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Salmonella spp. in Pigs Slaughtered in Small and Medium-Sized Abattoirs in Central Italy: Preliminary Results on Occurrence and Control Strategies

Sara Primavilla [1,†], Rossana Roila [2,*,†], Alessia Zicavo [1], Roberta Ortenzi [1], Raffaella Branciari [2,*], Tana Shtylla Kika [3], Andrea Valiani [1] and David Ranucci [2]

[1] Istituto Zooprofilattico Sperimentale Togo Rosati, via Salvemini 1, 06121 Perugia, Italy; s.primavilla@izsum.it (S.P.); a.zicavo@izsum.it (A.Z.); r.ortenzi@izsum.it (R.O.); a.valiani@izsum.it (A.V.)
[2] Department of Veterinary Medicine, University of Perugia, via San Costanzo 4, 06121 Perugia, Italy; david.ranucci@unipg.it
[3] Department of Veterinary Publich Health, Faculty of Veterinary Medicine, Agricultural University of Tirana, Kodër Kamëz, SH1, 1000 Tiranë, Albania; tana.shtylla@ubt.edu.al
* Correspondence: rossana.roila@unipg.it (R.R.); raffaella.branciari@unipg.it (R.B.)
† Sara Primavilla and Rossana Roila equally contributed and both are first-author.

Abstract: *Salmonella* in pork is still a relevant safety issue in the EU, and specific regulations are in force to control this hazard in the meat chain, in a from farm to fork perspective. In Italy, the control is mainly based on official sampling at the slaughterhouse level. The prevalence of *Salmonella*, and isolated serovars, was investigated during a three-year survey in small and medium slaughterhouses in central Italy. A total of 400 pig carcasses samples were collected by official authorities during the observation period. Data were also analyzed according to the year and season of sampling. The overall *Salmonella* prevalence in the five selected abattoirs was 13%, with no differences due to the slaughterhouses size and seasons of sampling. An increasing trend in *Salmonella* prevalence was registered over the years. The main serovars detected were *Salmonella enterica* 4,[5],12:i:- and Derby. The data emphasized that the number of contaminated carcasses is relatively high with respect to the level set by EU legislation, and equally distributed in the area, and therefore, appropriate monitoring and control strategies also need to be developed at the farm level.

Keywords: food safety; *Salmonella* serovars; slaughterhouse; pig carcasses

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1. Introduction

Among foodborne bacteria, *Salmonella* has in recent decades been globally considered a food safety issue. According to the 2021 European Center for Disease Control (ECDC)—European Food Safety Authority (EFSA) summary report on zoonoses, it represents the second most frequently reported zoonotic agent in the European Union (EU), with 87,923 confirmed cases in 2019 and a stable trend during 2015–2019 [1]. In the same year, *Salmonella* was identified in 926 food-borne outbreaks (17.9% of the total number of outbreaks) that together affected 9169 people in the EU, with 1915 hospitalizations and seven deaths. Outside the EU, the safety issue related to salmonellosis is characterized by different impacts on public health [1]. In Brazil, this pathogen was found to be responsible for 11% of investigated foodborne diseases between 2009 and 2018; 2.1% of reported cases were attributable to pork meat and meat products [2]. The U.S. Centers for Disease Control and Prevention (CDC) estimates that *Salmonella* causes 1 million cases of foodborne illness every year in the United States, with 113 outbreaks in 2017 accounting for 29% of confirmed single-pathogen outbreaks [3]. Data from Australia estimated more than 39,000 foodborne salmonellosis cases, of which 2.5% were attributable to a porcine source [4]; furthermore, the incidence of human salmonellosis continues to rise every year in Australia [5]. In China,

it has been calculated that 22.16% of bacterial foodborne illnesses between 1994 and 2005 were caused by *Salmonella* [6].

Although the highest levels of *Salmonella*-positive food samples are attributable to poultry production (meat products and eggs) [1], pork is considered the major source of infection in humans in some EU countries, including Italy [7–10]. About this, among 265 strong-evidence foodborne salmonellosis outbreaks (FSO), pig meat and products thereof were responsible for 9.8%, thus representing the type of meat most associated with salmonellosis in humans [1].

Salmonella can colonize pigs' gut, although most of them remain healthy carriers and the stress related to farm management, transportation, and early slaughtering stages (from unloading to stunning) may increase the prevalence of infected pigs entering the slaughtering process [11–13]. When animals are processed, contamination of pig carcasses can result from the skin, the intestinal contents, infected tissues, other carcasses, or slaughterhouse surfaces [14–16]. Consequently, the contamination/cross-contamination of carcasses, and therefore meat, represents a crucial way for the pathogen to enter the food chain and therefore a significant food-safety hazard [17].

The dynamic of the diffusion of *Salmonella* in the pork chain is often reported with an emphasis on the serotype most frequently involved, their origin, and correlation to animal source [1,18]. According to EFSA, the three most common serovars detected in pigs are *S. enterica* serovar 4,[5],12:i:- (the monophasic variant of Typhimurium) (28.8%), *S. enterica* serovar Derby (24.1%), and *S. enterica* serovar Typhimurium (12.7%) [1]. As regards pig meat, the top three serovars detected are *S. enterica* serovar 4,[5],12:i:- (26.6%), *S. enterica* serovar Derby (21,3%), and *S. enterica* serovar Typhimurium (14%) [1]. However, the majority (72.4%) of the FSO are caused by *S. enterica* serovar Enteritidis [1].

Food hazards, including *Salmonella*, can occur at any point from farm to fork, so food safety standards are needed to ensure the safety of the global food supply chain. In the pork meat chain, the management and monitoring of the most probable critical point are important to design preventive and corrective measures to decrease the frequency of contaminated carcasses at the end of the slaughtering process [12].

The contribution of different factors to the presence and prevalence of *Salmonella* in pigs has been investigated in depth at the farm level, during transport, and at the abattoir [19], and key areas to be monitored have already been proposed [14,20,21], mostly relating to industrial production that involves high-capacity slaughterhouses. Moreover, the literature also takes into consideration small slaughterhouses serving local farms [22].

Therefore, a chain approach is considered crucial to controlling *Salmonella* in pork production, as already secured for poultry [1]. In this context, following a request from the European Commission, the EFSA Panel on Biological Hazards was asked to deliver a Scientific Opinion on the public health biological hazards to be covered by the inspection of swine meat [21]. It was concluded that preventive measures and controls, applied both on the farm and at the abattoir, are the only way to ensure effective control of the main hazards and comprehensive pork safety. The EFSA has also provided technical assistance on harmonized epidemiological criteria for specific public health hazards in food and animals to be used by risk managers, including *Salmonella*. The epidemiological indicators can be used by the European Commission and the Member States in the pork safety assurance framework [23]. As regards *Salmonella* in pigs, seven harmonized epidemiological indicators have been selected, considering different food chain stages: farm, transport, and slaughterhouse. Microbiological testing and typing of *Salmonella* spp. in carcass swabs will also provide data on specific new zoonotic serovars, such as *S. enterica* 4,[5],12:i:- and new emerging serovars of special public health significance [23].

The present study was conducted to investigate, based on official control data and during a three-year observation, the prevalence of pig carcasses' contamination with *Salmonella* in small and medium-sized slaughterhouses located in central Italy devoted to the slaughtering of animals mainly from local farms. Data were also analyzed by seasons of the year to define a possible relationship between prevalence and environmental conditions.

Our hypothesis is that, in the lack of compulsory *Salmonella* monitoring and control plan at the preslaughter stage, the prevalence of *Salmonella* in the carcasses is unlikely to decrease. The diffusion of the *Salmonella* serovars was also investigated to define their evolution during the observed period.

2. Materials and Methods

2.1. Sample Collection

Pig carcass samples collected in Umbria (central Italy) between 2017 and 2019 were analyzed by the Istituto Zooprofilattico Sperimentale of Umbria and Marche "Togo Rosati". The samples came from the Competent Authority National Program for *Salmonella* undertaken in accordance with Regulation (EC) No. 2073/2005 and performed in three small-sized slaughterhouses (slaughtering fewer than 5000 animals per year from small, local farms) and two medium-sized slaughterhouses (slaughtering between 5000 and 50,000 animals per year, from both small and finishing farms) [24]. The sampling plan was defined according to Regulation (EC) No. 218/2014 with an average of 49 samples per year from the medium-sized slaughterhouse and 12 samples per year from the small ones [25]. A total of 400 carcass samples were collected during the observation period with sterile premoistened sponges rubbed over the half-carcass surface before chilling (EC regulation 2073/2005), and sponge samples were transported to the laboratory in thermal boxes, stored at 4 °C, and processed within 24 h [24].

2.2. Isolation, Identification, and Serotyping

Sponge samples were pre-enriched in 90 mL of buffered peptone water (Biolife Italiana s.r.l., Milan, Italy) and incubated for 16–22 h at 37 ± 1 °C. One hundred microliters of the pre-enrichment broth was transferred aseptically into 10 mL of SX2 broth (bioMérieux, Marcy-l'Etoile, France) and incubated for 22–26 h at 41.5 ± 1 °C. *Salmonella* detection was then performed through an alternative method validated by AFNOR (AFNOR BIO 12/10-09/02) based on enzyme-linked fluorescent immunoassay by means of VIDAS® SLM test (bioMérieux), following the instructions provided by the manufacturer [26]. According to UNI EN ISO 6579:2017, *Salmonella* was isolated from the positive broth cultures through selective and differential media (xylose lysine deoxycholate agar, Microbiol s.r.l., Cagliari, Italy; and *Salmonella* chromogenic medium, Biolife Italiana s.r.l.) [27]. After incubation at 37 ± 1 °C for 24 h, each medium was examined to identify the presence of typical *Salmonella* colonies: red colonies, with black center on xylose–lysine–deoxycholate agar and magenta colonies on *Salmonella* chromogenic medium. Biochemical tests (API 20E strips—bioMérieux) were used to confirm typical colonies. The *Salmonella* isolates, incubated at 37 ± 1 °C for 24 h in trypticase soy agar (TSA) (Oxoid Ltd., Basingstoke, UK) were finally serotyped according to the White–Kauffmann–Le Minor scheme by performing a slide agglutination test with polyvalent and monovalent antisera against somatic (O) and phase 1 and phase 2 flagellar (H) antigens (SSI diagnostics, Hillerød, Denmark) [28].

2.3. Statistical Analysis

Data were gathered on overall prevalence and analyzed by factors such as year (2017, 2018, and 2019), slaughterhouse capacity (small slaughterhouse (<5000 animals per year) = SMS; medium-sized slaughterhouse (between 5000 and 50,000 animals per year) = MDS), and season (spring and summer = SS; autumn and winter = AW). All the statistical analyses were determined by EpiInfo 7.2 free software [29]. Calculation of 95% confidence intervals (CIs) was performed by binomial distribution. The relation of the *Salmonella* prevalence and the different years, slaughterhouses, and seasons were calculated using Pearson Chi-square and Fisher's exact tests (significance set at $p < 0.05$) [30,31]. Odd ratios were also calculated according to user's manual [29]. The same tests were adopted for the two major Salmonella serotype detected, as the number of samples for the minor serotypes was not sufficient for a proper statistical analysis.

3. Results

A total of 400 carcasses were assessed over the three-year period considered; of these, 107 samples were referred to the SMS and 298 to the MDS. The distribution according to the season was 222 samples in SS and 178 in AW. The carcasses sampled in 2017, 2018, and 2019 numbered 133, 134, and 133, respectively.

The overall prevalence recorded in the carcasses was 13% (CI = 9.7–16.3%), with 52 samples positive for *Salmonella* spp. The results according to season and slaughterhouse capacity are reported in Figure 1. No difference was recorded in terms of the prevalence of *Salmonella* in relation to either the slaughtering capacity or season.

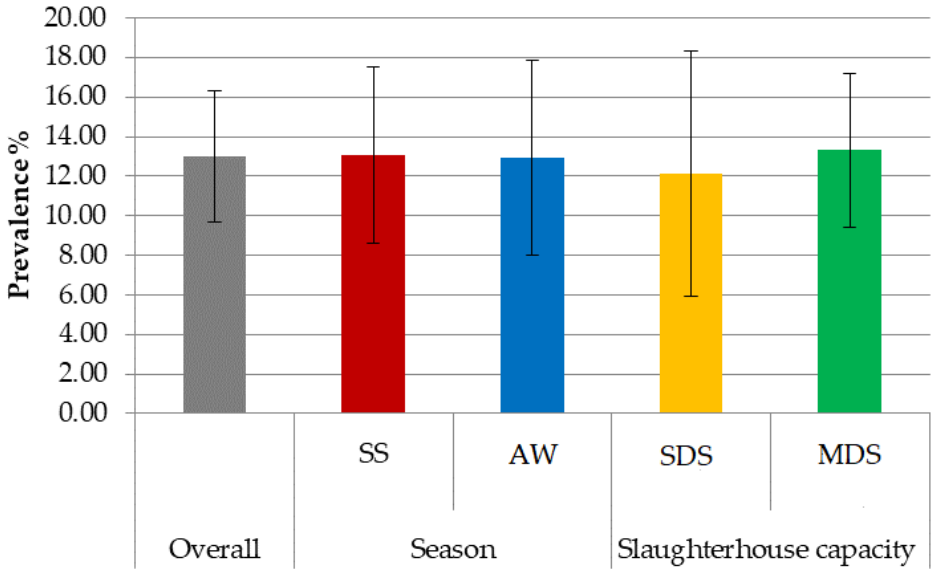

Figure 1. Overall prevalence (%) and 95% CI (bars) of pig carcasses positive for *Salmonella* according to season and slaughterhouse capacity. SS = Spring-Summer; AW = Autumn-Winter; SDS = small-sized slaughterhouse (<5000 pigs/year); MDS = medium-sized slaughterhouse (5000–50,000 pigs/year).

The prevalence results for each of the selected years are reported in Figure 2. A significant increase was recorded between 2017 and 2018 ($p < 0.05$) with an OR of 2.36, but not in 2019.

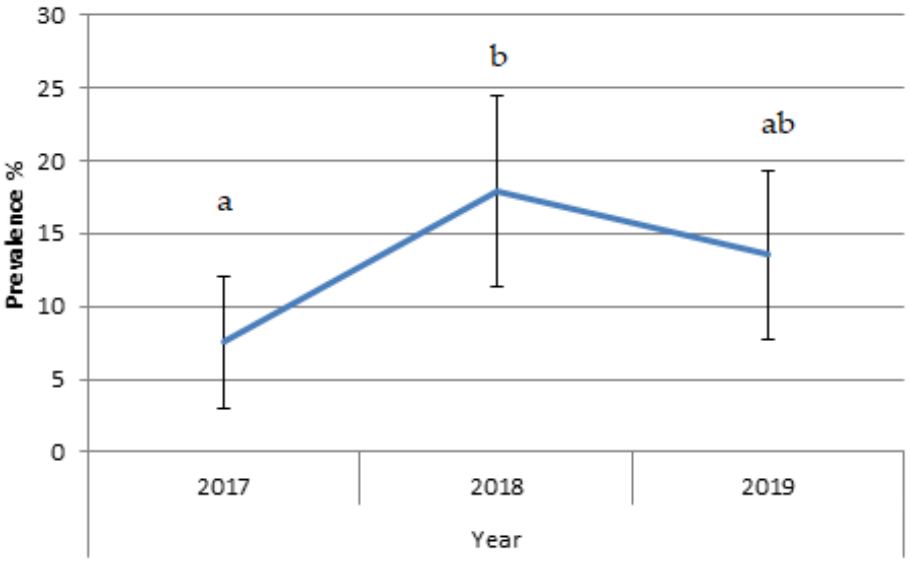

Figure 2. Prevalence (%, CIs) of pig carcasses positive for *Salmonella* according to the year of sampling. a, b = different letters indicate differences between years.

The prevalence of the different *Salmonella* serovars even according to season and abattoir capacity is reported in Table 1.

Table 1. Percentage of *Salmonella* serotypes isolated from pig carcasses according to season and slaughterhouse capacity.

Salmonella Serovars	Overall (%) (*n* = 45)	Season		Slaughterhouse Capacity	
		Spring/Summer (%) (*n* = 25)	Autumn/Winter (%) (*n* = 20)	<5000 Animals/Year (%) (*n* = 8)	5000–50,000 Animals/Year (%) (*n* = 37)
4,[5],12:i:-	33.33	36.00	30.00	50.00	29.73
Derby	40.00	44.00	35.00	25.00	43.24
Infantis	2.22	0.00	5.00	0.00	2.70
Bredeney	4.44	4.00	5.00	0.00	5.41
Typhimurium	2.22	4.00	0.00	12.50	0.00
Give	2.22	0.00	5.00	12.50	0.00
London	4.44	0.00	10.00	0.00	5.41
Rissen	6.67	4.00	10.00	0.00	8.11
Brandeburg	2.22	4.00	0.00	0.00	2.70
Goldcoast	2.22	4.00	0.00	0.00	2.70

Among the 52 positive samples, an identification was obtained for 45 isolates (7 remained unidentified). The most diffused serovars were *S. enterica* serovar Derby and *S. enterica* serovar 4,[5],12:i:-, together representing over 70% of the isolates. A similar prevalence was registered for these two serotypes according to abattoir capacity and sampling season. The prevalence of the two major *Salmonella* serovars was similar across the three years of observation.

The minor serovars detected were Infantis, Bredeney, Typhimurium, Give, London, Rissen, Brandeburg, and Goldcoast. Typhimurium and Give were detected only in small slaughterhouses and the other serovars were detected only in the medium-sized abattoirs. The distribution according to the seasons was Infantis, Give, and London only in AW and Typhimurium only in SS. Infantis was isolated only in 2017; Bredeney, Typhimurium, and Give only in 2018; and London, Rissen, Brandeburg, and Goldcoast in 2019.

The distribution of *Salmonella* serovar according to the year of sampling is reported in Table 2.

Table 2. Percentage of *Salmonella* serotypes isolated from pig carcasses according to the year of sampling.

Salmonella Serovars	2017 (%) (*n* = 8)	2018 (%) (*n* = 21)	2019 (%) (*n* = 16)
4,[5],12:i:-	50.00	23.80	37.50
Derby	37.50	57.10	18.80
Infantis	12.50	0.00	0.00
Bredeney	0.00	9.50	0.00
Typhimurium	0.00	4.80	0.00
Give	0.00	4.80	0.00
London	0.00	0.00	12.50
Rissen	0.00	0.00	18.80
Brandeburg	0.00	0.00	6.30
Goldcoast	0.00	0.00	6.30

4. Discussion

The results denote a level of contamination of the carcasses higher than that set by EU legislation as process hygiene criteria for pig slaughtering [24]. The results are even higher than those reported from pig carcasses by the ECDC and EFSA (3.88%) [1] but are in line

with results obtained by other authors in extra-EU, EU, and Italian abattoirs [14,22,32–35]. Nonetheless, where specific national guarantee plans are in force, including monitoring at the farm level (e.g., in Finland, Denmark, and Sweden) [19], the *Salmonella* prevalence drops to near zero [1].

When a high prevalence is detected by CA at a slaughterhouse, some concerns have to be considered. Even if the literature reports that the chilling of the carcasses could decrease the level of *Salmonella*-positive samples [15], the higher the prevalence in the carcasses, the more likely the presence of *Salmonella* after chilling [36], with a possible safety impact on the subsequent steps of the meat chain. In this context, the absence of *Salmonella* in the final products at the market level, as set by the EU legislation [24], may not be achieved, and the risk to consumers increases.

Nonetheless, the results consequent to the official control were sent each year to the FBOs to implement corrective action at the abattoirs, even if self-monitoring analyses always reveal compliance with the process hygiene criteria (<6% of positive carcass samples; data not shown). This discrepancy between the official results and FBOs' self-monitoring results is also stressed by ECDC EFSA reports and needs further investigation [1]. Despite the efforts and proper hygiene practices implemented at the selected abattoirs, inconclusive results were obtained in these three years. Moreover, the presence of a similar prevalence in small and medium-sized abattoirs, where a different approach to slaughtering procedures and HACCP implementation could affect the contamination level [22], brings the core of the problem back on the farm and the pre-slaughter level [13,22]. For this reason, a limited efficacy of preventive action (implementing good hygiene and manufacturing practices, and risk management procedures) could be obtained during slaughtering to control contamination, even though it is considered crucial to avoid carcass cross-contamination [36]. A combined farm–abattoir approach would likely have cumulative benefits [13,37]. Furthermore, the European approach to meat safety involves the prevention of carcass contamination through proper implementation of hygienic procedures as no pig carcass treatments are allowed [38]. Additionally, even if it is generally reported that large farms of finishing herds are more prone to high *Salmonella* prevalence than small farms (farrow to finish), other aspects, such as farm management and structures, and the implementation of biosafety measures, could have an impact on *Salmonella* prevalence and mitigate the differences, as reported in the present study [39]. Moreover, other important factors that have to be monitored, such as the transport and holding at the abattoir, could spread contamination among animals of the same batch before slaughtering [16].

The abovementioned hypotheses could be corroborated by the absence of seasonal differences, which could be due to the high presence of the hazard at the farm level [40]. These data are not in agreement with other authors who found a higher *Salmonella* prevalence in pig herds during the fall in the USA, compared to the spring and summer, but was due to seasonal contamination at a feed mill [41].

The results of the identification of the serovar confirm that the major ones are Derby and 4,[5],12:i:-, which are the two serovars typically isolated from pigs [1].

No difference was registered for the two main *S. enterica* serovars detected according to the considered factors; their presence was equally distributed between small and medium-sized abattoirs, and between the different seasons. Uneven distribution of minor strains, in terms of slaughterhouse size, season, and year, was observed. In particular, medium-sized abattoirs showed greater variability in the detected serovars than small ones. The larger number of animals slaughtered could be responsible for a greater probability of finding different serovars [42], but further investigation is needed.

Integrated surveillance based on the "One Health" approach, combined with effective containment measures along the entire food chain (based on the application of biosecurity measures, effective surveillance and vaccination at the farm level, good manufacturing, and hygienic practices during slaughtering and food processing, and in the retail and consumer phases) is crucial to controlling the spread of *Salmonella*, especially the current and emergent epidemic clones [10]. Prior to the implementation of such interventions to

prevent and manage *Salmonella* in pigs, it would be advisable to define specific, suitable, and harmonized sampling and testing strategies aiming to determine, with the highest possible degree of certainty, the real prevalence of *Salmonella* in the slaughterhouse [19]. In light of the above, the results of self-monitoring plans should be integrated with thorough surveillance by CAs. In this regard, following the EFSA suggested approach [23], the EU has set community targets for the reduction of the prevalence of zoonoses and zoonotic agents through national control programs [43], including pig breeding herds and herds of slaughtering pigs. The aims are to ensure that effective measures are taken to detect and control *Salmonella* at all relevant stages of production, processing, and distribution, in order to reduce its prevalence and public health risks. These control programs in pig herds are not harmonized between the Member States [19], so in some EU countries, such as Italy, to date, they are limited to the minimum sampling requirements: sampling only at slaughterhouse level [43] with a low number of samples collected and analyzed [44]. At this level, EU food safety legislation provides Food Safety Criteria and Process Hygiene Criteria, including for *Salmonella* [24], under the Food business operator (FBO), which has the primary responsibility for ensuring food safety and ought to verify compliance with these criteria through self-monitoring [45]. The FBO, therefore, has to guarantee that no more than 6% of carcasses are noncompliant with the process hygiene criterion (three nonconformities out of 50 samples) [46]. If the results are not compliant, the FBO must adopt corrective action on the overall production hygiene at the slaughterhouse level. As regards pork carcasses, the Competent Authority (CA) verifies whether the FBO has correctly implemented the Process Hygiene Criteria described by the EU legislation (in Regulation (EC) No. 2073/2005 (revised by Regulation (EC) No 217/2014) [24,46]. The Competent Authority must therefore perform sampling and analysis to verify this compliance by an official sampling using the same method and sampling areas as FBOs and taking at least 49 random samples from each slaughterhouse each year. If all are negative, 95% statistical certainty is achieved provided that the prevalence is below 6%. This number of samples may be reduced in small slaughterhouses (<300 pigs a week) based on a risk evaluation (one sample each month) [25]. Therefore, official monitoring at the slaughterhouse level is the only stronghold for the definition of the real situation in terms of *Salmonella* presence up to this point of the chain [47]. Nonetheless, the number of samples required to CA could be not sufficient to act properly as variations in *Salmonella* prevalence should be constantly monitored in order to carry out suitable counteractions [47]. However, it is of the utmost importance to assess the costs of possible preharvest interventions to control and manage *Salmonella* in pigs at different levels (feed, farm, transport, and slaughterhouse) and the benefits in terms of reductions in human and animal health costs [48].

5. Conclusions

The Salmonella prevalence in the examined context of both types of slaughterhouses in the central Italy area was higher than that allowed by EC regulation independent of the sampling year and seasons. The high levels detected throughout the years when research was conducted, confirms that in the present conditions, a Salmonella-free pig production chain is a difficult goal to achieve, and highlights the necessity of integrating a control plan at both the pre-harvest and slaughterhouse levels. The need for proper monitoring is also highlighted as CA sampling, provided by EU legislation only at the slaughterhouse level, could not be sufficient for rapid control intervention. Therefore, an integrated chain approach would be desirable to reduce the presence of these pathogens in pork and pork products.

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