Salmonella enterica prevalence in finishing pigs at slaughter plants in Northern Italy

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Abstract

Finishing pigs carrying *Salmonella enterica* are believed to be the main source of carcass contamination at the beginning of slaughtering. The aim of this study was to assess the *S. enterica* carrier status of finishing pigs at herd level by sampling pooled faeces on farm and mesenteric lymph nodes at slaughter in the North East of Italy. Environmental faecal samples belonging to 30 batches of pigs were collected on farm. At slaughter, mesenteric lymph nodes were collected from five randomly selected pigs per batch. *S. enterica* was isolated from 16 lymph nodes out of 150 (10.6%) and from seven out of 30 (23.3%) faecal samples. Four batches (13.3%) were positive to *S. enterica* both in lymph nodes and in faeces. The number of batches positive to *S. enterica* either in lymph nodes or in faeces was 13 out of 30 (43.3%). The most prevalent serovars from lymph nodes were *S.* Derby (25.0%) and *S.* Typhimurium monophasic variant 1, 4,[5],12:i:- (18.6%), which were also isolated from faecal material (14.3 and 42.8% respectively). Contaminated faecal material or lymph nodes could be a primary source of carcass contamination at slaughter during evisceration. *S. enterica* contamination is widespread on pig farms and carrier pigs pass undetected the inspection visits at slaughter, entering the food chain. Therefore, in order to control *S. enterica* in pigs, the need to quantify possible risk factors at slaughter and develop effective management strategies on farm is of paramount importance to ensure food safety.

Introduction

Food business operators involved at any level of the food chain have to comply with community and national legislative provisions related to the control of hazards in primary production, including programmes for the monitoring and control of zoonoses and zoonotic agents (Regulation No 852/2004/EC and Directive 2003/99/EC; European Commission, 2003, 2004). One of the main concerns arising from food safety issues is that carrier animals infected by foodborne pathogens may pass undetected the meat inspection procedures, thus entailing risks of contamination for the meat production chain (European Food Safety Authority, 2011b).

Based on surveillance system, the main microbiological threats in developed countries were identified among bacterial zoonoses, such as thermotolerant *Campylobacter*, *Salmonella*, *Yersinia enterocolitica* and verocytotoxin producing *Escherichia coli*, mainly excreted via faeces from sub-clinical animals (European Food Safety Authority, 2011b). Thus, the potential carcass contamination at slaughter becomes not only a major public health but also an economic concern for the food industries (Rostagno, 2009).

In 2011, the number of reported human *Salmonella* cases continued to decrease, and 95,548 confirmed cases (notification rate 20.7 cases per 100,000 population) were reported by 27 European Member States (European Food Safety Authority, 2013). Nevertheless, salmonellosis is still the second most frequently reported zoonosis in Europe. In 2011, 3,344 cases of *Salmonella* were reported in Italy (European Food Safety Authority, 2013). Finishing pigs carrying *Salmonella enterica* are believed to be the main source of carcass and pork contamination at slaughter.

Meat inspection, both *ante* and *post-mortem,* is a key factor of the overall surveillance system for pig health (European Food Safety Authority, 2011b). As part of the meat inspection process, the *ante mortem* inspection aims to prevent diseased animals entering in the food chain. Since sub-clinical animals often pass undetected through the ante and the post-mortem inspections, it is of paramount importance to enforce measures to control the *Salmonella* risk factors in pigs on farm and at slaughter plant in order to reduce the pathogen prevalence in the contaminated carcasses and viscera (Borch *et al*., 1996). In addition, surveillance programs aim to improve the understanding of the sources and the prevalence of *Salmonella* starting from the primary production. Although the eradication of the pathogen is rarely achievable, a reduction of the pathogen load by identifying which factors influence the animal carriage status could help in reducing the risk to human health in an integrated food chain plan (Milnes *et al*., 2009).

The aim of this study was to assess the *S. enterica* carrier status of finishing pigs at herd level and at slaughter by sampling pooled faeces on farm and mesenteric lymph nodes at slaughter in the North East of Italy.

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Materials and Methods

Study and design sample

The entire study was carried out according to the routine commercial activity of each slaughter plant without interfering with their operations either on farm or at slaughter plant facility. Only finishing pigs from the slaughter plants were included in the study. Sampling was carried out from April to November 2012.

Three pig slaughter plants, named as I, II and III, were selected in the North East of Italy on the basis of their willingness to cooperate and taking into account the variability of the distribution of the types of farming systems. From slaughter plant I and III, five farms were selected and two batches of pigs per farm were included in the study. From slaughter plant II four farms were selected instead of five, because we tested four batches of pigs from one farm y (Table 1). A total of 14 different farms (named A-P) were included in the study and 30 batches of pigs were tested. All the farms supplying pigs to slaughter plants I and II and one farm supplying pigs to slaughter plant III were intensive farms, rearing pigs for the Parma ham protected designation of origin (PDO) production system. The semi free range farms produced extensive *Nero di Parma* pigs. Each batch of pigs was composed by animals living in several pens. The average size of the farms was 6351.7 (SD 5020.8) and ranged from 13 to 16,000 pigs. The total number of pens that were included in the study was 159 with an average number of pens per farm of 5.3 (range: 1-11 pens).

On-farm environmental faecal samples collection

Environmental faecal samples belonging to the 30 batches of pigs were collected on farm the week before slaughtering. From each batch, one sample of faeces of at least 25 g was collected in each corresponding pen, according to the Commission Decision (EC) 55/2008 (European Commission, 2008). Thirty samples of faeces were collected in total. Faecal sampling was carried out by using sterile equipment. Sampling material was transported to the laboratory under controlled conditions and processed on the day of arrival.

Samples collection of the mesenteric lymph nodes at slaughter plant

On the day of slaughtering, the selected batch was sampled along the slaughter line. A minimum of 15 g of mesenteric lymph nodes (from at least five nodes) was collected from each of the five randomly selected pigs. Overall, 150 mesenteric lymph nodes samples were collected. Mesenteric lymph nodes were aseptically removed, placed in a sterile bag and transported to the laboratory under controlled conditions and processed on the day of arrival.

Isolation of *Salmonella enterica*

Mesenteric lymph nodes were first rinsed with sterile water. A 10 g- aliquot of mesenteric lymph nodes was processed according to the ISO 6579:2002 method (ISO; 2002a). A 10 g-aliquot of environmental faecal samples was analysed following the ISO 6579:2002/Amd 1:2007 method, Annex D (ISO, 2002b).

Xylose-Lysine-Desoxycholate (XLD) agar and Brilliant Green Agar were used as selective media showing typical *Salmonella* reactions were further tested with API® 20E micro-substrate system (bioMérieux, Marci l'Etoile, France). *Salmonella enterica* serotyping was performed according to the White-Kauffmann-Le Minor scheme by slide agglutination with O and H antigen specific sera by the Istituto Zooprofilattico della Lombardia ed Emilia Romagna (Parma, Italy).

Results

Results are shown in Table 2. A total of 16 lymph nodes out of 150 (10.6%) was positive for *S. enterica*. A total of 7 out of 30 (23.3%) batches tested positive for *S. enterica*. Four batches (13.3%) were positive to *S. enterica*, in lymph nodes and faeces. The number of batches positive to *S. enterica* either in lymph nodes or in faeces was 13 out of 30 (43.3%). The serovars isolated from the lymph nodes were the follow-

Table 1. Study design.

Slaughter plant	Farm	Batch	Type
I	A	2	Intensive
	B	2	Intensive
	C	2	Intensive
	D	2	Intensive
	E	2	Intensive
II	F	2	Intensive
	G	2	Intensive
	H	2	Intensive
	I	4	Intensive
III	L	2	Semi-free range
	M	2	Organic
	N	2	Semi-free range
	O	2	Organic
	P	2	Intensive
Total	14	30	

Table 2. Prevalence of *Salmonella enterica* in faeces and lymph nodes in finishing pigs.

Slaughter plant	Batch	Farm	Presence of *Salmonella* in faeces	No. of pigs positive to *Salmonella enterica* in lymph nodes	No. of *Salmonella enterica* positive batches
I	1	A	-	0	0
I	2	A	-	1	1
I	3	B	+	0	1
I	4	B	-	0	0
I	5	C	-	0	0
I	6	C	-	0	0
I	7	D	-	0	0
I	8	E	-	0	0
I	9	E	-	0	0
I	10	D	-	0	0
II	11	F	-	3	1
II	12	F	-	0	0
II	13	G	-	0	0
II	14	G	-	0	0
II	15	H	-	2	1
II	16	H	-	0	0
II	17	I	-	1	1
II	18	I	-	0	0
II	19	F	-	1	1
II	20	F	+	0	1
III	21	L	+	0	1
III	22	M	+	1	1
III	23	N	-	0	0
III	24	N	-	0	0
III	25	M	-	1	1
III	26	L	+	3	1
III	27	O	+	1	1
III	28	O	+	2	1
III	29	P	-	0	0
III	30	P	-	0	0
Total	30	14	7 (23.3%)	16 (10.6%)	13 (43.3%)

ing: 4 *S.* Derby, 2 *S.* London, 2 *S.* Give, 2 *S.* Rissen, 2 *S.* Typhimurium, 3 *S.* Typhimurium monophasic variant 1, 4,[5],12:i:- and 1 *S.* Braenderup. The serovars isolated from the faecal samples were: 3 *S.* Typhimurium monophasic variant 1, 4,[5],12:i:-, 2 *S.* London, 1 *S.* Derby and 1 *S.* Give.

Discussion and Conclusions

In our study 23.3% of the environmental faecal samples and 10.6% of the mesenteric lymph nodes were *Salmonella*-positive. Many authors reported different values of prevalence from ileocaecal lymph nodes in different countries (Methner *et al*., 2011; Vico *et al*., 2011; Visscher *et al.*, 2011). All *Salmonella*-positive finishing pigs tested in this study entered in the food chain. They were sub-clinical carriers of *S. enterica*, since they were subjected to the *ante* and *post mortem* inspection and no symptoms of salmonellosis were observed. It can be concluded that the *ante* and *post mortem* inspections is not accurate indicators of the *S. enterica* status on farm and at slaughter. *Salmonella*-positive faecal material or lymph nodes from these carrier animals could be a primary source of carcass contamination during evisceration at slaughter.

In line with Visscher *et al.* (2011), we consider that the types of sample used in our study are good epidemiologic indicator of the presence of *Salmonella enterica* both on farm (environmental faecal sampling representative of individuals living in a pen) and at slaughter plant (mesenteric lymph nodes representative of the entire batch). In fact environmental faecal samples are the most appropriate tool to assess the hygienic status of a herd since the ubiquitous *S. enterica* are resistant in pigs environment and can be representative of several individuals living in a pen (Belœil *et al*., 2004).

Berends *et al*. (1996), Borch *et al*. (1996) and Botteldoorn *et al*. (2003) estimated that ca 30% of the pork carcass contamination is due to the carrier pig itself, and the remaining is caused by cross-contamination at the slaughter plant. It may be due to the hygienic procedures of the slaughter plant (Botteldoorn *et al*., 2003), which determine the presence on the resident slaughter plant bacteria (Gebreyes *et al*., 2004).

In our study the higher rate of positive match between environmental faecal samples and lymph nodes for *S. enterica* was observed in organic straw bedded and semi free range farms only. Similarly, Smith *et al*. (2011) found a higher prevalence of *Salmonella* in outdoor farming systems rather than indoor ones. Jensen *et al*. (2004) in fact were not able to demonstrate that organic rearing conditions were protective for pigs against *S. enterica* infections, as a high infection rate was found by the authors. In the same study the pathogen survived in the paddock environment for several weeks, and even an estimated low level of *S. enterica* was unable to protect newly introduced animals (Jensen *et al*., 2004). Therefore, the high rate of positive match between *Salmonella*-positive faecal samples and lymph nodes observed in our study could be explained by the close contact between pigs in the hut and by their rooting behaviour, which likely pose pigs at a high risk of ingestion of *S. enterica* from the contaminated environment. Magistrali *et al*. (2008) recognised the importance of the *Salmonella* contaminated environment as source of *S. enterica* infections in pigs. In fact *S. enterica* is able to survive in soil, water and on a variety of surfaces which gives the bacterium an increased likelihood of infecting new hosts; enhanced survival of *Salmonella* is favoured by a cyclic transmission from external environment to a new host (Winfield and Groissman, 2003).

S. Typhimurium and *S.* Derby are widespread and relevant in most European Member States, while other serovars, such as *S.* London, *S.* Infantis or *S.* Rissen are frequently isolated from pigs in some countries only, therefore their relevance cannot be generalised to the whole European Union (European Food Safety Authority, 2011a). In our study the most prevalent serovars were *S.* Derby in mesenteric lymph nodes and *S.* Typhimurium monophasic variant 1, 4,[5],12:i:- in the faecal material, confirming previous data (European Food Safety Authority, 2011a). In line with our results, Wilkins *et al*. (2010) observed an association between *S.* Derby and the growing-finishing pigs in Canada. Similarly, Belœil *et al*. (2004) reported a higher prevalence of *S.* Derby (51.4%) and *S.* Typhimurium (37.8%) in finishing pigs in France; De Busser *et al*. (2011) identified as the predominant serotypes *S.* Typhimurium (58.7%) and *S.* Derby (17.4%) in pigs in Belgium; according to Visscher *et al.* (2011) *S.* Typhimurium (87.0%), was the predominant serovar in Germany. *S.* Typhimurium monophasic variant 1, 4,[5],12:i:-, which was detected both in animal and environment-related samples, has already been reported by many EU countries (European Food Safety Authority, 2010). In Spain, Vico *et al*. (2011) detected emerging monophasic variant *S. enterica* 1, 4,[5],12:i:- multi antibiotic-resistant isolates. Therefore, this emerging serotype requires a strict and continuous surveillance update in Europe.

S. enterica contamination is widespread on pig farms and carrier pigs usually pass undetected the inspection at slaughter, entering in the food chain. Therefore, in order to control *S. enterica* in pigs, we need to quantify possible risk factors at slaughter and to develop effective management strategies on farm to ensure food safety. Risk factors on farm include all-in/all-out systems, rodent control, no access of pets and birds, visitor hygiene and no close contact with other production animals. Moreover, farmers should avoid the introduction of subclinically infected pigs and implement the above mentioned *Salmonella*- control procedures. Nevertheless, hygiene standards of pork carcasses at slaughter continue to be essential to control the risk of *Salmonella* contamination.

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