to assess the molecular prevalence of these bacterial species in dogs.

Materials and Methods
A Taqman Real-Time PCR (qPCR), designed on 16S rRNA gene of *Borrelia* spp. and capable of identifying a broad spectrum of *Borrelia* species was developed, validated and used to test DNA extracted from blood samples of 70 dogs referred to the Veterinary Teaching Hospital of the University of Bologna.

Results
The qPCR developed showed a limit of detection of 10 copies/µL and correctly classified positive and negative controls. None (0/70) of the dogs tested positive.

Discussion and Conclusion
The assay developed showed excellent sensitivity and specificity for *Borrelia* spp. Considering the low molecular prevalences found in the few studies carried out, the absence of positive dogs in the analysed population was expected. Further studies will need to expand both the number of dogs and the animal species tested. The use of molecular diagnosis allows for epidemiological surveillance and assessment of the effectiveness of tick control and infection control measures, helping to reduce the risk of disease transmission to humans and animals.

Supported by EU funding within the NextGenerationEU-MUR PNRR Extended Partnership initiative on Emerging Infectious Diseases (Project no. PE00000007, INF-ACT).

References
1. Wolcott et al. Host association of *Borrelia* burgdorferi sensu lato: A review. Ticks Tick Borne Dis. 12: 101766, 2021.

P243

Zoonoses / vector borne diseases

PRELIMINARY ANALYSIS ON THE IMPACT OF CLIMATE CHANGE ON THE EMERGENCE OF VECTOR-BORNE DISEASES

F. Gargano[1], M. Ottaiano[1], R. Brunetti[1], M. Buonanno[1], C. De Martinis[1], G. Colarusso[1], A. Esposito[1], L. Baldi[1], E. De Carlo[1]

[1]*Istituto Zooprofilattico Sperimentale del Mezzogiorno, Italy*

Introduction
Vector-borne diseases represent a significant threat to global public health. The dynamic evolution of ecosystems together and climate change play an important role in the spread of these diseases, influencing their persistence in new areas; the impact of climate change indirectly causes changes in environmental ecology, also linked to the action of human activities. The aim of the research is to compare the prevalence of vector diseases with the trend of climate parameters over time.

Materials and Methods
The period considered is 2018 –2023. The data related to vector diseases were extracted from the Laboratory Management System (SIGLA) of the Istituto Zooprofilattico Sperimentale del Mezzogiorno while the environmental data from the archive of the Campania Region. The animal was considered positive if it presented at least one positivity (confirmed by the respective National Reference Center) to the diseases subjected to surveillance plans in the period considered. The data was processed through the software R version 4.1.0.

Results

The descriptive analysis (Figure 1 and Figure 2) would show that the prevalence of vector diseases follows the trend of environmental parameters: in 2021, a reduction in the number of days above the average temperature and humidity corresponds to a decrease in prevalence; in the following two years both increase.

Discussion and Conclusion

The analysis highlights the importance of conducting multidisciplinary studies, in order to estimate the risk of introduction and/or spread of vector-borne diseases. Surveillance of these diseases is important to understand their spread to adopt control measures and therefore implement prevention; to establish common actions that respond to the challenges and objectives in a One Health perspective, a shared strategic approach that integrates veterinary, human and environmental surveillance is essential.

References

G. Ferrara, E.Improda, F. Piscopo, R. Esposito, G. Iovane,U. Pagnini, S. Montagnaro. Bluetongue virus seroprevalence and risk factor analysis in cattle and water buffalo in southern Italy (Campania region) Veterinary Research Communications, 2023.

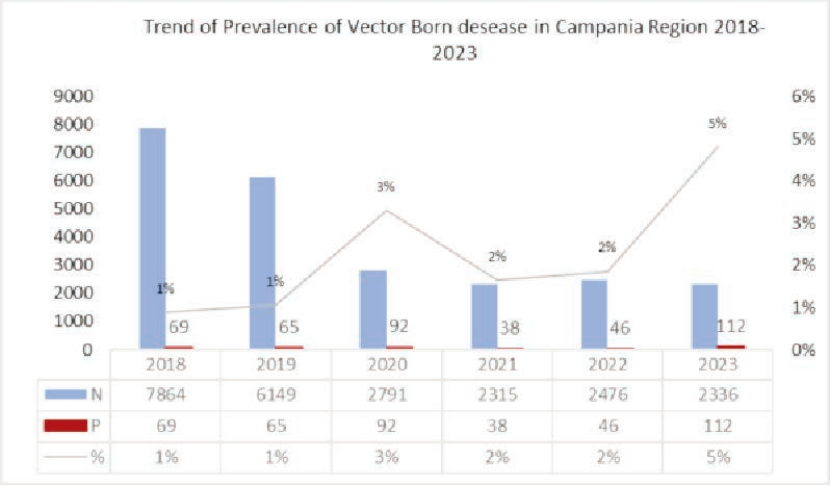


Figure 1. Trend of Prevalence of Vector Born disease in Campania Region 2018-2023.

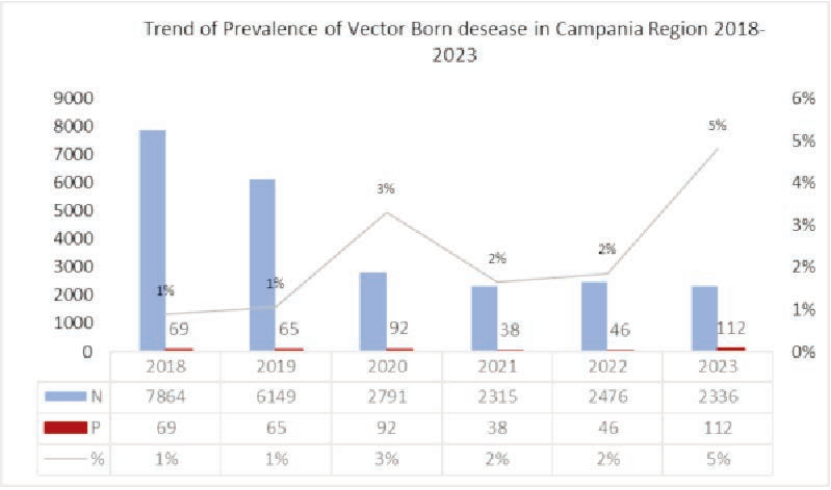


Figure 2. Distribution of the number of days with humidity and temperature above average in Campania Region 2018-2023.

P244

Zoonoses / vector borne diseases

INVOLVEMENT OF FORMYL PEPTIDE RECEPTOR 2 IN CANINE CORONAVIRUS INFECTION

R. Giugliano[4], L. Del Sorbo[4], V. Iovane[1], P. Cuomo[1], A. Gentile[1], M.M. Salvatore[2], C. Zannella[3], A. De Filippis[3], M. Galdiero[3], A. Pratelli[5], R. Capparelli[1], A. Andolfi[2], F. Fiorito[4]

[1]*Department of Agricultural Science, University of Naples Federico II, Naples, Italy*
[2]*Department of Chemical Science, University of Naples Federico II, Naples, Italy* [3]*Department of Experimental Medicine - University of Campania L.Vanvitelli, Naples, Italy*
[4]*Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy*
[5]*Department of Veterinary Medicine, University of Bari, Valenzano (Bari), Italy*

Introduction
In infection, a useful improvement mechanism is necessary to maintain appropriate cellular metabolic activity. Formyl peptide receptor 2 (FPR2) is a seven-protein transmembrane G-coupled receptor that plays an important role in modulating immune responses. Indeed, its expression is variably regulated not only by cell type but also by microbial infections. Interestingly, the expression of the anti-inflammatory FPR2 is reduced during coronaviruses (CoVs) infection, such as severe acute respiratory syndrome (SARS)-CoV-2. Canine coronavirus type II (CCoV-II) belongs to the alphacoronavirus genus and is responsible for mild enteritis, especially in puppies. But the remarkable plasticity of CCoV characterized by mutations and recombination, determines the emergence of more virulent variants, which can cause severe diseases in dogs but also in humans. Recently, it has been demonstrated that the replication and pathogenesis of human influenza A virus (IAV) also depend on FPR2 signaling.

Materials and Methods
The role of FPR2 during CCoV infection on A72, a canine fibrosarcoma cell line, by bioscreen, immunofluorescence staining and viral yield analyses.

Results
FPR2, expressed by A72 cells, was inhibited after CCoV infection. Moreover, in the presence of non-cytotoxic concentrations of WRW4, a specific FPR2 inhibitor, a further reduction in the level of FPR2 in infected cells was observed. These results were accompanied by alterations in cell morphology in the treated-infected groups, and in the cytopathic effect. Analysis of lysosomal environment showed an acidic environment, which was alkalinized by CCoV infection, but resulted markedly acidified by WRW4.

Discussion and Conclusion
In conclusion, in a cellular line expressing FPR2, according with other CoVs, our preliminary results indicate the involvement of this receptor during CCoV infection. However, based on the comparison of this CoV with IAV, a significant difference in the regulation of FPR2 was highlighted. Overall, these results showed that CCoV replication is related to FPR2, suggesting it as an interesting target to counteract CoVs infection.

P245

Zoonoses / vector borne diseases

BORRELIA MIYAMOTOI IN *IXODES RICINUS* TICKS RECOVERED FROM HOSTS IN LOMBARDY REGION, NORTHERN ITALY

E. Olivieri[3], S. Rigamonti[3], I. Karaman[4], N. Vicari[3], I. Bertoletti[4], A. Bianchi[4], C.M. Lodola[3], G.L. Polonini[3], M. Macrelli[1], A. Grassi[3], G. Maioli[2], P. Prati[3]

[1]*IZSLER sede Binago* [2]*IZSLER sede Bologna* [3]*IZSLER sede Pavia* [4]*IZSLER sede Sondrio, Italy*

Introduction
Borrelia miyamotoi is a relapsing fever spirochete recently identified as human pathogen in the Northern Hemisphere and transmitted by the tick Ixodes ricinus, the main vector of *B. burgdorferi*, the causative agent of Lyme disease. *B. miyamotoi* disease (BMD) includes influenza-like symptoms: high fever, fatigue, headache, myalgia and nausea (1). As BMD is an emerging public health concern and there is limited data about *B. miyamotoi* in Italy (2), the aim of this study is to investigate the presence of *B. miyamotoi* in *I. ricinus* collected from hosts in Northern Italy.

Materials and Methods
Between 2020-2023, *I. ricinus* ticks were collected from wildlife, domestic hosts and humans in Lombardy region, as a part of collaboration with the local health institutions in a one-health approach. The ticks were identified morphologically and tested for *Borrelia* spp. using Real-Time PCR. Positive samples were further screened for *B. miyamotoi* by targeting the glpQ gene with Real-Time PCR. For phylogenetic analysis, the glpQ gene was amplified and sequenced.

Results

A total of 3,887 *I. ricinus* ticks were collected from 2,019 hosts including chamois, deer, roe deer, wild boar, wolf, fox, badger, hedgehog, weasel, goat, dog and human. The majority were nymphs (53.5%), followed by adult females (33%), adult males (10.4%) and larvae (3.2%). Molecular screening identified 39 ticks positive for *B. miyamotoi* (15 adult females, 12 nymphs, 10 adult males and 2 larvae). Positive ticks were found on human (15), deer (8), roe deer (8), chamois (4) and a wolf (1). Sequence analyses of the glpQ gene confirmed 100% nucleotide identity with *B. miyamotoi* and the phylogenetic analyses placed the sequences within the European clade (95% bootstrap).

Discussion and Conclusion

Although the rate of *B. miyamotoi*-positive samples is low, the detection of this pathogen highlights a significant potential infection risk. *B. miyamotoi* should be considered in the differential diagnosis for patients with flu-like symptoms after a tick bite. Given that this region is endemic for Lyme disease, and ticks can transmit both *B. miyamotoi* and *B. burgdorferi* spirochetes simultaneously, this finding has important public health implications.

References

Krause P.J, et al. 2015 Ravagnan S, et al 2018

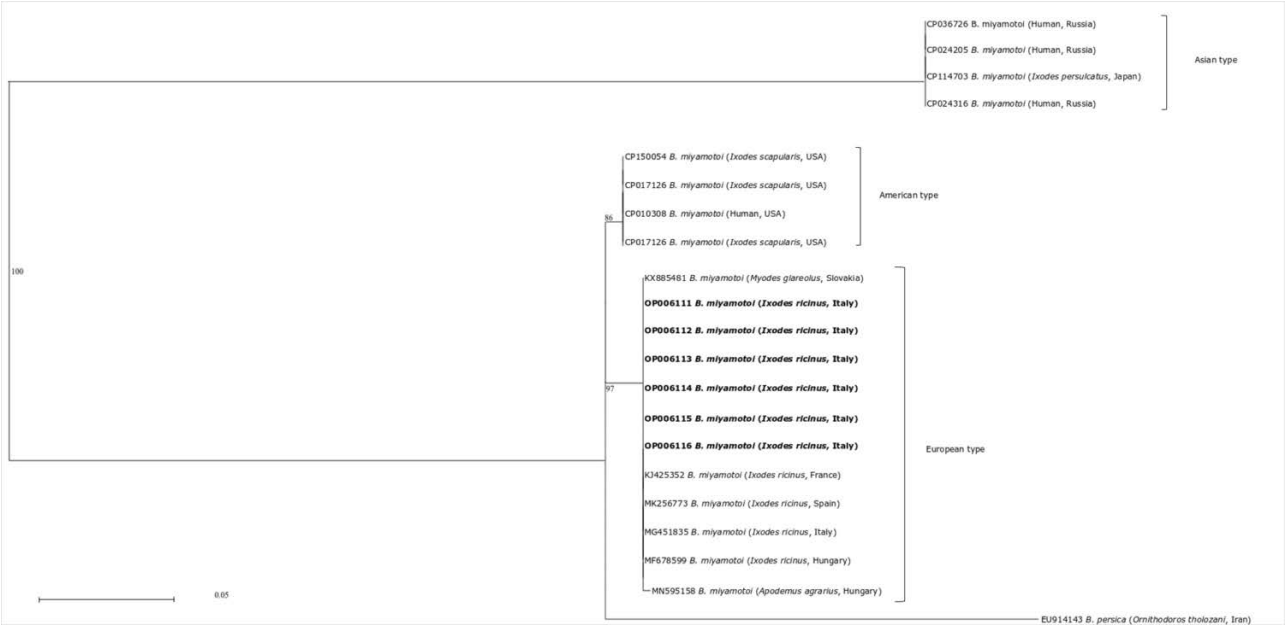


Figure 1. Phylogenetic analysis of B. miyamotoi isolates.

P246

Zoonoses / vector borne diseases

IDENTIFICATION OF A NEW SUBTYPE OF HEPATITIS E VIRUS IN WILD BOARS

G. La Bella[1], A.M. D'Antuono[2], M.G. Basanisi[1], G. Nobili[1], R. Coppola[1], A.M. Damato[1], G. Occhiochiuso[1], S. Faleo[1], P. Di Taranto[1], F. Pellegrini[3], G. Lanave[3], V. Martella[3], G. La Salandra[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata; PhD National Programme in One Health approaches to infectious diseases and life science research, Department of Public Health, Experimental and Forensic Medicine, University of Pavia, Italy*
[3]*Università degli Studi di Bari - Dipartimento di Medicina Veterinaria, Italy*

Introduction

Hepatitis E is an emerging zoonotic disease in industrialized countries where food-borne transmission plays a key role. Domestic pigs, wild boar, and deer represent the main reservoir of hepatitis E virus (HEV) for humans. The genetic variability and the presence of numerous animal hosts contribute to its spread and potential host range expansion. In this study, we investigated the prevalence and the genetic variability of HEV in wild boar samples in the Apulia region (south-east of Italy).

Materials and Methods

Two hundred and sixteen muscle samples of wild boar from the Apulia region were collected in 2022. Tissue samples were collected during post-mortem inspections from animals hunted for domestic consumption and analyzed to detect the presence of HEV by RT-qPCR assay. Genotyping was carried out by sequencing of HEV partial ORF1 and ORF2 regions, using nested RT-PCR protocols. The obtained sequences were analyzed using BLAST and the HEV Typing Tool (http:// www.rivm.nl/mpf/typingtool/hev/).

Results

Out of the 216 samples, 22 tested positive for HEV RNA (10.2%) by RT-qPCR. Seventeen of the 22 positive samples (77.3%) were successfully amplified by nested RT-PCR. On sequencing of the amplicons, all these samples belonged to genotype 3. In detail, 14 samples belonged to the subtype 3c while 3 sequences were characterized as genotype 3 but could not be assigned to any known subtype (Table 1). These sequences showed nt identity >94% with the closest HEV sequence available in GenBank (KP294371), unclassified by ICTV (https:///ictv.global/sg_wiki/hepeviridae/ orthohepevirus).

Discussion and Conclusion

This study confirms the circulation (10.2%) of HEV in samples in wild boar hunted for domestic consumption in Southeast Italy. Moreover, a possible new clade/subtype of HEV was identified in the animals surveyed in this study. Importantly, the emergence of new subtypes could have implications for human health, determining changes in the patterns of HEV infection and disease in humans. Gathering epidemiological and molecular data is important to depict a baseline of HEV circulation in animals and of HEV genetic diversity.

Sample	Hevnet Assignment		Identity in the NCBI Database					
			ORF1 (RdRp)			ORF1 (methyltransferase)		
	Genotype	Subtype	Accession nr	Reference Strain	nt Identity %	Accession nr	Reference Strain	nt Identity %
ITA/2024/wild boar/291-1	3	c	MG020042	Paslahepevirus balayani isolate DE/16-0145	94.3	MK355827	Hepatitis E virus isolate NL_Patient_191	98.5
ITA/2024/wild boar/291-2	3	c	MG020042	Paslahepevirus balayani isolate DE/16-0145	94.4	MK355815	Hepatitis E virus isolate NL_Patient_179	97.0
ITA/2024/wild boar/291-4	3	c	MH504135	HEV_29_Southampton_UK_021214	95.8	MK355815	Hepatitis E virus isolate NL_Patient_179	97.0
ITA/2024/wild boar/291-5	3	c	KX073466	Hepatitis E virus isolate ww9	94.6	MK355827	Hepatitis E virus isolate NL_Patient_191	98.5
ITA/2024/wild boar/291-7	3	c	MG020048	Paslahepevirus balayani isolate DE/16-0150	94.6	MK355815	Hepatitis E virus isolate NL_Patient_179	93.3
ITA/2024/wild boar/291-8	3	c	MG020048	Paslahepevirus balayani isolate DE/16-0150	95.7	MK355827	Hepatitis E virus isolate NL_Patient_191	97.8
ITA/2024/wild boar/291-9	3	c	MG020048	Paslahepevirus balayani isolate DE/16-0150	95.7	MK355827	Hepatitis E virus isolate NL_Patient_191	98.5
ITA/2024/wild boar/291-10	3	c	MG020048	Paslahepevirus balayani isolate DE/16-0150	95.7	MK355827	Hepatitis E virus isolate NL_Patient_191	95.6
ITA/2024/wild boar/291-11	3	c	MG020048	Paslahepevirus balayani isolate DE/16-0150	95.7	MK355827	Hepatitis E virus isolate NL_Patient_191	97.0
ITA/2024/wild boar/291-12	3	c	MG020048	Paslahepevirus balayani isolate DE/16-0150	96.4	MK355827	Hepatitis E virus isolate NL_Patient_191	98.5
ITA/2024/wild boar/291-13	3	c	MG020048	Paslahepevirus balayani isolate DE/16-0150	96.7	MK355827	Hepatitis E virus isolate NL_Patient_191	96.3
ITA/2024/wild boar/291-14	3	c	MG020048	Paslahepevirus balayani isolate DE/16-0150	96.1	MK355827	Hepatitis E virus isolate NL_Patient_191	97.8
ITA/2024/wild boar/291-15	3	c	MG020048	Paslahepevirus balayani isolate DE/16-0150	94.3	MK355827	Hepatitis E virus isolate NL_Patient_191	96.3
ITA/2024/wild boar/291-17	3	NA	JQ807506	Hepatitis E virus strain WS09-292	96.2	AF279122	Hepatitis E virus strain Au1	94.8
ITA/2024/wild boar/291-19	3	c	MG020048	Paslahepevirus balayani isolate DE/16-0150	95.7	MK355827	Hepatitis E virus isolate NL_Patient_191	97.8
ITA/2024/wild boar/291-20	3	NA	JQ807506	Hepatitis E virus strain WS09-292	95.8	AF279122	Hepatitis E virus strain Au1	94.0
ITA/2024/wild boar/291-21	3	NA	JQ807506	Hepatitis E virus strain WS09-292	94.1	AF279122	Hepatitis E virus strain Au1	96.3

RdRp: RNA-dependent RNA polymerase; NA: not assigned

Table 1. Results of data analysis on the sequences generated in this study. The source, year of collection and the best match using the Blast Nucleotide interrogation tool from the National Center for Biotechnology Information database (accessed on 25th June 2024) are shown. Classification at the genotype and subtype level is based on NCBI taxonomical and HEVnet typing tool assignments.

P247

Zoonoses / vector borne diseases

A NOVEL MULTIPLEX PCR APPROACH FOR *ANAPLASMA* SPP, *BORRELIA BURGDORFERI* AND *RICKETTSIA* SPP SCREENING IN TICKS COLLECTED FROM HUMANS

J. Lai[1], C. Tessarolo[1], V. Carta[1], A. Ferrari[1], M. Gallo[1], C. Palmitessa[1], M. Lo Faro[1], A. Favole[1], S. Peletto[1], M.G. Maniaci[1], T. Avanzato[1], C. Pasqualini[2], M. Caramelli[1], C. Casalone[1], C. Corona[1]

[1]*Istituto Zooprofilattico Sperimentale del Piemonte Liguria e Valle d'Aosta, Torino, Italy*
[2]*SeREMI ASL AL, Alessandria, Italy*

Introduction

The increasing prevalence of tick-borne diseases (TBDs) is a significant public health issue. To speed up pathogen detection, in order to aid clinicians in TBDs' diagnosis, there is a need for a method able to simultaneously detect different pathogens. We developed a SYBR Green-based multiplex PCR for a rapid detection of *Anaplasma* spp., *Rickettsia* spp. and *Borrelia burgdorferi*. A comparison of this novel method with three end-point PCR assays used in routine analysis was also performed.

Materials and Methods

Total DNA from 40 ticks, collected from humans in 2023, was extracted with the AllPrep DNA/RNA Mini Kit (Qiagen). Samples were tested by multiplex SYBR Green Real-Time PCR (iTaq Universal SYBR Green Supermix, Biorad) performed on a Biorad CFX96 thermal cycler (Table 1). All samples were also amplified with respective simplex end-point PCRs [1-3]. Amplification results (Real-Time and end-point PCRs) were visualized on 2.5% agarose gel through transilluminator (ChemiDoc, Biorad). Positive samples were Sanger sequenced and sequences were submitted to Blast analysis versus the NCBI Nucleotide database.

Reagents	Concentration	µl
H_2O		2,5
Mix Sybr Green 1X	1x	12.5
Primer Anaplasma Forward 10µM	10µM	2
Primer Anaplasma Reverse 10µM	10µM	2
Primer Borrelia b. Forward 10µM	10µM	2
Primer Borrelia b. Reverse 10µM	10µM	2
Primer Rickettsia Forward 10µM	10µM	2
Primer Rickettsia Reverse 10µM	10µM	2
Template	/	3
Total amount		30

Thermocycling profile

- 95°C for 5'
- 95°C for 15''
- 60°C for 30'' } 40 cycles
- 72°C for 30''
- 72°C PER 7 MINUTI
- CFX96 Biorad MELTING CURVE

Table 1. Termocycling conditions.

Pathogens	Positive sample number confirmed
Anaplasma spp	1
Borrelia burgdoferi	1
Rickettsia spp	17
Anaplasma spp and Borrelia burgdoferi coinfected samples	1

Table 2. Positive sample pathogens.

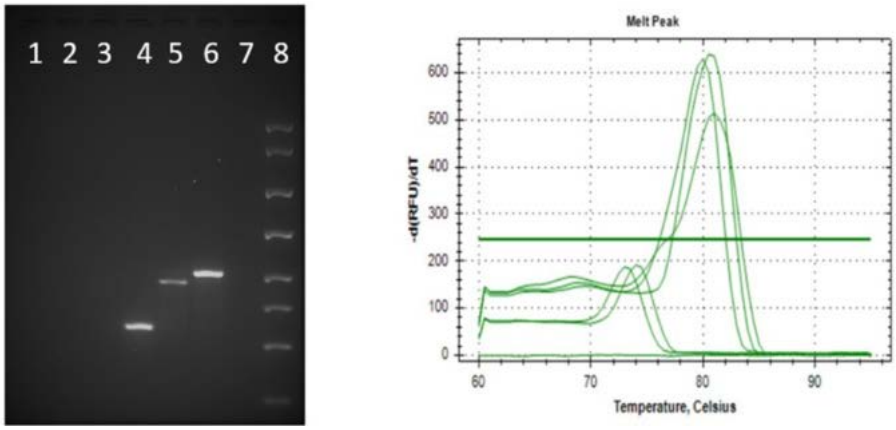


Figure 1. Positive and negative samples on agarose gel and positive sample melting curves

Results

The novel multiplex Real-Time PCR resulted positive for at least one pathogen for 20 out of 40 samples (Table 2). End- point PCRs showed concordant results. In positive samples, SYBR Green Real-Time PCR melting temperatures were between 79.5°C and 82°C. The amplification product lengths, visualized on agarose gel, were 334 bp for *Anaplasma* spp, 482 bp for *Borrelia* b., and 511 bp for *Rickettsia* spp. Sequencing and Blast analysis confirmed the specificity of pathogen amplification.

Discussion and Conclusion

The genera *Anaplasma*, *Borrelia* and *Rickettsia* include many pathogenic species that can infect humans and animals. Our multiplex SYBR Green Real-Time PCR method could be useful for a rapid screening between positive and negative samples. Followed by an electrophoret-

ic run on agarose gel, this method could also permit the identification of the specific pathogen according to the band's molecular weight. Further experiments are required to optimize the assay procedures in order to discriminate the pathogens relying on melting temperatures.
Supported by PNRR PE00000007 INF-ACT.

References
Skotarczak B. et al. Ann Agric Environ Med 2002, 9, 25–28
DOI: 10.1111/j.1749-6632.2003.tb07407.x
DOI: 10.1128/jb.173.5.1576-1589.1991

P248

Zoonoses / vector borne diseases

SWINE NOROVIRUS: FROM OVERLOOKED AGENT TO POSSIBLE ZOONOTIC CONCERN. MOLECULAR IDENTIFICATION AND CHARACTERIZATION IN SPANISH SWINE HERDS.

S. Lazaro[1], C. Martinez[1], J.L. Arnal[1], A. Benito[1]

[1]*EXOPOL SL.*

Introduction
Norovirus is the first non-bacterial cause of gastroenteritis in humans, but it can infect other animal species, including swine. Swine norovirus (SwNoV) has been found in both diseased and healthy pigs, but experimental infections showed it causes diarrhea[1]. This highly diverse agent is classified into genogroups/genotypes (VP1 gene) and P-groups (RdRP gene). SwNoV has not been reported in Spain, despite being identified in several EU countries. Moreover, the detection of human genotype (GII.4) in pigs[1] raised concerns about swine as a potential reservoir. This work evaluates and characterizes SwNoV in digestive samples from Spanish swine herds.

Materials and Methods
480 clinical cases from 38 Spanish provinces were evaluated (2020-2022). Samples included organ swabs (55%), faeces (30%) or intestinal tissue (15%) from mainly white (81%) but also Iberian pigs (15%). SwNov was identified by RT- qPCR and genotyped by sequencing using reported oligos2, 3 and in-house methods. Genotyping was achieved with the Norovirus Typing Tool V2. A phylogenetic tree was constructed using MAFFT and Neighbor-Joining method.

Results
Out of 480, 52 samples tested positive (11%). In Iberian pigs, 31% of the samples were positive for SwNoV, compared to 7% in white pigs. Results by breed and production stage are shown in Table 1 and region distribution in Figure 1. Six samples were sequenced, with two assigned to genotype GII.18 and four to GII.11 (Figure 2).

Discussion and Conclusion
SwNoV was detected in digestive clinical cases from Spanish swine herds, with similar frequencies (11%) to those reported in other European countries[1]. Iberian pigs had a higher infection rate than white pigs. SwNoV was detected in 14/38 provinces analyzed, with 43% of positive samples found in Extremadura, the region with the highest density of Iberian pigs. This is the first report of the detection of SwNoV in Spain. The genotypes found, together with GII.19, are commonly found in swine[1]. Nevertheless, further studies are needed to understand the pathogenesis of this virus in pigs and to evaluate, from the One Health perspective, its role as a potential host species.

References
1. https://doi.org/10.3390/v14030537
2. https://doi.org/10.1128/AEM.01428-07
3. https://doi.org/10.1016/j.meegid.2017.03.011

Table 1. SwNoV positive samples per breed and production stage

Breed	SwNoV positive/ Total samples	Percentage	Production stage	SwNoV positive/ Total samples	Percentage
Iberian	23/74	31,1%^{a***}	Suckling	0/3	0,0%
			Weaning	3 /7	42,9%
			Post-weaning	7/26	26,9%
			Growing-finishing	13/35	37,1%
			Adults	0/3	0,0%
White pig	29/390	7,4%^{b}	Suckling	0/33	0,0%
			Post-weaning	2/88	2,3%^{b}
			Growing-finishing	23/200	11,5%^{a}
			Adults	1/52	1,9%^{b}
Unknown	0/16	0%	-	-	-

Note: Superscript letters (a, b) indicate significant differences between categories within each group, determined using the Chi-square test (for large samples) or Fisher's exact test (for small samples). Values with different superscript letters indicate a significant difference at $p < 0.05$. The triple asterisks (***) indicate a highly significant difference at $p < 0.001$.

Table 1.

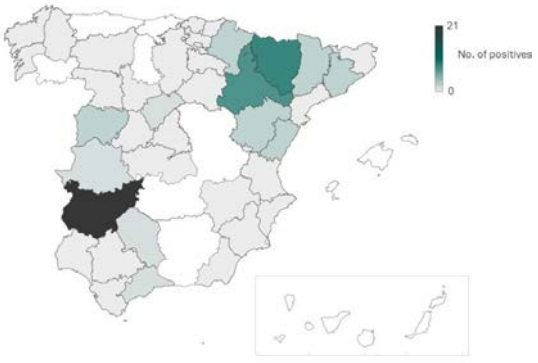


Figure 1. Spanish provinces where SwNov was detected coloured by number of positive samples.

1. https://doi.org/10.3390/v14030537
2. https://doi.org/10.1128/AEM.01428-07
3. https://doi.org/10.1016/j.meegid.2017.03.011

Figure 1.

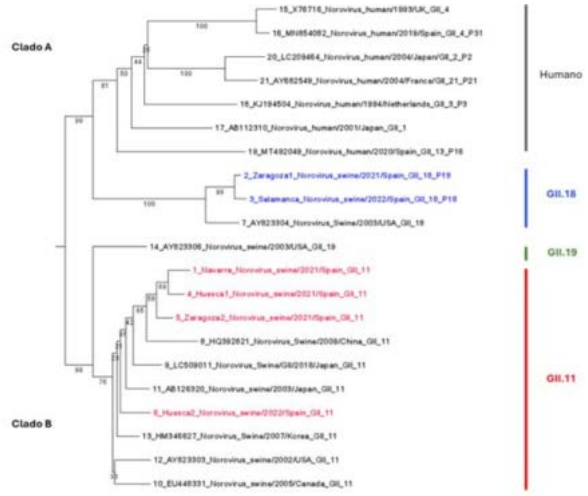


Figure 2. Phylogenetic tree including the 4 samples of genotype GII.11 (red) and two of genotype GII.18 (blue) obtained in this work. GenBank reference sequences of SwNoV genotypes GII.11, GII.18 and GII.19 and human NoV genotype GII.4 and others occasionally detected in pigs (GII.1, GII.2, GII.3, GII.21 y GII.31) are also included (black).

Figure 2.

P249

Zoonoses / vector borne diseases

INFECTION WITH *MYCOBACTERIUM MICROTI* IN SOUTH AMERICAN CAMELIDS IN SWITZERLAND

J. Lienhard[1], U. Friedel[1], M. Schneeberger[1], S. Schmitt[1]

[1]*Section of Veterinary Bacteriology, Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich, Switzerland*

Introduction
South American camelids (SAC) are becoming increasingly popular in Switzerland with a population of over 6700 animals in the year 2023, compared to only 1000 animals at the beginning of this century. Moreover, a new animal health law regulation was implemented in 2021 in Europe, where infections with *Mycobacterium tuberculosis* complex (MTBC) in SAC are listed as a category D disease. Tuberculosis in SAC is mostly caused by *Mycobacterium (M.) bovis or M. microti.* Hence, we evaluated the data collected from mycobacterial culture from SAC in Switzerland collected between 2014 and 2024 to check for the presence of MTBC.

Materials and Methods
43 lymph nodes or organ samples from llamas and alpacas were tested for mycobacteria using culture. If growth was detected, the isolate was submitted to a real-time PCR for detection of MTBC. PCR positive samples were further identified using GenoType MTBC, spoligotyping or HRM. Out of all samples, 33 samples were also directly tested with a real-time PCR for MTBC.

Results
Out of the 33 samples directly tested by PCR, nine samples were positive. All of these nine samples could be further identified as *M. microti*. Two of these samples could not be isolated by culture, the results of the other seven samples were confirmed by mycobacterial culture. While no information on macroscopic changes was available for two PCR positive animals, the other PCR positive animals showed typical tuberculous lesions, such as granulomas and enlarged or pus-filled lymph nodes.

Discussion and Conclusion
In the present study, we detected several *M. microti* positive SAC with typical tuberculous lesions, while *M. bovis* was not detected. *M. microti* is an important differential diagnosis for tuberculosis in SAC in Europe. As *M. bovis* is eradicated in Switzerland, *M. microti* seems to be a more likely cause of tuberculous lesions in SAC in Switzerland. *M. microti* is a fastidious bacterium, which might explain, why we were not able to culture all PCR positive samples. As a conclusion, *M. microti* is the most important differential diagnosis for tuberculosis in SAC in Switzerland.

References
DOI: 10.1128/JCM.42.4.1818-1821.2004 DOI: 10.1111/j.1939-1676.2009.0377.x DOI: 10.1128/JCM.02518-09
DOI: 10.1371/journal.pone.0054253

P251

Zoonoses / vector borne diseases

HUMAN-ANIMAL LEPTOSPIROSIS OUTBREAK. DIAGNOSTIC APPROACH IN A ONE HEALTH PERSPECTIVE

E. Mazzotta[1], N. Menegotto[4], V. Biscaro[4], E. Vian[4], C. La Spisa[3], E. Pascotto[2], L. Ceglie[1], L. Bellinati[1], L. Lucchese[1], C. Zanardello[1], G. Foiani[1], A. Busa[1], A. Natale[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy*
[2]*Servizio Veterinario Sanità Animale, AULSS2 Marca Trevigiana, Treviso, Italy*
[3]*UOC Anestesia e Rianimazione, Ospedale Ca' Foncello, AULSS2 Marca Trevigiana, Treviso, Italy*
[4]*UOC Microbiologia e Virologia Ospedale Ca' Foncello, AULSS2 Marca Trevigiana, Treviso, Italy*

Introduction
Leptospirosis is a widespread zoonosis. The authors present the findings of a comparative and integrated diagnostic approach following a case of human leptospirosis in Northeastern Italy (pig farmer) and an investigation in pigs.

Materials and Methods
After the symptoms onset, the farmer was admitted to the hospital for further investigations, including serology Enzyme-Linked Immuno Assay (ELISA) and Microagglutination test (MAT), real time PCR (rPCR). Accordingly, a total of 60 samples were collected from 20 pigs slaughtered within 60 days of the onset of clinical signs of the farm owner. MAT was performed on 20 sera (1). Twenty kidney and urine

samples were subjected to rPCR targeting the 16S rDNA gene of pathogenic leptospires (2). Isolation (3) was attempted on 24 kidney/urine samples. The kidneys of 12 pigs were histologically examined.

Results

The farmer exhibited hepatorenal syndrome, acute kidney injury, coagulopathy, pneumonia and Acute Respiratory Distress Syndrome. ELISA reported positivity for anti-*Leptospira* (L.) antibodies (IgM) and MAT titres for serovar Icterohaemorrhagiae (1:100), Copenagheni (1:100), and Pomona (1:100). rPCR on urine was negative, as well as, other viral, bacterial and immunological tests. MAT positivity was reported in 16 pigs (80%) (Table 1). Three urine samples exhibited weak positivity to rPCR, precluding the identification of the *L. strain* (4). Histologically, kidney samples showed mild (58%) to moderate (42%) congestion associated to multifocal, mild (50%) to moderate (50%) interstitial and perivascular lymphoplasmacytic infiltrate, and rare foci of tubular necrosis (33%).

Discussion and Conclusion

This case study highlights the significance of the One Health approach to the management of zoonotic diseases. The comparison of serological reactivity between the farmer and the pigs indicates environmental exposure to the same *L. strains,* circulating among synanthropic or wild reservoirs (5). It is crucial to foster the collaboration between doctors and veterinarians for a more effective approach to zoonoses.

References

1. doi: 10.3390/pathogens9060484
2. doi: https://doi.org/10.1186/1471-2334-2-13
3. WOAH Manual for Terrestrial Animals, Cap. 3.1.12, par. B.1.1, 2021 – Leptospirosis
4. doi: 10.1371/journal.pntd.0001954
5. doi.org/10.3390/ijerph20053783

Antigen panel	Swine serology						Human patient serology
	Prevalence (CI 95%)	MAT titres					MAT titres
	N = 20 (100%)	1:100	1:200	1:400	1:800	1:3200	1:100
L. interrogans serogroup Canicola serovar Canicola	1 (5%; 0.0–14.5)	1	0	0	0	0	0
L. kirschneri serogroup Grippotyphosa serovar Grippotyphosa	5 (25%; 6.0–43.9)	4	1	0	0	0	0
***L. interrogans* serogroup Icterohaemorrhagiae serovar Copenhageni**	**11 (55%; 33.2–76.8)**	**4**	**4**	**2**	**1**	**0**	**1**
***L. interrogans* serogroup Icterohaemorrhagiae serovar Icterohaemorrhagiae**	**16 (80%; 62.5–97.5)**	**4**	**6**	**2**	**3**	**1**	**1**
***L. interrogans* serogroup Pomona serovar Pomona**	**1 (5%; 0.0–14.5)**	**0**	**1**	**0**	**0**	**0**	**1**
L. interrogans serogroup Australis serovar Bratislava	0	0	0	0	0	0	0
L. borgpetersenii serogroup Ballum serovar Ballum	0	0	0	0	0	0	0
L. borgpetersenii serogroup Sejroe serovar Hardjo	0	0	0	0	0	0	0
L. interrogans serogroup Tarassovi var Tarassovi	0	0	0	0	0	0	0

Table 1. A comparison of the serological investigation between the human patient and the animals. MAT titres of the 20 serum samples from sampled pigs (prevalence, (%), and CI 95%) and MAT titres from the farm-owner diagnosed with leptospirosis.

P252

Zoonoses / vector borne diseases

EXPLORING THE POTENTIAL OF MURIDAE AS SENTINELS FOR HUMAN AND ZOONOTIC VIRUSES

I. Di Bartolo[1], L. De Sabato[1], G. Ianiro[1], G. Vaccari[1], F.M. Dini[2], F. Ostanello[2], M. Monini[1]

[1]*Department of Food Safety, Nutrition and Veterinary Public Health, Istituto Superiore di Sanità, Rome, Italy*
[2]*Department of Veterinary Medical Sciences, University of Bologna, Bologna, Italy*

Introduction

Transmission of viruses from wildlife to humans has raised public health concerns as exemplified by the COVID-19 pandemic [1]. Human activities play a substantial role in increasing the risk of zoonotic virus transmission from wildlife to humans. Rats and mice may act as reservoirs for various pathogens. The presence of zoonotic viruses in wild rats and mice in urban and rural areas was evaluated, focusing on well-known zoonotic viruses as betacoronavirus (beta-Covs), along with viruses occasionally detected in rats and mice. These viruses can be species-specific, zoonotic, or acquired through reverse zoonoses from other hosts. Muridae have been identified as host for non-zoonotic beta-Covs such as Rat Coronavirus [2]. Furthermore, some SARS-CoV-2 Variants of Concern (VOCs) are capable of infecting laboratory rats [3] and urban rats are exposed to the virus [4, 5].

Materials and Methods

Animals were captured during 2020-2023 from rural and urban areas in 2 regions in North-Central Italy (4.156 km2) as part of pest control programs. Among the 128 captured animals 70 were brown rats (*Rattus norvegicus*), 45 black rats (*Rattus rattus*), and 13 house mice (*Mus musculus*). Livers, lungs and intestinal contents were collected (Table 1). Real-time RT-PCR was used for the detection of betaCoV, RVs and NoVs GI-GII. Conventional RT-PCR/PCR were used for coronavirus, kobuvirus, astrovirus arenavirus hantavirus and monkeypox virus.

Specie	Sex	Age class (%)		Weight (g)			Samples			
		Adult	Subadult	Min	Max	Median	Fecal	Lung	Liver	Total
Mus musculus (House mice) (n=13; F=2, M=11	F	2 (100)	0 (0.0)	17	20.4	18.7	2	2	2	6
	M	10 (90.9)	1 (9.1)	7	70	22.1	6	9	4	19
Rattus norvegicus (Brown rats) (n=70; F=24, M=46)	F	18 (75.0)	6 (25.0)	45	390	195.2	22	23	20	65
	M	22 (47.8)	24 (52.2)	28.4	470	156.5	41	36	34	111
Rattus rattus (Black rats) (n=45; F=17, M=28)	F	12 (70.6)	5 (29.4)	29	195	97.6	16	15	14	45
	M	15 (53.6)	13 (46.4)	18	165	87.6	24	18	11	53
Total (n=128)		79 (61.7)	49 (38.3)	-	-	-	111	103	85	299

Table 1. Description of the characteristic of examined animals.from sampled pigs (prevalence, (%), and CI 95%) and MAT titres from the farm-owner diagnosed with leptospirosis.

Specie*	Sample type	No. of Samples Tested	Detected viruses (no. of positive samples)
Rattus norvegicus (Brown rats)	Faecal/rectal swab	63	MukV (6) AstV (1)
	Liver	54	- (0)
	Lung	59	MukV (1)
Rattus rattus (Black rats)	Faecal/rectal swab	40	MukV (7)
	Liver	25	- (0)
	Lung	33	- (0)

* The results for *Mus musculus* were not reported since all samples tested negative.

Table 2. Number of animals studied and types of samples analysed per animal. For each sample type, the detected viruses and the number of positive samples are provided.

Results
Among brown rats, 1 faecal sample tested positive for astrovirus RNA. Nucleotide sequencing revealed high sequence similarity to both human and rat astrovirus, suggesting copresence of these viruses in the faeces. Murine kobuvirus was detected in faecal samples from both black and brown rats, primarily from urban areas, as confirmed by sequence analysis (Table 2).

Discussion and Conclusion
The study highlighted a low prevalence or absence of the investigated viruses among Italian rats and mice, reflecting the limited transmission of such pathogens in this specific population or geographic area (Table 2). Findings highlight the importance of surveillance to mitigate the risks associated with the potential transmission of pathogens by rodents.

References
1. Andersen et al., 2020
2. Decaro et al., 2020
3. Montagutelli et al., 2021
4. Colombo et al., 2022
5. Fisher et al., 2023

P253

Zoonoses / vector borne diseases

TICKS AND ASSOCIATED PATHOGENS FROM WILD ANIMALS IN VALLE D'AOSTA REGION (NW ITALY): DATA FROM 2017 TO 2022

C. Nogarol[2], S. Robetto[1], I.N. Miceli[2], S. Brusadore[2], L. Domenis[1], R. Spedicato[1], S. Di Bella[3], A. Guercio[3], V. Blanda[3], R. Orusa[1], M.L. Mandola[2]

[1]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, S.S. Patologia della Fauna Selvatica, Centro di Referenza Nazionale Malattie Animali Selvatici (CeRMAS), Italy*
[2]*Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, S.S. Virologia Specialistica, Italy*
[3]*Istituto Zooprofilattico Sperimentale della Sicilia, Centro di Referenza Nazionale per Anaplasma, Babesia, Rickettsia e Theileria, Italy*

Introduction
Wild animals serve an important mission in the ecology of tick-borne diseases both as suitable hosts for tick vectors and as reservoirs of pathogens. Global climate and environmental changes had led to modified geographical expansion of ticks. Over recent years, I. ricinus have been reported in NW Italy at altitudes greater than 1000–1200 m.a.s.l, which were previously considered as the max altitudinal limits of the tick's geographic range. This colonization of new areas is partially facilitated by migratory bird and some mammals. We examined the diversity of tick species infecting wildlife and the presence of major tick-borne pathogens.

Materials and Methods
Ticks were collected in Valle d'Aosta region (NW Italy) from wild animals during the period 2017-2022. After collection, ticks were examined using a stereo-microscope for morphological identification. Tick homogenates were prepared by mechanical disruption with Tissue Lyser and ceramic beads, followed by the Maxwell® RSC viral TNA Kit procedure. Final extracts were tested with different PCR protocols (Table 1). All positive samples were sent to the CRABaRT (NRC, IZS Sicilia) for sequencing.

Pathogen	Primers	Target gene	Reference
Anaplasma spp.	16SANA-F 5'-CAGAGTTTGATCCTGGCTCAGAACG-3' 16SANA-R 5'-GAGTTTGCCGGGACTTCTTCT GTA-3'	16S rRNA	Stuen et al., 2003
Borrelia burgdorferi s.l.	FLA1 5'-AGAGCAACTTACAGACGAAATTAAT-3' FLA2 5'-CAAGTCTATTTTGGAAAGCACCTAA-3'	FLA	Skotarczak et al., 2002
Coxiella burnetii	Trans1 5'-TATGTATCCACCGTAGCCAG C-3' Trans2 5'-CCCAACAACACCTCCTTATTC-3'	IS1111	Berri et al., 2000
Rickettsia spp.	RpCS.877p 5'-GGGGGCCTGCTCACGGCGG-3' RpCS.1258n 5'-ATTGCAAAAAGTACAGTGAACA-3'	citrate synthase	Regnery et al., 1991
TBE	F-TBE 5'-GGGCGGTTCTTGTTCTCC-3' R-TBE 5'-ACACATCACCTCCTTGTCAGACT-3' TBE-Probe-WTTGAGCCACCATCACCCAGACACA	--	Schwaiger et al., 2003

Table 1. Details of primers, target genes and references of PCRs.

	POS screening	Confirmed species	POS screening to be confirmed
Anaplasma spp.	7	*Anaplasma phagocytophilum* n=4	1
Borrelia burgdorferi s.l.	1		
Coxiella burnetii	0		3
Rickettsia spp.	14	*R. monacensis* n=4 *R. helvetica* n=8 *R. slovaca* n=1	19
TBE virus	0		

Table 2. Results of TBPs detected (confirmed and to confirm).

Results
Overall, 160 tick pools (1–5 individuals each) were collected from wild animals and *I. ricinus* was the most prevalent tick specie found (87.5%). To date, two set of samples have been analyzed: the first one, on wich sequences confirmation has been done; the second was screened and positivities are going to be confirmed. Regarding pathogens, the most prevalent were *Anaplasma s*pp. and *Rickettsia* spp. (Table 2). *D. marginatus* was only found on wild boars, supporting this tick-host association and only one tick was identified as *R. sanguineus*, coming from a stone marten.

Discussion and Conclusion
Updated surveillance programs are essential to have a broader awareness of TBPs circulation and to adopt proper prevention and control strategies. About *A. phagocytophilum*, our screening showed a higher occurrence in ticks taken from ungulates: this is in accordance with other studies in Europe. The two confirmed *R. helvetica* and *R. monacensis* are the most prevalent *Rickettsia* species found in *I. ricinus* in NW Italy. To note, several of these positivity's came also from small wild carnivores, relevant data for *Rickettsia* ecology.

P254

Zoonoses / vector borne diseases

WILD BOAR AS A POTENTIAL RESERVOIR OF FOODBORNE ZOONOTIC AGENTS IN CENTRAL ITALY: A 2-YEAR MONITORING

S. Primavilla[1], M. Borghi[1], R. Ortenzi[1], R. Branciari[2], R. Roila[2], D. Ranucci[2], C. Altissimi[2], S. Gavaudan[1], S. Di Lullo[1], B. Morandi[1], A. Valiani[1]

[1]*Istituto Zooprofilattico Sperimentale dell'Umbria e delle Marche "Togo Rosati", Italy*
[2]*University of Perugia, Department of Veterinary Medicine, Italy*

Introduction
Wild boar populations worldwide have increased dramatically in recent decades, causing damages and remarkable losses to agricultural income (1). On the other hand, game meat represents an important food resource with high nutritional value (2). The possibility of a controlled and guaranteed supply chain with this high value food resource is of considerable interest and requires in depth analysis regarding the main zoonoses potentially transmissible from wild boar meat to consumers. The aim of this study was to investigate the presence of different bacterial and viral foodborne pathogens in haunted wild boars in Central Italy, over a 2-year monitoring period.

Materials and Methods
Samples were collected in two different meat processing centres located in Central Italy, recognized according to Regulation (EC) No 853/2004. Microbiological determinations included detection of: *Salmonella* spp. from feces, livers and carcasses, ail-positive Yersinia enterocolitica from tonsils and carcasses, *Campylobacter* spp. from feces and carcasses and hepatitis E virus (HEV) from livers and muscles.

Results
A total of 116 livers, 112 tonsils, 65 carcasses and 53 muscles were analyzed. The food safety hazards most commonly found were: HEV (in liver and with low prevalence in muscle), *Campylobacter* spp. (feces) and ail-positive Y. enterocolitica (tonsils), while *Salmonella* spp. was found with low prevalence (Table 1).

Discussion and Conclusion
No positivity was found for *Campylobacter* spp., ail-positive *Y. enterocolitica* and *Salmonella* spp. in carcasses, despite the positivity found in feces, tonsils and livers. This suggests that good slaughter practices significantly reduce the risk of pathogenic bacterial contamination of carcasses. Conversely, for HEV, the lower viral presence not only in the liver but also in the muscle, could suggest the possibility of cross-contamination of the muscle from the liver during the evisceration process.

In conclusion, this work provides preliminary findings about the presence of foodborne pathogenic microorganisms in haunted wild boars. On this basis, further investigations are needed to better characterize the highest prevalence dangers, including also microbiological analyses in wild boar meat products.

References

1. Fredriksson-Ahomaa M., 2019
2. Pedrazzoli et al., 2017

Pathogen	Matrix	Number of positives/Total (Prevalence% ± 95% Confidence Interval)
Salmonella spp.	feces	1/ 62 (1.61% ± 2.37)
	liver	3/116 (2.59% ± 2.89)
	carcass	0/65
ail-positive *Yersinia enterocolitica*	tonsils	10/112 (8.93% ± 5.28)
	carcass	0/65
Campylobacter spp.	feces	6/62 (9.68 % ± 5.57)
	carcass	0/65
hepatitis E virus	muscle	2/53 (3.78 ± 5.16)
	liver	28/116 (24.14% ± 7.79)

Table 1. Results of analytical determinations performed on wild boar samples.

P255

Zoonoses / vector borne diseases

FIRST *CANDIDATUS MIDICHLORIA MITOCHONDRII* INVESTIGATION TROUGHT REAL-TIME PCR AND DROPLET DIGITAL PCR

A. Pucciarelli[1], L. Cardillo[1], M. Viscardi[1], G. Picazio[1], N. D'Alessio[4], G. Sgroi[4], V. Veneziano[2], G. Fusco[3], C. De Martinis[1]

[1]*Unit of Exotic and Vector-Borne Diseases, Department of Animal Health, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Naples, Italy*
[2]*Unit of Translational Medicine, Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy*
[3]*Unit of Virology, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Naples, Italy*
[4]*Unit of Wildlife, Department of Animal Health, Istituto Zooprofilattico Sperimentale del Mezzogiorno, Naples, Italy*

Introduction

Vector-borne diseases (VBDs) are a growing concern in public health. *Candidatus Midichloria mitochondrii (Ca. M. m.)* is a symbiotic bacterium of several ixodid tick species, and after a blood meal, it can be horizontally transmitted to vertebrate hosts, including humans. The movement of wildlife into human-inhabited areas may contribute to an increased circulation of ticks and tick-borne pathogens in suburban and rural regions. To date, no quantitative molecular protocol is available for *Ca. M. m.* Therefore, the purpose of this study was to develop a Real-Time PCR (rt- PCR) and Droplet Digital PCR (dd-PCR) to quantify the presence of *Ca. M. m.* in different wildlife species, comparing the performance of these two diagnostic tools.

Materials and Methods

Both PCR protocols, Real-Time and Droplet Digital, were designed using a positive control of *Ca. M. m.* DNA previously obtained from a human blood sample via combined endpoint PCR/sequencing approach. The DNA was subjected to base ten serial dilutions down to 10-6, each one, together with three negative controls, wats analyzed in triplicate, for a total of 30 samples. Next we conducted molecular screening on 87 spleen samples from wildlife (Figure 1).

Results

Both techniques were able to detect *Ca. M. m.* DNA up to a dilution of 10-5 whereas only Droplet Digital PCR detected the target DNA at higher dilutions. The K-Cohen value (45%) revealed a moderate agreement between the techniques, indicating the Droplet Digital as a more suitable and sensitive tool for the detection of *Ca. M. m*. DNA (Tables 1- 2).

Discussion and Conclusion

The study reveals fot the first time, the circulation of *Ca. M. m.* in foxes, badgers, otters, porcupines, hares, and alpacas, suggesting tick infestations and potential pathogen transmission. Besides serving as a useful tick-bite marker, the potential role of *Ca. M. m.* infection in vertebrates, including humans, remains to be clarified. It is important to implement control strategies to promote animal welfare and human health from a One Health perspective.

References

Duron O et al., (2017) Evolutionary changes in symbiont community structure in ticks. Mol Ecol.
Sgroi et al.,(2022) Detection of endosymbiont Candidatus Midichloria mitochondrii and tickborne pathogens in humans exposed to tick bites, Italy. Emerg Infect Dis.

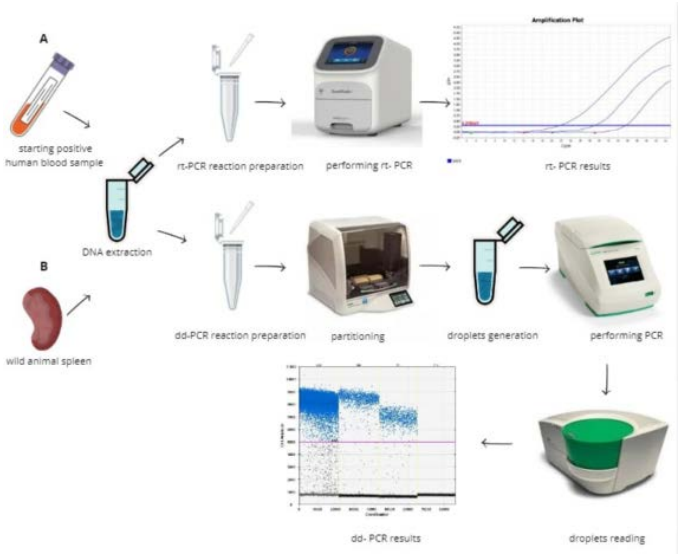


Figure 1. Molecular investigation workflow. Real time PCR and Droplet Digital PCR. Stage A) Starting positive human blood samples for the design of the rt-PCR and dd-PCR. Stage B) Wildlife spleens analysis.

Samples	rt-PCR				dd-PCR				
	#1 (Ct)	#2 (Ct)	#3 (Ct)	Mean (Ct)	#1 (copies/µl)	#2 copies/µl)	#3 (copies/µl)	Mean (copies/µl)	Mean (copies/reaction)
PC tq	16	16,5	16	16.2	saturation	saturation	saturation	saturation	saturation
1:10	18.3	19	18.7	18.6	saturation	saturation	saturation	saturation	saturation
1:100	24	24.7	24.8	24.5	saturation	saturation	saturation	saturation	saturation
1:1000	32	32	32	32	4200	3800	4300	4100	18040
1:10000	36	36	35.3	35.7	174	143	138	151.6	667
1:100000	38.9	39	39.2	39	16.2	14.4	14.5	15.0	66.1
1:1000000	nd	nd	nd	nd	1.3	1.1	1.4	1.3	5.7
NC	nd	nd	nd	nd	0	0	0	0	0
NC	nd	nd	nd	nd	0	0	0	0	0
NC	nd	nd	nd	nd	0	0	0	0	0

Table 1. Real-time PCR and Droplet Digital results of the starting controls. Abbreviations: cycle threshold, Ct; positive control, PC; negative control, NC.

Positive species	rt-PCR (Ct)	dd-PCR (copies/µl)	dd-PCR copies/reaction)
Red fox 1	38.7	0.5	2.2
Red fox 2	38.6	0.5	2.2
Badger	37.0	78.0	343.2
Otter 1	nd	0.2	0.9
Porcupine 1	nd	0.2	0.9
Porcupine 2	38.0	1.8	7.9
Alpaca	nd	0.8	3.5
Red fox 3	nd	0.9	3.9
Red fox 4	nd	2.0	8.8
Otter 2	nd	0.6	2.6
Hare 1	nd	0.2	0.8
Porcupine 3	nd	0.3	1.3
Red fox 5	nd	2.0	8.8

Results of analytical determinations performed on wild boar samples.

Table 2. Comparative results obtained by real-time PCR and dd-qPCR. Abbreviations: cycle threshold, Ct; not determined, nd.

P256

Zoonoses / vector borne diseases

WEST NILE ENCEPHALITIS CLAIMS THE LIFE OF A HORSE IN THE APULIA REGION, ITALY.

D.A. Raele[1], L. Marino[1], N. Cavaliere[1], M.A. Cafiero[1], R. Catanzariti[1], E. Macolino[1], M.S. Cappucci[1], E. De Simone[1], L. Del Sambro[1], L. Capozzi[1], A. Petrella[1]

[1]*Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Italy*

Introduction

The West Nile disease is an arboviral infection transmitted by mosquitoes, primarily affecting birds but also posing serious health risks to humans and horses. According to Italy's National Arbovirus Plan 2020-2025, the Apulia region adopted a comprehensive strategy aimed at preventing, surveilling, and responding to arboviral diseases. In 2023, in Apulia region, a total of 11 WNV infections were notified (6 human cases and 5 cases in horses). The presence of WNV was also molecularly confirmed in one pool of screened mosquitoes[1]. Here we report a fatal case of WND in a horse exhibited neurological symptoms in Apulia region.

Materials and Methods

In November 2023, IZSPB were alerted about the presence of a horse that had died after showing neurological symptoms. During the survey and the subsequent necropsy veterinarians collected serum, spleen, kidney, brain, obex, cerebellum, spinal cord and lung. These organs underwent histological analysis, the serum was tested using a commercial WND IgM Capture ELISA kit and genomic material was extracted for molecular testing. The examinations aimed to detect the presence of Equine herpesvirus, Borna Virus, WNV and USUTUV using RT-qPCR assays. In addition, all samples seed on Blood Agar for bacteriological testing at 37°C.

Results

Although no gross lesions were found in exanimated materials, the nervous tissue samples showed histological lesions characterized by the presence of perivascular lymphocytic infiltrates, gliosis and spongiosis (Figure1). Serum tested positive for IgM antibodies. Samples were negative for both molecular and bacteriological tests. Only RNAs extracted from the obex and spinal cord tested positive in RT-qPCR specific for the West Nile Virus Lineage 2.

Discussion and Conclusion

Even if the correlation between nervous symptoms and the presence of circulating IgM antibodies is sufficient to support a diagnosis of WND, histological characteristic findings of a non-septic viral infection have been valuable. In conclusion, the molecular detection, far from obvious, allowed to identify in the WND the cause of death of the horse.

References

1. Loconsole D et al.2023.Abrupt Increase in Detection of Locally Acquired West-Nile-Virus-Lineage-2-Mediated Neuroinvasive Disease in a Previously Non-Endemic Area of Southern Italy.Viruses.16:53.

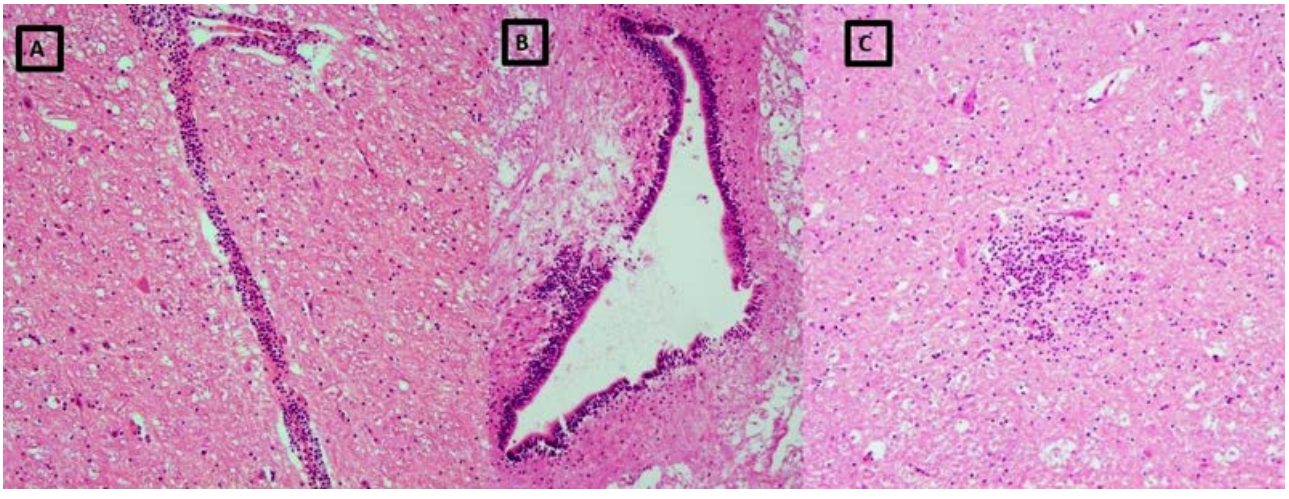


Figure 1. Histologic findings of brain and ependymal canal stained by hematoxylin-eosin: A-B: Details of perivascular lymphocytic infiltrates; C: focal gliosis and spongiosis.

P257

Zoonoses / vector borne diseases

SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI* FROM ROE DEER (*CAPREOLUS CAPREOLUS*), FALLOW DEER (*DAMA DAMA*) AND RED DEER (*CERVUS ELAPHUS*) IN EMILIA ROMAGNA REGION, NORTHERN ITALY

M. Sampieri[2], P. Chiani[1], S. Lauzi[4], R. Tozzoli[1], C. Garbarino[2], A. Prosperi[2], G. Pupillo[2], G. Rugna[2], S. Rubini[2], M. Frasnelli[2], L. Fiorentini[2], P. Bassi[2], A. Padovani[3], S. Palminteri[3], G. Galletti[2], G. Scavia[1], R. Taddei[2]

[1]*Istituto Superiore di Sanità, Laboratorio di Riferimento Europeo per Escherichia coli, Roma, Italy* [2]*Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna, Brescia, Italy*
[3]*Regione Emilia Romagna, Settore Prevenzione Collettiva e Sanità Pubblica, Bologna, Italy*
[4]*Università degli studi di Milano, Dipartimento di Medicina Veterinaria e Scienze Animali, Lodi, Italy*

Introduction

Shiga-toxin-producing *Escherichia coli* (STEC) are zoonotic pathogens capable to induce serious diseases in humans, including the life-threatening haemolytic uremic syndrome (HUS), and represent a significant public health concern (1). Although domestic ruminants are recognized as the main reservoirs of STEC (2), wildlife plays an important role as carriers of these pathogens (3). The aim of the study was to investigate the occurrence and characteristics of STEC strains in wild ruminant populations in the Emilia Romagna Region (RER) (North Italy).

Materials and Methods

111 faecal samples from roe deer (n=101), fallow deer (n=8) and red deer (n=2) were collected from February to May 2024. Samples were analysed according to ISO/TS 13136:2012. stx1 and stx2 gene subtyping was performed according to the EURL for *E. coli* procedure (EU-RL VTEC_Method 006).

Results

Cultivable STEC strains (n=46) were isolated from 42 out of 111 (38%) wild ruminants, representing all three deer species included in the study and collected from all the RER provinces (Figure 1). stx1 and stx2 genes were equally prevalent: 21 strains (46%) were positive for stx1 gene, 21 strains (46%) were positive for stx2 gene, and 4 (8%) were positive for both (Table 1). The eae gene was detected in an stx2 positive strain, identified as serogroup O26 and isolated from a roe deer. The isolates carried all the investigated stx1 subtypes, with stx1a predominating (n=11; 44%); stx2a, stx2b and stx2g subtypes were identified, with stx2b prevailing (n=21; 84%) and stx2a detected in two strains. Remarkably, STEC O26 carried the highly virulent stx2a subtype.

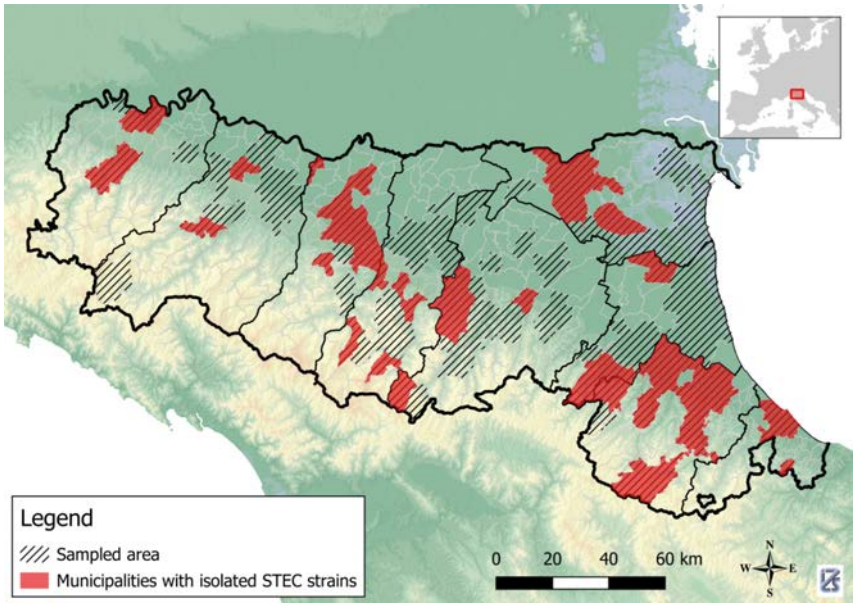


Figure 1. Map of RER displaying the sampled area and the municipalities with isolated STEC strains

Discussion and Conclusion

The present study confirms that STEC strains circulate and spread in the environment through wild ruminants, including strains with high pathogenic potential for humans. The O26 serogroup has spread rapidly throughout Europe in recent decades, and it is now the most commonly identified serogroup in HUS cases in Italy (4). In-depth molecular investigation of the isolates will aid in better defining the role of wild ruminants as a possible source of STEC infection in humans.

References

1. EFSA, ECDC, 2023. EFSA J.; 21(12): e8442.
2. Caprioli A. et al., 2005. Vet. Res.; 36: 289.
3. Lauzi S. et al., 2022. Transbound Emerg. Dis.; 69(4): 1902. 4.Michelacci V. et al., 2022. Front Cell Infect Microbiol.; 12: 842508.

Virulence Gene/s (number of strains)	Subtype *stx1* (number of strains)	Subtype *stx2* (number of strains)	Subtypes *stx1* + *stx2* (number of strains)	Top Five Serogroup
STEC *stx1* (21)	*stx1a* (8)	-	-	NT (21)
	stx1c (4)	-	-	-
	stx1d (8)	-	-	-
	stx1a, stx1c (1)	-	-	-
STEC *stx2* (20)	-	*stx2a* (1)	-	NT (20)
	-	*stx2b* (17)	-	-
	-	*stx2g* (2)	-	-
STEC *stx1* + *stx2* (4)	-	-	*stx1a, stx2b* (2)	NT (4)
	-	-	*stx1c, stx2b* (2)	-
STEC *stx2 eae* (1)	-	*stx2a* (1)		026 (1)

Table 1. Virulence genes, *stx* subtypes and serogroups of the 46 STEC isolates. (NT: non-typeable)

P258

Zoonoses / vector borne diseases

EXPOSURE OF RED FOXES TO *TOXOPLASMA GONDII, ANAPLASMA PHAGOCYTOPHILUM, LEISHMANIA INFANTUM*, AND *TRICHINELLA* SPP. IN GREECE: A SEROLOGICAL INVESTIGATION

L.V. Athanasiou[2], S. Boutsini[1], E.G. Katsogiannou[2], K.E. Tasioudi[4], D.P. Papatheodorou[4], G. Markantonatos[4], S. Tampach[5], M. Bisia[2], D. Stougiou[1], C.N. Tsokana[3]

[1]*Department of Parasitology-Parasitic Diseases, Entomology and Bee Health, Directorate General of Veterinary Services, Ministry of Rural Development and Food, Athens, Greece*
[2]*Diagnostic Laboratory, Clinic of Medicine, Faculty of Veterinary Medicine, University of Thessaly, Karditsa, Greece*
[3]*Laboratory of Parasitology and Parasitic Diseases, School of Veterinary Medicine, Aristotle University of Thessaloniki, Greece*
[4]*Virology Laboratory – National Reference Laboratory for Rabies in animals, Department of Molecular Diagnostics, FMD, Virological, Rickettsial & Exotic Diseases, Directorate General of Veterinary Services, Ministry of Rural Development and Food, Athens, Gr*
[5]*Wildlife Ecology & Health Group, Universitat Autónoma de Barcelona, Barcelona, Spain*

Introduction

The red fox (Vulpes vulpes) is widely distributed and inhabits rural and urban areas. Interactions with humans and domestic animals and the occurrence of zoonotic pathogens in vulpine populations are reported worldwide1,2. This study assessed the exposure of red foxes in Greece to *Toxoplasma gondii, Anaplasma phagocytophilum, Leishmania infantum,* and *Trichinella* spp. and defined the risk factors for seropositivity.

Materials and Methods

The study included 180 sera from red foxes hunted and delivered by hunters and gamekeepers to the Regional veterinary services during the monitoring phase of the Oral Rabies Vaccination campaign from 2013 to 2016. Location data were marked with handheld global posi-

tioning system units. Sera were tested by indirect fluorescence antibody test for IgG antibodies against T. gondii, A. phagocytophilum, and L. infantum and by enzyme-linked immunosorbent assay for anti-Trichinella IgG antibodies. Map generation was performed using QGIS version 3.32.3 Lima, and statistical analyses using MedCalc Statistical Software version 14.8.1 and Fisher's Test for Exact Count Data.

Results

Overall, 31.67%, 15%, 13.33%, and 11.11% vulpine sera were positive for *T. gondii, A. phagocytophilum, L. infantum* and *Trichinella* spp, respectively. Multivariate analysis indicated that A. phagocytophilum-seropositive foxes were more likely to be L. infantum-seropositive. The latter were more likely to be A. *phagocytophilum* or *T. gondii*-seropositive while *T. gondii*-seropositive foxes were more likely to be *L. infantum*-seropositive and to be older.

Discussion and Conclusion

This study provides compelling evidence of red fox exposure to the above-mentioned pathogens in Greece, notably with higher seroprevalences than those in other wild mammals2. Co-exposure to certain pathogens with higher probability was also identified. Our findings underscore the role of the red fox as a key sentinel for environmental contamination and pathogen circulation between rural and urban areas.

References

1. IUCN. Vulpes vulpes: Hoffmann, M. & Sillero-Zubiri, C.: The IUCN Red List of Threatened Species 2016: 2016 [cited 2024 Jun 23]. Available from: http://www.iucnredlist.org/details/23062/0
2. Tsokana CN et al. Wildlife and parasitic infections: A One Health perspective in Greece. Curr Res Parasitol Vector- Borne Dis. 2024;6:100184.

P259

Animal health

EXTENDED SPECTRUM BETA-LACTAMASES AND CARBAPENEM RESISTANCE IN ENVIRONMENTAL STRAINS OF *KLEBSIELLA* PNEUMONIAE IDENTIFIED IN VETERINARY HOSPITAL

D. Dellamaria[1], S. Deotto[1], L. Viel[1], M. Corrò[1], M. Vedana[1], E. Spagnolo[1], A. Masiero[1], P. Danesi[1], K. Capello[1], A. Pinto[1], G. Capelli[1], M. Vascellari[1], M. Cocchi[1]

[1]*Istituto Zooprofilattico Sperimentale delle Venezie, Italy*

Introduction

The antimicrobial resistance (AMR) is increasing worldwide and represents a significant threat for the public health. In human medicine, one of the most important and known source of the diffusion of AMR includes the hospital environment through contact with contaminated surfaces. Carbapenem-resistant (CR) and extended spectrum beta- lactamases (ESBL) Enterobacterales have been considered as critical priority pathogens.
In veterinary, limited data regarding resistant pattern of isolates from hospital are available.
This study aimed at the identification of ESBL and CR-*Klebsiella* (K.) *pneumoniae* isolates from veterinary clinical settings.

Materials and Methods

14 strains, randomly selected and previously identified by MALDI-TOF (MALDI Biotyper® Sirius System, Bruker) were submitted to the minimum inhibitory concentration (MIC) using the Sensititre™ EU Surveillance ESBL EUVSEC2 AST plate (Thermofisher). The breakpoints of the CLSI M100-ED34:2024 were adopted for the interpretation, where available. These strains were obtained by sampling defined points of the surfaces of the different rooms (visiting rooms, the hospitalization area, and the operating room) in 15 veterinary clinics, located in the north-eastern part of Italy.

Results

The results are summarized in the Table 1 and in Figure 1. Phenotipically, all the strains are resistant to at least three cephalosporins. No resistant strains to carbapenems were found.

Discussion and Conclusion

In humans, both the carbapenem and the ESBL resistant Enterobacterales has become significantly more prevalent in the last decades, representing one of the major cause of hospital-acquired infections, associated with high morbidity and mortality. In this study, ESBL *K. pneumonia*e were found, but no CR strains were identified. Although veterinary hospitals are regarded as a high risk places due to the potential contaminated environment, few data are available. Scarpellini et al. (2023) reported low percentage of ESBL and CR Enterobacterales from veterinary hospital. According to other authors, veterinary clinic should be monitored. Collecting data in a frame of an harmonized surveillance system should be useful to properly monitor the AMR phenomenon.

The Italian Ministry of Health [IZSVE 11/21 RC] supported this work.

Antimicrobial	Antimicrobial concentration (mg/l)											
	0.03	0.063	0.125	0.250	0.5	1	2	4	8	16	32	64
Cefepime											14	
Cefotaxime												14
Cefotaxime/clavulanic acid		13		1								
Cefoxitin								8	5		1	
Ceftazidime										7	7	
Ceftazidime/clavulanic acid			11	2	1							
Ertapenem	5	8		1								
Imipenem			6	7	1							
Meropenem	12	1	1									
Temocillin								8	4	1	1	

Table 1. MIC distribution of tested antimicrobials towards *K. pneumonia*e identified from different surfaces in the veterinary hospital settings.

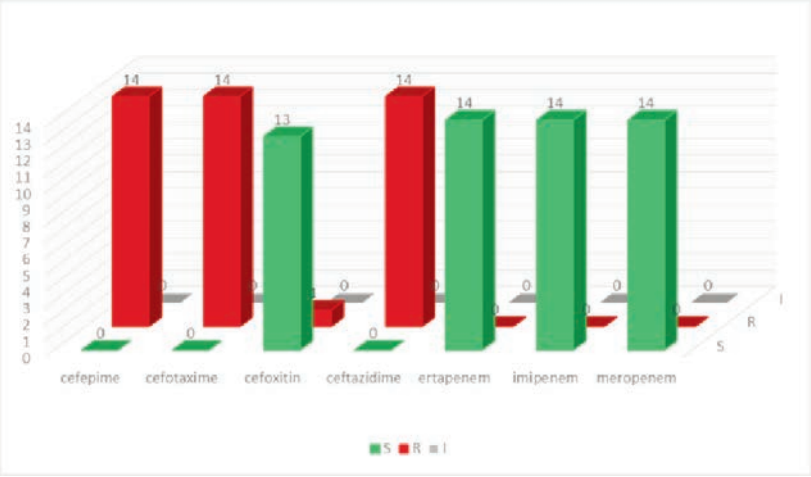


Figure 1. Susceptible pattern of *K. pneumonia*e strains toward cephalosporins and carbapenems. S, susceptible; I, intermediate; R, resistant.

P260

Epidemiology / Risk analysis and communication

VARIABILITY OF THE SIPA GENE IN *SALMONELLA SEROVARS* ISOLATED FROM SWINE: STUDY OF ITS CORRELATION WITH VIRULENCE IN HUMANS

M. Tambassi[1], M. Berni[1], C. Bracchi[1], I. Menozzi[1], A. Dodi[1], L. Mazzera[1], M. Morganti[1], E. Scaltriti[1], S. Pongolini[1]

[1]*Risk Analysis and Genomic Epidemiology Unit, Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia- Romagna (IZSLER), Parma, Italy*

Introduction

Human salmonellosis is a high priority foodborne disease (1). The most frequent source of salmonellosis is food of animal origin, with pork being among the most relevant. However, the most prevalent *Salmonella* serovars in swine differ in their ability to cause disease in humans. For instance, *S. Typhimurium* and its monophasic variant are more frequently responsible for human salmonellosis than *S. Derby* and *S. Rissen.* The genetic basis of this difference remains largely unexplained. *Salmonella* induces enteritis through effectors encoded by the *Salmonella* Pathogenicity Island 1 (SPI-1), which promote intestinal epithelial invasion and the production of pro-inflammatory mediators by the infected cells (2). The effector SipA has a major role in *Salmonella*-induced enteritis. We therefore evaluated whether the difference in virulence among *Salmonella* serovars from swine correlated with the fixation of mutations in the gene encoding the SPI-1 effector SipA.

Materials and Methods

A bioinformatic analysis was performed using the Enterobase database. Genomes belonging to the serovars of interest were selected and

their core genome MLST data were retrieved to identify the allelic variants of sipA. The alleles were compared with the reference strain *S. Typhimurium* SL1344 through multiple alignment.

Results
The sipA allelic variants of *S. Derby* and *S. Rissen*, with reduced ability to cause disease in humans, exhibited a higher number of missense mutations compare to the reference strain of the virulent S. Typhimurium serovar and its monophasic variant. Interestingly, *S. Derby* and S. Rissen sipA alleles share ten missense mutations.

Discussion and Conclusion
Our results support the hypothesis that diversity in the ability of *Salmonella* serovars from swine to cause disease in humans correlates with the accumulation of missense mutations in sipA. We will further investigate whether these mutations influence *Salmonella* pathogenicity and, thus, if the analysis of sipA polymorphism could be a useful tool for genotype-specific *Salmonella* risk assessment.

References
1. DOI: 10.2903/j.efsa.2023.8442
2. DOI: 10.1016/j.mib.2021.08.007

P261

Antimicrobial resistance / susceptibility testing

METHICILLIN-RESISTANT STAPHYLOCOCCI IN THE BULK TANK MILK OF A LIVESTOCK-DENSE AREA IN NORTHERN ITALY

S. Fusar Poli[3], C. Locatelli[3], V. Monistero[3], F. Santandrea[3], C. Lecchi[3], C. Allievi[3], L. Villa[3], P. Cremonesi[1], B. Castiglioni[1], A. Guaraglia[4], C.M. Longheu[2], S. Tola[2], M.T. Manfredi[3], M.F. Addis[3]

[1]*IBBA-CNR, Lodi, Italy*
[2]*Istituto Zooprofilattico Sperimentale della Sardegna G. Pegreffi, Sassari, Italy*
[3]*University of Milan, Milano, Italy*
[4]*University of Sassari, Sassari, Italy*

Introduction
Staphylococcus aureus is a major cause of contagious mastitis in dairy cows, while non-aureus staphylococci and mammaliicocci (NASM) are frequently isolated from milk [1]. NASM may carry antimicrobial resistance (AMR) genes and pose a risk for bidirectional transfer with *S. aureus* [2].

Materials and Methods
This study investigated MRSA and MR NASM isolated from the bulk tank milk (BTM) of 88 dairy farms in northern Italy. For *S. aureus* isolation BTM was seeded on Baird Parker agar, while MR NASM were detected via enrichment in Mueller Hinton broth and Chromogenic MRSA agar. Isolates were identified by MALDI-TOF MS. *S. aureus* and MRSA were typed by RS-PCR and assessed for biofilm-related genes by qPCR. Presumptive MR were tested for mecA/mecC genes by PCR and characterized for the SCCmec cassette. Antimicrobial resistance profiles were determined by agar disk diffusion.

Results
S. aureus was isolated in 32.95% of the BTM samples, all carrying ica genes for biofilm production. Isolates belonged to 10 different genotypes, with GTB being the most common. MR NASM were detected in 56.81% of farms, with *S. epidermidis* being the most frequent (35.59%), followed by *S. aureus*, *M. sciuri, S. saprophyticus, S. borealis, S. haemolyticus, M. fleurettii, S. cohnii,* and *S. pettenkoferi.* Most MR isolates carried the mecA gene, while none carried mecC. The majority of MRSA belonged to genotype S and carried the SCCmec type V cassette (45.45%). For MRSE, 61.90% carried the type IV, followed by type II and V. Considering all MR NASM, the most prevalent SCCmec was type IV, followed by V.

Discussion and Conclusion
The study revealed a significant presence of MRSA and MR NASM in dairy herds, highlighting their resistance characteristics. Considering the potential for bidirectional AMR transfer, monitoring AMR in all NASM would be preferable to focusing solely on MRSA for a comprehensive understanding of MR in dairy herds, within a One Health perspective.

References
1. De Buck et al. 2021. Non-aureus Staphylococci and Bovine Udder Health: Current Understanding and Knowledge Gaps. Front Vet Sci.
2. Schnitt et al. 2021. The occurrence of methicillin-resistant non-aureus staphylococci in samples from cows, young stock, and the environment on German dairy farms. J Dairy Sci.