Trend of *Salmonella enterica* occurrence and serotypes in Sardinian pig slaughterhouses

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Abstract

The aim of this study was to evaluate *Salmonella* prevalence and serotypes in four Sardinian pig slaughterhouses. Moreover, a population study was conducted with pulsed field gel electrophoresis (PFGE). The results were compared with previous investigations carried out during years 2008 and 2014. A total of 147 samples were collected, 117 from slaughtered pigs (lymph nodes, colon content and carcass surface) and 30 from the slaughterhouse environment (surfaces in contact and not in contact with meat). *Salmonella* was isolated from 3.4% pig samples and was not detected from environmental samples. Comparing the results with those of previous investigations, occurrence showed a sharp decrease through the years in both animals (18.8% in 2008, 10% in 2014 and 3.4% in 2020) and environmental samples (34.1% in 2008, 3.7 in 2014, and 0% in 2020). At the same time, prevalence of carriers (pigs positive at lymph nodes and/or colon content level) showed a reduction through the years and was always lower in animals coming from local farms rather than those coming from other European Member States, probably indicating the role of stressful factors as transport in increasing *Salmonella* susceptibility and shedding.

Salmonella serotypes were monophasic Typhimurium, Rissen and Muenchen. Overall, 13 different *Salmonella* serotypes were identified during the three surveys with the most prevalent being serotypes often isolated from slaughtered pigs and during human salmonellosis cases: *S.* Derby and *S.* Typhimurium in 2008, *S.* Anatum and *S.* Rissen in 2014, monophasic *S.* Typhimurium in 2020. Population study with pulsed field gel electrophoresis showed a high similarity between *Salmonella* strains belonging to the same serotype. The results of the investigations showed a decrease of *Salmonella* occurrence during twelve years in Sardinia, probably due to the improvement in the application of correct GMPs and GHPs at slaughterhouse and also to a reduction of the rate of carrier pigs at farm level.

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Introduction

According to the European Food Safety Authority (EFSA) and European Center for Disease Prevention and Control (ECDC) *Salmonella* was the second most common zoonotic pathogen in 2018, with 91.857 cases of human infection, 1.580 outbreaks originated from food (EFSA & ECDC, 2019). Among food of animal origin, pig meat and related products represent an important source of salmonellosis. According to the EFSA and ECDC report, 6.8% serotypes isolated in salmonellosis outbreaks in Europe in 2018 originated from pigs and pig meat, with a prevalence of *S.* Derby and monophasic *S.* Typhimurium. The same serotypes, along with *S.* Enteritidis, *S.* Typhimurium, and *S.* Infantis, are among the most frequently associated with human salmonellosis cases (EFSA & ECDC, 2019).

Salmonella can contaminate meat and meat products directly or indirectly, from both the animal itself and the processing environment (Arguello *et al.*, 2012; Pires *et al.*, 2014). Pigs become infected via the fecal-oral route and infected animals usually asymptomatically host the pathogen in the palatine tonsils, in the intestine and in the Gut-Associated Lymphoid Tissue, GALT (Bonardi *et al.*, 2013).

The prevalence of infected pigs can increase during the transport from the farm to the slaughterhouse (Nowak *et al.,* 2007). During these phases, both a renew of latent infections and an increased susceptibility to infection are observed (Hurd *et al.,* 2002). It is assumed that this phenomenon is due to the release of catecholamines in association with stress, with a consequent reduction in gastric acid production and an increase in intestinal motility, which raise the probability of survival of *Salmonella* and its replication in the intestine (Ferrer Savall *et al.*, 2016; Zimmerman *et al.*, 2019). Feed withdrawal, handling, loading, mixing with unfamiliar pigs, high densities and changes in the environment are important factors that promote stress during the transport phases (Isaacson *et al.,* 1999; Arguello *et al.,* 2013). Considering the highly contaminated transport environment and high stocking densities, the contact with excretory pigs and contaminated surfaces is an important source of contamination for healthy pigs (Arguello *et al.,* 2013). The transport time may also play a role: various authors found an increase in the prevalence of *Salmonella* in faeces after different transport times, concluding that the longer the transport time, the higher the level of excretion of *Salmonella* (Kasbohrer *et al.,* 2000; Arguello *et al.,* 2013; Massacci *et al.,* 2020).

Afterwards, the rest period in holding pens is a further moment in which *Salmonella* contamination can occur: during lairage, many of the same stressors present during transport (unfamiliar environment, high densities, handling) are exacerbated; newly acquired infections or recrudescence of existing infections can result in high shedding of *Salmonella* during this period (Swanenburg *et al.,* 2001). These factors result in an increase in the prevalence of infected pigs and rises the probability of introducing *Salmonella* into the slaughter line (Hurd *et al.,* 2002). Different studies showed that *Salmonella* could be isolated from lymph nodes and intestinal contents as early as 3 hours after infection, which allows animals to become infected during transport and/or lairage (Swanenburg *et al.*, 2001; Mannion *et al.*, 2012). Consequently, contaminated pigs at slaughter are most likely carrier pigs that are experiencing a recrudescence of infection and/or pigs that became infected during transport or in the lairage through contact

with *Salmonella*-shedders (Boughton *et al.,* 2007; Massacci *et al.,* 2020). *Salmonella*-shedding increases the risk of carcass contamination in the slaughterhouse, thus increasing the risk of meat contamination (Pesciaroli *et al.,* 2017).

The slaughtering operations themselves can also influence the carcass contamination level: hair removal, brushing and evisceration represent critical phases that can easily determine pathogen spread on the same carcass and to others, as well as on the slaughtering equipment and environment (De Busser *et al.*, 2013). Besides, the slaughtering process does not include any point where the risk can be totally eliminated (Borch *et al.*, 1996). *Salmonella* is also capable of forming biofilms and environmental strains may be responsible for carcass contamination along the slaughter line (O'Leary *et al.*, 2013).

Moreover, carcass contamination could occur during sanitary meat inspection: palpation and incision performed during post mortem inspection can cause the spreading of the pathogen. For this reason, in 2011 EFSA adopted a Scientific Opinion which concluded that, because of the risk of microbial cross-contamination, palpation/incisions laid down in Regulation (EC) No. 854/2004 should be omitted in pigs subjected to routine slaughter and limited to suspect pigs identified through food chain information/ante-mortem inspection and/or post-mortem visual detection of relevant abnormalities and where it would lead to risk reduction (EFSA, 2011). Therefore, Annex 1 of Regulation (EC) No. 854/2004 was first amended by Regulation (EU) No. 219/2014, as regards the specific requirements for post-mortem inspection of domestic swine, and subsequently repealed by Regulation (EU) 2017/625. At the present, the post-mortem inspection performed by an official veterinarian is exclusively visual in pigs, with the application of palpation/incisions when there are indications of a possible risk to human health, animal health or animal welfare.

Considering the significant worldwide importance of *Salmonella* as a food-borne pathogen, the control of this agent in the pig food chain is a crucial food safety element. With these considerations, the slaughterhouse is the most appropriate point of the food chain to carry out epidemiological studies on zoonotic agents. Moreover, it allows the collection of many and different types of samples such as lymph nodes, colon content, carcasses and environmental surfaces both in contact and not in contact with meat (Astorga Marquez *et al.*, 2007; Bolton *et al.*, 2013).

Molecular typing methods such as pulsed field gel electrophoresis (PFGE) and sequencing can be used to obtain information on meat contamination sources and trace the strains spreading along the slaughter line.

Pig slaughtering plants in Sardinia are medium-small sized, with a capacity between 50 and 100 pigs/hour; most of the slaughterhouses work for 1-2 days a week and usually a single batch of pigs is processed during one working day, considering a group of animals coming from the same farm as a batch. To the best of our knowledge, little studies on the *Salmonella* occurrence in pig samples collected at slaughterhouses in Sardinia exist. Therefore, the main objective of this study was to evaluate *Salmonella* prevalence and serotypes in four Sardinian pig slaughterhouses. With the aim to evaluate *Salmonella* trend during the years in Sardinia, the results were compared with previous investigations carried out during years 2008 (Piras *et al.*, 2011) and 2014 (Fois *et al.*, 2017). Moreover, a population study using pulsed field gel electrophoresis (PFGE) was conducted.

Materials and methods

Slaughterhouses selections

The study was carried out in 2020. The samplings were conducted in four slaughterhouses (SA, SF, SH, SL) located in different areas of Sardinia (Italy).

Abattoirs were comparable in terms of slaughtering amount and adopted techniques and procedures: the scalding procedure was performed in tanks using recirculating water with a temperature of 62-63°C; the flambing was carried out at temperatures of 1000°C for 10-15 sec, so that the temperature of the carcass surface reached about 100°C. Finally, intestines were manually removed.

Samples were collected during each sampling session from slaughtered adult pigs randomly chosen in the middle of a workday and belonging to the same batch.

Samples collection at the slaughterhouse

Different kind of samples were collected with different objectives. Lymph nodes and colon content give information about the carrier status of the pig population. Carcass surface allows assessing the proportions of self- and cross-contamination during carcass dressing operations. Finally, samples taken from slaughterhouse surfaces in contact and not in contact with meat provide data about environmental contamination caused by incorrect application of hygiene and slaughtering procedures.

In accordance with the EFSA Opinion "Risk assessment and mitigation options of *Salmonella* in pig production" (2006), the following pig samples were collected immediately after the evisceration:

- mesenteric lymph nodes: at least five lymph nodes in the ileo-caecal regions were cut out with a sterile, disposable scalpel and collected in a sterile plastic bag;
- colon content: colon was incised with a sterile, disposable scalpel and at least 25 g of its contents were collected in a sterile plastic bag;
- carcass surface: samples were taken after evisceration but before chilling by means of a non-destructive method with a sterile sponge pre-moistened with 10 ml of sterile Buffered Peptone Water (BPW, 3M Health Care, Milan) at the following points: ham, loins, abdomen and throat (ISO 17604: 2015, Reg. CE No. 2073/2005). Sampling was carried out using the same sponge for the four points, with a sterile 10x10 cm^2 delimiter (Copan, Brescia, Italy), proceeding from the least contaminated point (ham) to the most contaminated (throat). The sponges were handled with a sterile glove and placed inside sterile sponge bags.

As regards environmental samplings, the following samples were collected at the end of the slaughtering operations:

- food contact surfaces: including cutting equipment (knives) and hair removal equipment (brushes or whips). These surfaces were sampled using a sterile sponge.
- non-food contact surfaces: including walls near stunning and killing area, walls and drain surface of the pre-chilling area. These surfaces were sampled using a sterile sponge and a sterile delimiter (10x10 cm^2). The drain surface in the prechilling area was sampled using a sterile sponge.
- scalding water: approximately 100 mL of scalding water were collected using a sterile sampler (Bibby Scientific Limited, Stone, UK).

All the samples were transported to the laboratory at +4 °C and processed within 24 h after collection. Overall, 147 samples were collected (117 from adult pigs and 30 from slaughterhouse environment).

With regard to the origin and the farming method of the animals, in 2020 all tested pigs came from Sardinia, and between these, 10/39 (25.6%) were from intensive farms, while 29/39 (74.3%) were from extensive farms.

Salmonella enterica isolation and serotypes

All the samples were tested for the presence of *Salmonella* according to ISO 6579-1:2017. At least three typical *Salmonella* colonies were selected and tested for biochemical properties with the API ID 32E system (bioMerieux, Marcy l'Etoile, France). Isolates confirmed as *Salmonella* was serotyped by slide agglutination test as reported in ISO/TR_6579-3 and the name attributed according to the Kauffmann-White-Le Minor scheme.

Comparison of results with previous investigations

Results about *Salmonella* prevalence and serotypes were compared with two previous investigations carried out in Sardinia: the first investigation (Piras *et al*., 2011) was conducted between 2006 and 2008 in five slaughterhouses (SA, SC, SD, SE, SI), the second one (Fois *et al.*, 2017) between 2013 and 2014 in 9 slaughterhouses (SA, SB, SD, SF, SG, SH, SI, SL, SM).

Pulsed field gel electrophoresis (PFGE)

PFGE analysis was performed using the international standardized protocol from PulseNet US (http://www.cdc.gov/pulsenet/pathogens/salmonella.html). In total, n. 59 *Salmonella* strains were selected: four from the present investigation, 30 from 2008 survey and 17 from 2014 survey. Moreover, eight *Salmonella* strains isolated during 2016 from wild boars' population, coming from Asinara National Park (an island off the coast of Sardinia) and regularly slaughtered, were submitted to the analysis in order to evaluate possible epidemiological correlation among strains isolated from pigs and boars. In order to avoid over representation, strains were selected between the serotypes that were more frequently isolated over the three investigations, including isolates from both pig and environmental samples and excluding clones. Strains were submitted to PFGE and the comparison performed by BioNumerics software v7.1 (Applied Maths, Saint-Martens-Platen, Belgium). Cluster analysis was performed by the Dice similarity coefficient, with 1% optimization and 5% tolerance using the unweighted pair group method with arithmetic mean (UPGMA).

Results

Salmonella occurrence and serovars in pig and environmental samples

Overall, *Salmonella* was isolated in 4/117 (3.4%) pig samples. The microorganism was detected in 1/39 (2.6%) lymph nodes samples, 2/39 (5.1%) colon content, each one isolated from three different carrier pigs, and 1/39 (2.6%) carcass surface. *Salmonella* was never detected from environmental samples.

Serotypes

Three serovars were detected among n. 10 strains: monophasic *S.* Typhimurium was the most prevalent (4/10; 40%) followed by *S.* Muenchen and *S.* Rissen (both 3/10; 30%).

Comparison of prevalence and serotypes results with previous investigations

Comparing the results about *Salmonella* prevalence with the previous investigations conducted in Sardinia, it was noticed a decrease during the years. In fact, the highest occurrence was observed in 2008 with 64/340 positive samples (18.8%), in 2014 *Salmonella* was isolated in 65/644 samples (10%) and in 2020 in 4/117 (3.4%). The decrease was observed for all the analysed samples. In fact, in lymph nodes samples occurrence was 26/85 (30.5%), 16/161 (9.9%) and 1/39 (2.6%) respectively during 2008, 2014 and 2020. As regard colon content, *Salmonella* was isolated with an occurrence of 14/85 (16.4%) in 2008, 19/161 (11.80%) in 2014 and 2/39 (5.1%) in 2020. Carcass surface showed a prevalence of 12/85 (14.1%) in 2008, 14/161 (8.7%) in 2014, and 1/39 (2.6%) in 2020 (Table 1).

In parallel, prevalence of carriers gradually decreased during the surveys: in 2008, 31/85 (36.5%) pigs were identified as carriers because of identification of *Salmonella* in lymph nodes and/or colon content. During 2014, *Salmonella* was detected in lymph nodes and/or colon content samples of 27/161 (16.7%) carrier pigs and during 2020, 3/39 (7.7%) pigs were identified as carriers.

As regard the origin of the carriers, during 2008, 11/31 (35.5%) pigs were from local farms and 20/31 (64.5%) were from other European Member States (France, Spain, The Netherlands). The same trend was confirmed during 2014 when, between 27 carriers, 4/27 (14.8%) were from local farms and 23/27 (85.2%) were from farms located in other European Countries. During 2020, 3/39 (23%) pigs were carriers and all were from local farms.

The decrease in *Salmonella* prevalence also included the environmental contamination level. In 2008 *Salmonella* was detected in 14/41 samples with an occurrence slightly higher in non-food contact surfaces (37.5%), rather than in food contact surfaces (35.2%). In a slaughterhouse, *Salmonella* was isolated in all the drain water samples, in one dehairing equipment sample and in one carcass splitter sample; in another abattoir the pathogen was detected in all environmental samples, including a scalding water sample. In 2014 *Salmonella* was detected only in 4/108 (3.7%) environmental samples: two samples collected from the clean zone (wall and drain), one from the splitting equipment and one drain sample. In 2020, no positive sample was collected from slaughterhouse environment.

Overall, 13 serotypes were identified through the years, with several of them isolated during more than one survey. In particular, *S.* Rissen was identified during the three surveys, *S.* Derby, *S.* Typhimiurium and *S.* Bredeney during 2008 and 2014 surveys. Finally, monophasic *S.* Typhimurium was identified during 2014 and 2020 inves-

Table 1. Positive samples/total and occurrence (%) of *Salmonella* in the tested samples during the three surveys.

Samples	Pos/Tot (%)
Pig samples	4/117 (3.4)
Lymph nodes	1/39 (2.6)
Colon content	2/39 (5.1)
Carcass surface	1/39 (2.6)
Environmental samples	0/30
Surfaces in contact with meat	0/10
Surfaces not in contact with meat	0/15
Scalding water	0/5
Total	4/147 (2.7)

tigations. Table 2 shows the distribution of the *Salmonella* serotypes detected in pig and environmental samples during the three investigations.

PFGE

*Xba*I digestion yielded from 12 to 15 bands. Between 21 *S.* Derby strains, eleven PFGE profiles were identified. Dendrograms based on similarity value are presented in Figures 1 and 2. The 21 strains were grouped into two major clusters designated as Derby 1 and Derby 2, with a similarity value above 88.6%. The Derby 1 cluster comprised 14 strains isolated from the surveys 2008 and 2014 (animals and slaughterhouse environment) from four different slaughterhouses: SA, SC and SD in 2008 and F in 2014. In the same cluster were included three strains isolated during 2016 in the wild boars survey (animals and slaughterhouse environment) at slaughterhouse SA (data not published). in the Derby 2 cluster were included 7 strains isolated from three different slaughterhouses: SA and SD during 2014 and SF during 2014.

Among n. 16 *S.* Typhimurium strains, fourteen PFGE profiles were identified; a dendrogram is shown in Figure 2. Strains were grouped in three major clusters (Typh 1, Typh 2, Typh 3) with a similarity value above 86.1%. Typh 1 cluster included six strains isolated from three different slaughterhouses: SE and SC in 2008 and SH in 2020. Typh 2 cluster comprised five strains isolated only during 2008 from slaughterhouses SC, SD and SE. Finally, cluster Thyp 3 comprised five strains: one detected during 2008 from slaughterhouse SC and the others during 2014 from slaughterhouses SD and SG.

Between 10 *S.* Rissen strains, five profiles were identified and grouped into two major clusters (Ris 1 and Ris 2) with a similarity value above 85.8 %. Cluster Ris 1 comprised five strains that were detected during 2008 from slaughterhouse SC and during 2014 from slaughterhouse SD. Cluster 2 comprised five strains detected during 2014 from slaughterhouse SD and during 2020 from slaughterhouse SF. Among six *S.* Newport strains, two different profiles were identified, one from a strain detected during 2008 and the other from strains detected from wild boars in 2019. The three *S.* Bredeney strains were assigned to three different PFGE profiles, while the three *S.* Muenchen strains showed the same PFGE pattern.

Discussion

The results of this survey demonstrate that the overall occurrence of *Salmonella* in slaughtered pigs and environmental abattoir samples in Sardinia showed a progressive reduction through the years. In fact, occurrence decreased from 16.8% in 2008 to 8.8% in 2014 and 3.4% in 2020. This finding is probably due to two main reasons: the correct application of GMPs and GHPs in slaughterhouses and the decrease of carrier pigs.

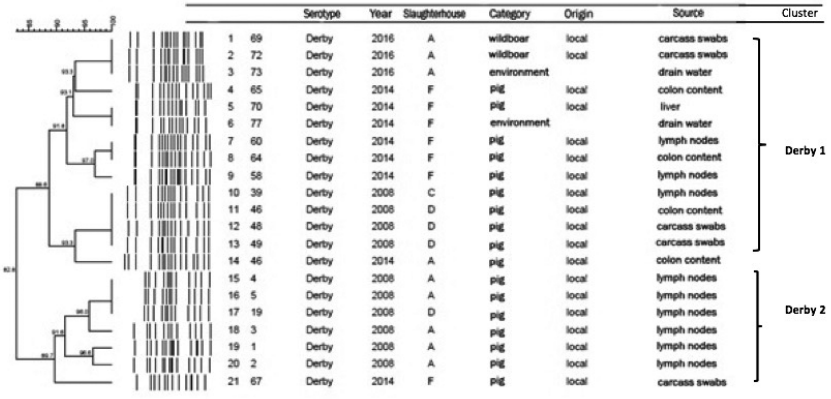

Figure 1. *S. Derby* dendrogram based on similarity value.

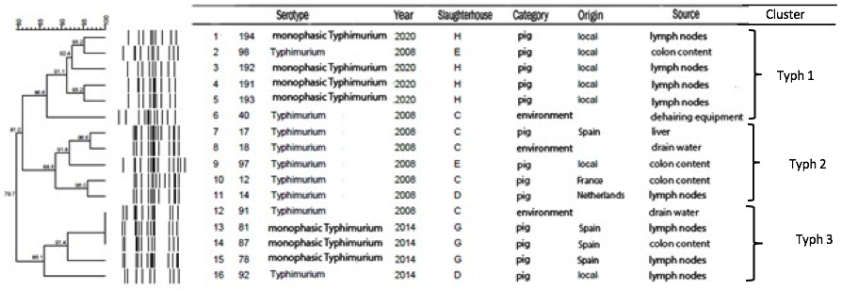

Figure 2. *S. Typhimurium* dendrogram based on similarity value.

Table 2. Distribution of *Salmonella* serotypes in pig and environmental samples during the three surveys.

Survey	Pig samples				Environmental samples	
	Lymph nodes	Colon content	Carcass	Liver	SC	SNC
2008	Derby Typhimurium Livingstone Bredeney Newport	Derby Typhimurium Panama Infantis Rissen	Derby Typhimurium Panama	Derby Typhimurium Panama Rissen	Derby Typhimurium	Derby Typhimurium
2014	Typhimurium mono Typh Rissen Anatum	mono Typh Rissen Anatum Derby	Rissen Anatum Bredeney	Rissen Anatum Holcomb	Anatum Bredeney	Anatum Bredeney
2020	mono Typh	Rissen Muenchen	0	nt	0	0

SC: surfaces in contact with meat; SNC: surfaces not in contact with meat; mono Typh.: monophasic Typhimurium nt: not tested.

The first one is the ever-growing application of correct GMPs and GHPs in slaughterhouses. As the main source of *Salmonella* contamination in the slaughterhouse are the conferred pigs, the implementation of correct hygiene measures is of great importance to obtain a reduction of *Salmonella* prevalence in carcasses and meat. Therefore, the adoption of adequate control measures are effective to counteract *Salmonella* spreading and persistence in the plant and in the slaughter line (Reg. EC No. 2073/2005). As we could observe through the years, important improvements have been made by FBOs as regard both GMPs and GHPs, such as slaughtering of clean animals only, frequent substitution (even if partial) of the scalding water during the slaughtering day, accurate staff training which reflects in more accurate operations during slaughtering and evisceration steps. This was evident in the reduction of *Salmonella* prevalence at carcass level in slaughterhouses tested during different surveys. The percentage of positive pig carcasses at slaughterhouse SA was 13.3% in 2008, 2.7% in 2014 and 2.2% in 2020. Similarly, slaughterhouse SF showed a prevalence of positive pigs of 46.2% in 2014 and 3.3% in 2020. At the same time, *Salmonella* prevalence in environmental samples showed a substantial reduction, going from an occurrence of 31.7% in 2008, to 3.7% in 2014 to 0% in 2020. Moreover, the post-mortem inspection performed by official veterinarians exclusively through visual examination in pigs (Reg. EC No. 854/2004 as amended by Regulation EU No. 219/2014; Reg. EU No. 2017/625) could have played a role in the reduction of *Salmonella* spread and cross-contamination through inspection cuts.

The second reason explaining the reduced *Salmonella* occurrence over time could be a decrease of the numbers of carrier pigs (*i.e.* positive at lymph nodes and/or colon content level), which in our investigations corresponded to 36.5% in 2008, 16.7% in 2014 and 7.7 % in 2020. The reduction in the occurrence of carrier pigs may depend on better application of biosecurity measures at farm level.

According to the survey conducted on pig farms in the EU in 2008 (EFSA, 2009), Italy was with the highest prevalence of the pathogen in pig's population both in breeding (51.2%) and in production farms (43.9%). However, during the past years numerous steps forward have been made in the pig farming practices, particularly in management (implementation of closed farms, batch farrowing and all–in/all–out housing, no mixing of pigs from different sources, appropriate cleaning and sanitizing practices, nutrition and vaccination) (Andres & Davies, 2015; De Lucia & Ostanello, 2020). These measures have been implemented in Sardinian pig farms in the context of the African Swine Fever eradication plan 2015–2018 that aimed to eliminate free–ranging pigs and incentivize good practices of swine breeding (PE–ASF15–18; Regional Decree Number 5/6, 6 February 2015) and that may have resulted sideways in a successful control of *Salmonella* and in a lesser occurrence of the pathogen at the slaughterhouse.

In our survey, the highest *Salmonella* occurrence was always observed in lymph nodes samples, followed by colon content and carcass surface. These results are in accordance with data reported by other authors that showed *Salmonella* occurrence in lymph nodes ranging between 7.4% (Arguello *et al.*, 2013) and 19.9% (Bonardi *et al.*, 2015) in EU countries. Other studies reported an occurrence in colon content samples from 19% (Botteldoorn *et al.*, 2004) to 24.9% (Visscher *et al*., 2011). Subclinically infected animals typically exhibit intermittent excretion of low numbers of *Salmonella* in their faeces and in those animals the pathogen can be isolated from intestinal lymph nodes, reflecting a localized intestinal infection or a previous exposure. Therefore, the detection of *Salmonella* in these samples is indicative of the pig carrier state at slaughter (EFSA, 2006). Moreover, all animals had shown no symptoms during ante and post-mortem inspection. These results showed that the ante and postmortem inspection are not adequate measures to indicate the pig status regarding *Salmonella* infection, in accordance to what stated by EFSA (2011).

As observed for the other investigated samples, *Salmonella* occurrence on carcass surface showed a constant decrease, from 14.1% in 2008 to 8.7% in 2014 and 2.6% in 2020. Different levels of prevalence have been detected in pig carcasses in EU countries, ranging from 3.1% (Pala *et al.*, 2019) to 17.8% (Bonardi *et al.*, 2016). Carcasses of pigs may be cross-contaminated by either *Salmonella*-positive pigs previously slaughtered on the same day, or by contaminated slaughter equipment (EFSA, 2006); such equipment can also be contaminated from *Salmonella* positive pigs slaughtered on the same day, but data strongly suggest that residual and/or persistent contamination of the equipment is an important source, also considering the biofilm development ability of *Salmonella* strains (O'Leary *et al.*, 2013; Piras *et al*., 2015). Taking into account that contaminated carcasses are the main source of *Salmonella* in the pork meat processing industry, as well as meat cutting laboratories and sausage production plants (Pala *et al.*, 2019), the development and implementation of effective hygiene and processing strategies in abattoirs is of paramount importance to ensure food safety. Cross-contamination of pig carcasses at slaughterhouse could have occurred in the 2014 survey, in which 21.5% of slaughtered pigs were positive at carcass level but not in their lymph node and/or colon content. In our most recent study (2020), only one pig carcass was found positive, thus probably indicating the effectiveness of a correct application of GMP and GHP.

As said before regard the origin and the farming method of the animals, in 2020 all tested pigs came from Sardinia, while in our previous surveys also pigs coming from Member States were tested, with an overall rate of 210/285 (73.7%) of tested pigs coming from Sardinia and, between these, 40/210 (19%) from intensive farms, and 170/210 (81%) from extensive farms. Finally, 75/285 (26.4%) pigs were imported from Member States (France, Netherlands, Spain) and all of them were from intensive farms. In 2008 and in 2014 there was an evident prevalence of *Salmonella* carrier pigs coming from other European Member States (France, Spain, Holland), thus travelling for long distances and time, respect to pigs coming from local farms. Different studies conducted through the years (Fravalo *et al.*, 1999; Verbrugghe *et al.*, 2016; Pesciaroli *et al.*, 2017) demonstrated that stressful factors occurring during transport and waiting in lairage (rough handling, high stock densities, unfamiliar animals and environment, long travels) increase pigs sensitivity to *Salmonella* and the number of shedding animals from farm to slaughtering plant.

The most prevalent serotypes have been isolated during our survey along the years, such as *S.* Derby (2008 and 2014), *S.* Typhiumurium, monophasic *S.* Typhiumurium (2008, 2014 and 2020) and *S.* Rissen. This finding is consistent with other studies (Bonardi *et al.*, 2015; Barilli *et al.*, 2018; EFSA and ECDC, 2019; Pala *et al.*, 2019) and is most likely due to the ability of these *Salmonella* serotypes, especially Typhimurium, to persist in lymphatic tissue and survive along the slaughtering process (Stevens *et al.*, 2009). Moreover, these serovars are widespread and relevant in most European countries and were between the most frequent in confirmed cases of human salmonellosis in the EU in 2018 (EFSA and ECDC, 2019). With this regard, pigs and pork products play a potential role in the epidemiology of human salmonellosis, as 7.1% of the outbreaks in the period 2010-2017 in the EU were caused by pork

meat and derived products (EFSA and ECDC, 2019).

Other *Salmonella* serotypes, such as Anatum and Panama, although detected only during one of the surveys, have been already detected in pigs at both farm (Vico *et al.*, 2020) and slaughterhouse level (Arguello *et al.*, 2012 and 2013; Bonardi *et al.*, 2013).

Although it was not possible to detect the same PFGE profile through the years, this technique allowed showing high similarity between *Salmonella* strains belonging to the same serotypes, with a similarity value above 88.6% in *S.* Derby, above 86.1% in *S.* Typhimurium and above 85.8% in *S.* Rissen, thus indicating common ancestors. Nevertheless, different combinations of *Salmonella* clonal types indicate that each group of animals introduces new clonal types of the pathogen into the slaughterhouse environment and that each lot of animals is, therefore, a potential source of contamination for other carcasses and the plant environment itself.

Conclusions

The results of these investigations provide information about the decrease of *Salmonella* occurrence during twelve years in Sardinia showing the reduction of carrier pigs at farm level and an improvement in the application of correct GMPs and GHPs in slaughterhouses. In this context, the Food Business Operator (FBO) plays a crucial role ensuring that the meat complies with the microbiological criteria established by the EU regulations.

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