Intradiaphragmatic abscesses in a wild boar (*Sus scrofa*): Inspective implications based on anatomopathological evidences

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Abstract

The intradiaphragmatic localization of an abscess is rarely described in humans and in other animal domestic and wild species, and can be caused by penetrative traumas (*i.e.*, firearm injuries). Here we describe two intradiaphragmatic abscesses in a hunted adult male wild boar (*Sus scrofa*) pluck, associated with adhesion phenomena with the contiguous anatomical structures (pleural, phrenic, and glissonian serosas) and observed during the *post mortem* inspection, in accordance with the Reg. EU 627/2019. One of these lesions also presented a phreno-abdominal fistula. We found in cytopathological evaluation of the neoformations' content the presence of spheroidal bacterial *soma*, characterized by linearly concatenated "*Streptococcus*-like" aggregation pattern. Furthermore, microbiological assays revealed a polymicrobial pattern characterized by the presence of telluric microorganisms, some of which have a marked pyogenic action (*Streptococcus suis* type I, *Sphingomonas paucimobilis*, *Carnobacterium divergens*, and *Lactobacillus sakei*). Our results and collected evidence demonstrate the pathogenetic hypothesis of bacterial contamination secondary to penetrative trauma caused by a not-mortal projectile's wound, defining the inspective behaviour according to the cogent legislation. These lesions, in the reason of their potential relation to toxemia, bacteremia and septicemia phenomena, represent a sanitary risk that impose, from a normative point of view, the total condemnation of the carcass. These inspective implications, originating from the observation and interdisciplinary description of the anatomopathological and microbiological features of the lesions, are directly projected on the normative scenario, providing useful elements to guarantee the health of the consumer.

Introduction

Understanding the intimate pathogenetic mechanisms of a lesion and its anatomopathological features' interpretation represent a fascinating scientific process, showing the power of the inductive method based on observation.

Abscesses are lesions characterized by neutrophilic exudate collection in a neoformed cavity (Kumar *et al*., 2014). The intradiaphragmatic localization of an abscess is rarely described in humans and in other animal domestic and wild species, and can be caused by penetrative traumas (*i.e*., firearm injuries) (Mercer and Hill, 1985).

Here we describe two intradiaphragmatic abscesses in a hunted adult male wild boar (*Sus scrofa*) pluck, associated with adhesion phenomena with the contiguous anatomical structures (pleural, phrenic, and glissonian serosas) and observed during the *post mortem* inspection, in accordance with the Reg. EU 627/2019. One of these lesions also presented a phreno-abdominal fistula.

The *post mortem* exam inspection represents an essential phase for identification of these lesions, showing the high diagnostic sensitivity of this inspective method.

From the normative point of view, the obligation to confer the carcass (eviscerated) to the game handling establishment and the Competent Authority is limited to the hunted game ungulates destined to the commercialization circuit. Furthermore, in absence of signs detectable during the exam conducted by the "formed person" on hunted game ungulates and implicating a risk for human health, the pluck may be classified as animal by-product and not sent to the game handling establishment and the Competent Authority. In this case, the *post mortem* exam first evaluation is demanded to the hunter, as "formed person". For all types of consumption (self-consumption, direct sale, commercialization) described by the law, in all animals ascribed to species receptive to *Trichinella* spp., the confer-ment of a single aliquot of diaphragmatic muscle, sampled in diaphragm pillars, is expected for *Trichinella* spp. screening.

With a multidisciplinary approach, from macroscopic anatomy to microbiological observations, all the findings presented in this study were collected to rebuild the "pathogenetic puzzle", defining the inspective behaviour, through an evidence-based law interpretation.

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Materials and methods

Gross pathology examination

A hunted adult male wild boar (*Sus scrofa*) pluck was inspected during the *post mortem* examination, in accordance with the Reg. EU 627/2019.

Anatomical dissection, conducted through the manual digitoclasia method and surgical instruments, was also performed to characterize topographical relations between lesions and anatomical structures and organs.

Radiology

The polispancnic unit, constituted by trachea, oesophagus, lungs, diaphragm, heart, liver, and lymph nodes, was radiologically screened to investigate the potential presence of radio opaque foreign bodies (*i.e.,* projectiles).

Cytopathology

Neoformations' content was collected in sterility by the fine needle aspiration (FNA) method. Collected material was smeared on glasses, colored with Diff Quick stain, and observed by optical microscopy.

Microbiology

According to cytopathological observations, the lesions' content was plated on tryptic soy agar (TSA) and incubated at 37°C for 24 hours.

Bacterial identification was performed by two methods: biochemical and mass spectrometry assays. The first one was conducted using the automatized biochemical system VITEK® 2 (bioMèrieux, France), and the second one by mass spectrometry MALDI-TOF (Matrix Assisted Laser Desorption Ionization - Time Of Flight).

Results

Gross pathology examination

Based on macroscopic observation and anatomical dissection, gross pathology examination revealed two focal abscess lesions at the right hemidiaphragm, characterized by phrenic intramural localization (Figure 1). The most lateral lesion was 7.2x5.0 cm and the most medial one was 4.5x3.7 cm. Both lesions were associated with adhesion phenomena involving contiguous pleural, phrenic, and glissonian anatomical structures. One of them, the most lateral, also presented a phreno-abdominal fistula (Figure 2).

Anatomical tissue dissection, executed

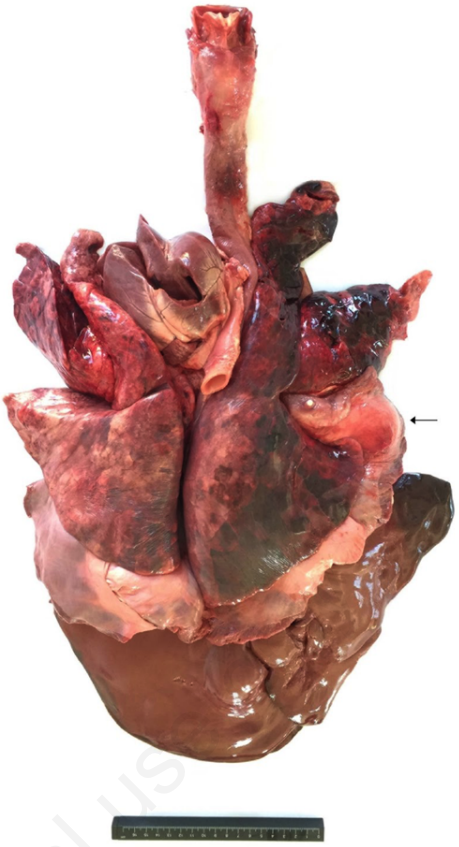

Figure 1. Trachea, oesophagus, lungs, diaphragm, heart, liver, and lymph nodes (macroscopic dorsal view). Intradiaphragmatic abscesses (arrow) localized at the right hemidiaphragm, in association with adhesion phenomena involving contiguous pleural, phrenic, and glissonian anatomical structures. One of the lesions also presented a phreno-abdominal fistula.

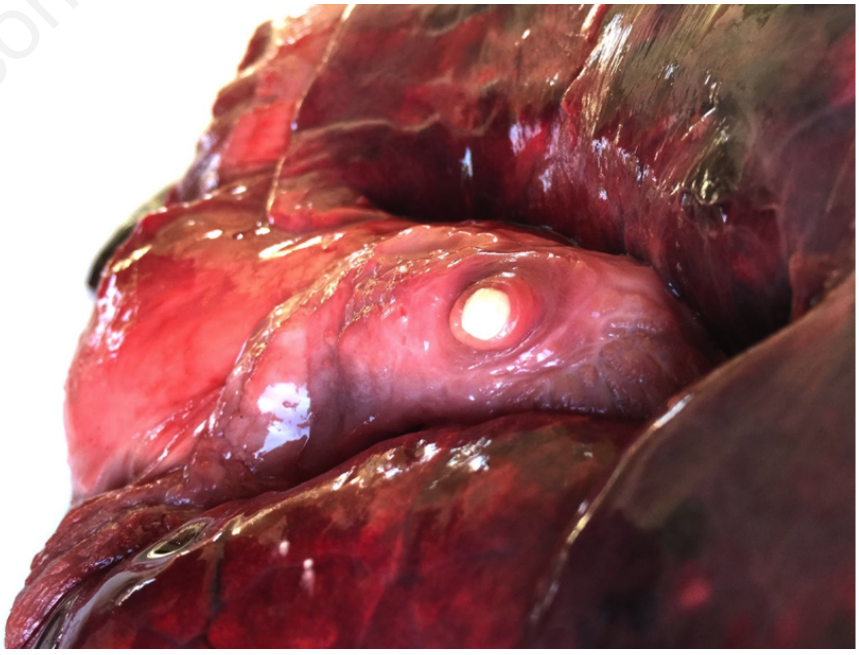

Figure 2. Intradiaphragmatic abscess with phreno-abdominal fistula (macroscopic view). Gross pathology morphological observation of the most lateral lesion reveals an umbilicated area with central phreno-abdominal fistula.

through digitoclasia, revealed lesions' paravascular localization to the cranial vena cava, the right superior phrenic artery and the right pericardiophrenic artery at the level of their diaphragmatic insertion.

Radiology

Radiography showed no radiopaque signs referable to metallic projectile fragments.

Cytopathology

Cytological evaluation showed the presence of spheroidal bacterial *soma*, characterized by a single, coupled and linearly concatenated "*Streptococcus*-like" aggregation pattern, on a diffuse eosinophilic amorphous material background (Figures 3 and 4).

Microbiology

Microbiological identification assays (VITEK®2 and MALDI-TOF) revealed a polymicrobial pattern characterized by: *Streptococcus suis* type I, *Sphingomonas paucimobilis*, *Carnobacterium divergens*, and *Lactobacillus sakei*.

Discussion

From macroscopic observations to microscopic details, our results unveil the pathogenetic "story" of the lesions and define the inspective behaviour.

Based on macroscopic observation and anatomical dissection, gross pathology examination revealed two focal abscess lesions at the right hemidiaphragm, characterized by phrenic intramural localization (Figure 1). The intradiaphragmatic localization of an abscess is rarely described in humans and in other animal domestic and wild species, and can be caused by penetrative traumas (*i.e.*, firearm injuries) (Mercer and Hill, 1985). This anatomical and topographical feature represent one of the main pillars at the basis for the formulation of the pathogenetic hypothesis of bacterial contamination secondary to penetrating trauma caused by a not-mortal projectile's wound.

Radiography showed no radiopaque signs referable to metallic projectile fragments. This evidence is perfectly compatible with the ballistic and physical features of the most commonly used projectiles for wild boar hunting, characterized by a high kinetic energy, causing a transfixing wound that crosses the animal body.

The lesions' association with adhesion phenomena involving contiguous pleural, phrenic, and glissonian anatomical structures and the phreno-abdominal fistula, observed in the most lateral lesion (Figure 2), may represent the result of the projectile

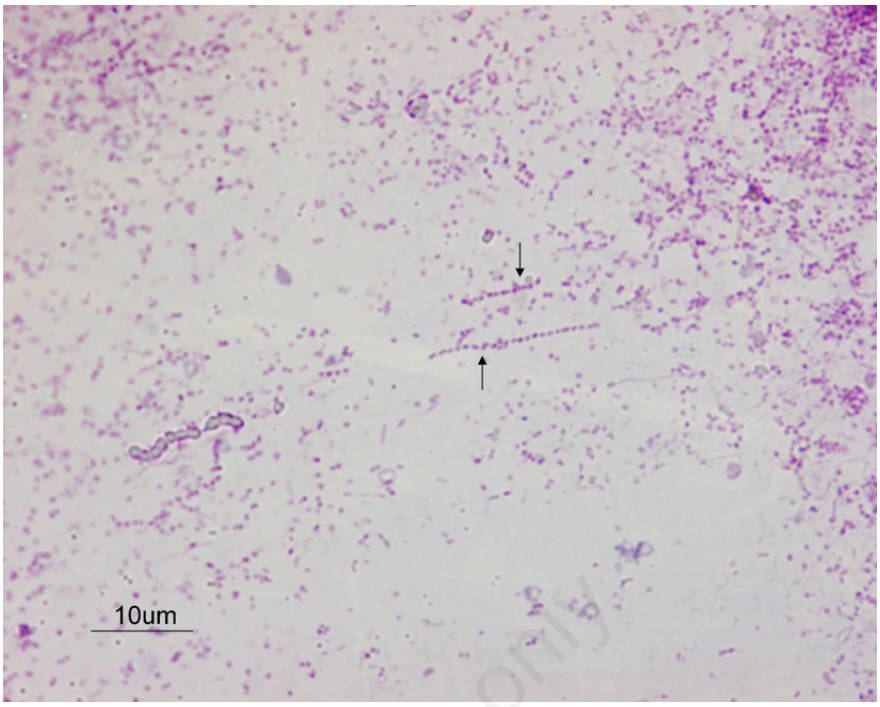

Figure 3. Lesions' content cytopathology (lateral lesion, Diff Quick stain, optic microscopy, 100x). Lesion's cytological biopsy obtained by fine needle aspiration (FNA) method reveals the presence of spheroidal bacterial *soma* characterized by a single, coupled and linearly (arrow) concatenated *Streptococcus*-like aggregation pattern, on a diffuse eosinophilic amorphous material background.

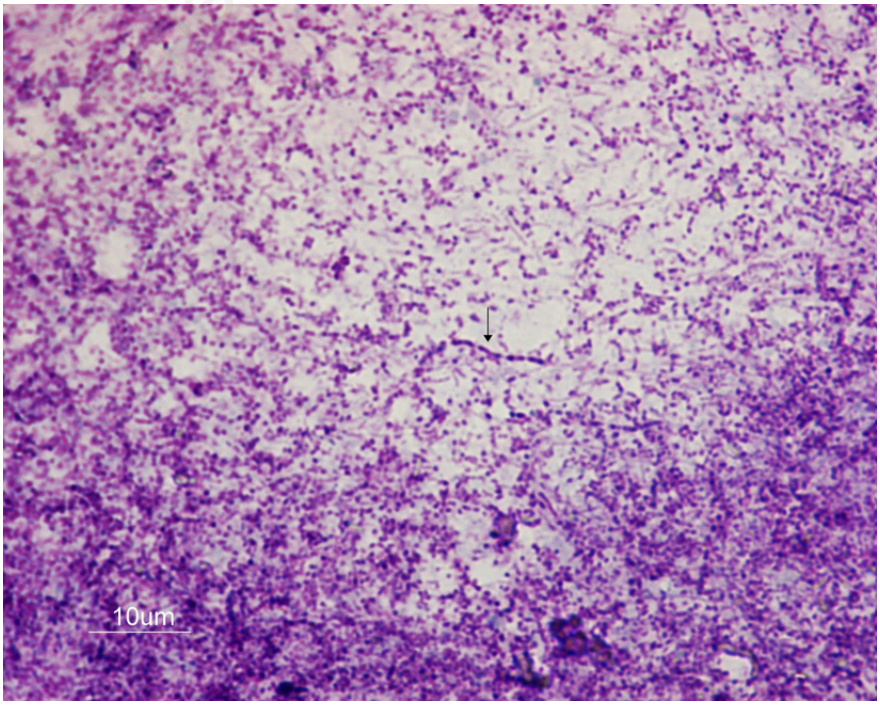

Figure 4. Lesions' content cytopathology (medial lesion, Diff Quick stain, optic microscopy, 100x). Cytology of the medial lesion's content revealed a similar cytopathological inflammatory pattern with presence of numerous spheroidal bacterial *soma*, also in a concatenated aggregation pattern (arrow), and diffuse eosinophilic amorphous material background.

internal injuries disseminated along the ballistic trajectory.

Cytological evaluation reveals the presence of spheroidal bacterial *soma* characterized by a single, coupled and linearly concatenated "*Streptococcus*-like" aggregation pattern, confirming the bacterial pathogenetic hypothesis. The diffuse eosinophilic amorphous material background is constituted by cellular necrosis debridement and purulent exudate originating from a prevalent and massive neutrophil chemotaxis and their *in situ* apoptosis.

Neutrophils as the prevalent cytotype in inflammatory pattern lesions, also in combination with other inflammatory cells, and their cytomorphological characterization are one of the "conducting" signs in the diagnostic hypothesis formulation and lesions' etiology identification, the "narrating voices" of a pathogenetic history, representing another precious element composing the lesion' ontogenesis "puzzle".

Neutrophil chemotaxis represents the result of a complex and elegant immunodynamic orchestrated by the organism in response to specific molecular "triggers", generally constituted by lipidic complex molecules (*i.e.* lipopolysaccharides, glycosphingolipids) and particularly expressed in Gram-negative bacteria (Heaver *et al*., 2018). These molecular signals are ascribed to the Pathogen Associated Molecular Pattern (PAMP), inducing a cellular innate immune response. Eukaryotic cells, leukocytes included, are biologically programmed to recognize, and respond to specific PAMP signals. This phenomenon is the mirable result of a close co-evolutive relationship between host's cell and pathogens, dialoguing together with the same language of biochemical intelligence. Cells evolved Pattern Recognition Receptors (PRRs), ubiquitously distributed in cellular membrane and cytoplasm, functioning as pathogens sensor molecules (Thomas and Schroder, 2013). Once the Gram-negative bacterial PAMP binds to cellular PRR, the infected cell (*i.e.* epithelial cells, airway smooth muscle cells, striated muscle cells, endothelial cells) and phagocytes (*i.e.* macrophages) activate a biochemical waterfall signaling response, with the aim to block and fight bacterial invasion. For these specific molecular "triggers", the organism's defense strategy starts with cellular gene induction and massive synthesis of one of the most powerful neutrophil-tropic molecules: the interleukin 8 (IL-8), also known as *neutrophil chemotactic factor*. This is the molecular main "actor" explaining our findings.

Guided by the cytological microscopical observations, the presence of spheroidal bacterial *soma* was further investigated using microbiological identification assays (VITEK®2 and MALDI-TOF), revealing a polymicrobial pattern characterized by two main subsets of bacterial populations.

The first one ascribes: *Carnobacterium divergens* and *Lactobacillus sakei*. These are meat-associated, Gram-positive lactic acid bacteria species, typical of the altering bacterial microbiota colonizing tissues during the *post mortem* phase (Chaillou *et al*., 2005; Rieder *et al*., 2012).

The second one, the most diagnostically suggestive, revealed the following bacterial species: *Streptococcus suis* and *Sphingomonas paucimobilis*. A recent study demonstrated *Streptococcus suis* as one of the species composing the pig oral and salivary microbiota in normal conditions (Murase *et al*., 2019). The other isolated bacterial species, *Sphingomonas paucimobilis*, is a Gram-negative bacterium constituting the soil and water microbiota.

Focusing our attention on *Streptococcus suis* and *Sphingomonas paucimobilis* and linking together their microbiota-ecological features with the ethology of rooting and grooming behaviour of wild and domestic suids, it is possible to suppose the presence of these two bacterial species on the host's tegument surface (Murase *et al*., 2019). A penetrating trauma, caused by a not-mortal projectile's wound, may have caused a secondary bacterial contamination, justifying isolation of these two species from lesions, representing another proof element to the pathogenetic hypothesis sustained in our study.

Furthermore, one of the isolated bacterial species, *Sphingomonas paucimobilis*, as evidenced by the genus' etymology, expresses sphingosine-1-phosphate, a parietal glycosphingolipid. This molecule represents one of the most powerful molecular "triggers" inducing the synthesis and release of IL-8, the neutrophil chemotactic factor, by the host's cells. This causes neutrophil chemotaxis from blood torrent periphery to the lesion's core, generating an abscess (Heung *et al*., 2006).

The chemotactic effect induced by certain microorganisms is a clear result of the pathogen-host coevolution, another mirable example of biochemical intelligence, which guided the microorganism to develop an intriguing invasion strategy: after pathogen penetrates in the host organism, its molecular "recalls" induce neutrophils' chemotaxis and phagocytosis, inhibit leukocytes cytotoxic phagolysosomal fusion, guaranteeing pathogen' masking, protection and systemic dissemination and transforming neutrophils in a perfect immunological "Trojan horse". A similar mechanism is also used by other bacterial species of inspective interest, such as *Salmonella* spp. and *Mycobacterium* spp. (Kumar *et al*., 2014).

In accordance with the Reg. EU 627/2019, meats obtained from animals with generalized disease, septicemia and toxemia signs are excluded from human consumption. These conditions, diagnosed during the *post mortem* inspection, represent a sanitary risk that impose the total condemnation of the carcass.

In the described lesions, this sanitary risk sensibly increases in the reason of lesions' anatomical and topographical features, which revealed paravascular localization to the cranial vena cava, the right superior phrenic artery and the right pericardiophrenic artery, posing the basis for potential microbial translocation, bacteremia and septicemia.

Furthermore, one of these lesions presented a phreno-abdominal fistula. This condition causes a transperitoneal endotoxic reabsorption, introducing another element further increasing the related sanitary risk.

Conclusions

With a multidisciplinary approach, from macroscopic anatomy to microbiological observations, all the findings presented in this study were collected to rebuild the "pathogenetic puzzle".

The *post mortem* examination represents an essential phase for identification of intradiaphragmatic abscesses, showing the high diagnostic sensitivity of this inspective method.

From the normative point of view, the obligation to confer the carcass (eviscerated) to the game handling establishment and the Competent Authority is limited to the hunted game ungulates destined to the commercialization circuit. Furthermore, in absence of signs detectable during the exam conducted by the "formed person" on hunted game ungulates and implicating a risk for human health, the pluck may be classified as animal by-product and not sent to the game handling establishment and the Competent Authority.

Therefore, for the reasons discussed and argued above, it would be appropriate to integrate and harmonize the mandatory cogent regulations on the inspection of large game carcasses, introducing the obligation to confer the whole pluck to the Competent Authority for all animals destined to any type of consumption (self-consumption, direct sale, commercialization) described by the law.

From the moment that the health of

every single citizen is a collective asset, precious constitutive element of the Public Health, it must be protected in every context, going beyond the private and commercial law's logics, in perfect harmony with the constitutional value of safeguarding Public Health, interpreted as an individual right and an inestimable asset of the Society.

These inspective implications, originating from the observation and interdisciplinary description of the anatomopathological and microbiological features of the lesions, are directly projected on the normative scenario, providing to the legislator useful elements to guarantee the health of the consumer.

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