Salmonella Infection in Mesenteric Lymph Nodes of Breeding Sows

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Abstract

Salmonellosis is one of the main foodborne diseases worldwide. Breeding sows asymptotically infected with *Salmonella* can transmit the pathogen to piglets and humans. The isolation of *Salmonella* from mesenteric lymph nodes (MLNs) is considered a demonstration of asymptomatic infection in swine. As previous breeding sow studies have been performed using feces, the aim of this work was to study the occurrence of *Salmonella* infections by sampling MLNs, in comparison to their serological status. First, *Salmonella* fecal shedding was studied in 12/16 large breeding farms to establish the framework of study. Then, MLN ($n=264$) and blood ($n=237$) samples were obtained at an abattoir from sows of 15 of these 16 breeding farms. Additionally, risk factors associated with *Salmonella* MLN infection were analyzed. A total of 6.1% (16/264) sows, distributed in 40% (6/15) of the farms, had the pathogen in MLN. *Salmonella* Typhimurium was the most frequent serovar isolated. Interestingly, 43.8% (7/16) of MLN isolates were susceptible to all the antimicrobials tested and were found distributed throughout all farms with at least one sow positive. As well, one isolate of the emerging DT195 clone was detected and found to be resistant to six antibiotic families (ASSuTNx-Cfx). The serovars and the resistance profiles of the *Salmonella* isolates from feces were completely different to those obtained from MLNs. The seroprevalence (41.8% of sows and 100% of farms) was higher than that of MLN infections, showing no concordance ($k=0.15$) between these two diagnostic tests in sows. Strategies directed to correct two risk factors (i.e., administration of dry food and old premises) would most likely help to reduce *Salmonella* infections in breeding sows.

Keywords: *Salmonella*, prevalence, sows, lymph nodes, shedding, antimicrobial resistance, risk factors

Introduction

NONTYPHOIDAL SALMONELLOSIS is a worldwide-distributed zoonosis caused by *Salmonella*, a pathogen of public health relevance. After initial control in fowl, pig products are emerging as an important source of *Salmonella* for humans in the European Union (EU) (EFSA-ECDC, 2015; EFSA-ECDC, 2018). To protect the health of the consumers, the EU is legislating for the reduction of *Salmonella* in pigs and derivate food, including breeding sows as a vertical source of infection of the pathogen to the litter (DOUE, 2003).

Spain is the fourth largest country in pig production, after China, United States, and Germany, showing an increase of 228.5% sow offal (i.e., entrails and organs used for food processing) export from 2008 to 2017 (MAPA, 2018). Results of the salmonellosis EU baseline studies indicated that

Spain was on the top of *Salmonella* prevalence at pig herd level, detecting the bacteria in pooled fecal samples (PFS) from 64% of breeding holdings (EFSA, 2008, 2009). *Salmonella* can be present in feces after an active infection of enterocytes and lymphatic system and/or by passive transmission of the bacteria through the gut after ingestion. Sows infected asymptotically can shed the pathogen intermittently contaminating the environment (Scherer *et al.*, 2008) and transmitting it to humans and piglets (Casanova-Higes *et al.*, 2019). Reliable diagnostics of *Salmonella* infection can only be assessed by the pathogen isolation in the gut wall or lymphatic system, being mesenteric lymph nodes (MLNs) the sample of choice (DOUE, 2003; EFSA, 2006). However, no information about *Salmonella* prevalence in sow MLN is available, probably owing to practical limitations for sampling sows in the abattoir. In some EU countries, serological

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diagnosis is considered an alternative in *Salmonella* control programs in fattening pigs (Mousing *et al.*, 1997; Merle *et al.*, 2011; Meroc *et al.*, 2012). Nevertheless, no correlation was observed between serology and the presence of *Salmonella* in MLN or fecal samples of these animals (San Román *et al.*, 2018).

The objectives of this study were to determine: (1) prevalence and type of *Salmonella* present in sow MLN; (2) seroprevalence and the usefulness of the enzyme-linked immunosorbent assay (ELISA) as a tool to assess *Salmonella* MLN infection in sows, at individual and at herd levels; and (3) main risk factors associated to MLN infection.

Materials and Methods

Sampling design

The research was performed in Navarra, a region in Northern Spain with moderate–low prevalence of *Salmonella* in fattening pigs (San Román *et al.*, 2018). In this context, a total of 65,308 breeding sows kept on 763 farms were registered in 2014 (INTIA, personal communication). Most of these animals (37,964 sows) were kept on only 16 large farms, which contained more than 1200 sows each and were managed by 7 integrator companies. These large herds were based on a closed replacement system using gilts born in the farm, with a replacement rate of around 50%.

To know the framework of the research, we determined the prevalence of *Salmonella* shedding at herd level, in comparison to that observed in the EU baseline study (EFSA, 2009). For this, individual fecal samples were obtained in 12 out of the 16 large farms of Navarra and processed in pools of 5 (25 g/PFS), as previously described (San Román *et al.*, 2018). The fecal samples were obtained at farm proportionally to: (1) the number of rooms of each reproductive unit (i.e., gestation, farrow, replacement, and confirmed gestation); and (2) the number of sows kept in each room, representing the different reproductive cycle stages. Accordingly, 340 sows housed in gestation units and 205 sows housed in farrowing units were sampled in 12 farms, however, only 30 sows housed in replacement units in 4/12 farms and 25 sows housed in confirmed gestation units in 3/12 farms could be analyzed. Thus, a total of 600 individual fecal samples from 5 or 10 rooms/farm were collected from the rectum by using the double-glove method and individual sterile containers to avoid cross-contamination.

Within this framework, 15 out of the 16 large farms containing a total of 33,545 sows were included in the MLN infection and serological study. The sows sampled were selected from those regularly sent by the farmer to the abattoir for breeding replacement, thus, all sows were multiparity, but the parity number was unknown. Owing to the lack of previous information on *Salmonella* MLN infection in sows, 15–20 animals/farm (depending on the herd size) were considered representative to detect the presence of *Salmonella* infection, assuming an estimated minimum prevalence of 20% by farm and a 95% confidence interval (CI_{95%}). The pooling was not considered for sample size calculations, since pooling was highly efficient compared with individual sampling in previous studies (Arnold *et al.*, 2009). Accordingly, a total of 264 sows were sampled for MLN and 237 of them (one farm failed) were also bled at the abattoir and used as paired samples for concordance studies.

Salmonella isolation and characterization

Both MLN ($n=264$) and fecal ($n=120$) samples were processed by the ISO 6579:2002/Amd 1:2007 (hereafter, ISO 6579) (ISO, 2007), as previously detailed (San Román *et al.*, 2018). Presumptive *Salmonella* isolates were sent to the National Center for Animal Salmonellosis (Algete, Madrid, Spain) for confirmation and serotyping by the Kauffman–White Scheme (Grimont and Weill, 2007). Thereafter, *Salmonella* Typhimurium isolates were phage typed by standardized protocols in the National Center of Microbiology at the Instituto de Salud Carlos III (Madrid, Spain) according to standard protocols (Echeíta *et al.*, 2005).

All *Salmonella* isolates were analyzed by the Kirby–Bauer disk diffusion test (CLSI, 2013) in cation-adjusted Mueller–Hinton plates against 12 antimicrobials (all from BD, Spain) of 7 different families, as previously detailed (Garrido *et al.*, 2014). The antimicrobials tested were: ampicillin and amoxicillin/clavulanic acid (aminopenicillins; A); chloramphenicol (phenicol; C); streptomycin and gentamicin (aminoglycosides; S); sulfisoxazole, trimethoprim, and trimethoprim/sulfamethoxazole (sulfonamides; Su); tetracycline (tetracyclines; T); nalidixic acid (quinolones; Nx); ciprofloxacin (fluoroquinolones; Cip); and cefotaxime (third-generation cephalosporins; Cfx). *Escherichia coli* ATCC 25922, *Salmonella* Typhimurium ATCC 14028 and DT104 were used as controls in each experiment. Clinical breakpoints to classify isolates as susceptible or resistant were defined by the Clinical and Laboratory Standards Institute (CLSI, 2013). Isolates were considered multidrug resistant when exhibiting resistance to at least three antimicrobial families.

Serological study

Blood samples ($n=237$) were obtained at abattoir, individual sera were extracted by centrifugation (4°C, 10 min, 1500×g) in a Multifuge 3L-R (Sorvall, Heraeus), and analyzed by Herd-Check® Swine *Salmonella* indirect ELISA test (IDEXX™ Laboratories, Switzerland). This test had shown an optimal sensitivity and specificity in fattening pigs (88% and 74%, respectively) compared with other commercial kits (Vico *et al.*, 2010). Optical density (O.D.) values were normalized and analyzed at different cutoff values (i.e., 10%, 20%, and 40%), according to the manufacturer's instructions.

Concordance test

Considering the 100% specificity of bacteriology, a farm was considered positive when *Salmonella* was confirmed in at least one MLN sample. Concordance analysis between infection and serology was performed by Kappa test (k) using MLN and blood paired samples ($n=237$). Descriptive statistics and prevalence were estimated with a CI_{95%}. Statistical comparison of percentages was performed by a Chi-square test with Fisher's correction ($p \leq 0.05$) when required, using the SPSS 15.0.1 statistical software (SPSS, Inc., Chicago, IL).

Questionnaire data and statistical analysis

A questionnaire consisting of 70 variables was used to assess possible risk factors associated to *Salmonella* MLN infection. Questions were divided into five main sections in

the farm survey: (1) farm general characteristics related to herd size, number of gestation units, and number of full-time workers; (2) biosecurity aspects such as existence and maintenance of outside fence and footpath, use of specific clothes, entrance restrictions, rodent control programs, and presence of cats, dogs, and wild birds; (3) feeding: type of feed, number of diets, and water supplier; (4) use of antimicrobial agents: type, number and length of treatments; and (5) farmer's personal information: age, educational level, and additional training on pig production. To provide reliable information, all the surveys were checked for reliability and filled out with the assistance of the farm veterinarian.

For statistical analysis, an initial risk factors' screening was carried out by a univariable Chi-square test. The variables detected as significant ($p \leq 0.05$) were further considered in a multivariable random-effect logistic regression model in which the outcome variable was the "culture positive"; the explanatory variables included in the model as fixed effect were those from the questionnaire; and the random effect was the farm. Multivariable analysis was performed by the STATA software (StataCorp, L.P., College Station, TX). An odds ratio (OR) >1 indicated that animal exposure to the factor increases the risk of *Salmonella* positivity, whereas an OR <1 indicates a reduced risk of animal positivity due to exposure to the factor.

Results

Salmonella prevalence at farm level in fecal samples

The framework of the study was defined by the presence of *Salmonella* in 13/120 (10.8%) PFS, which were from 50% (6/12) of farms studied (Table 1). Farms with at least one positive PFS showed a 21.6% mean prevalence, but 58.3% of these farms exhibited low levels of contamination, since they had $\leq 10\%$ positive PFS (Table 1). As shown in Table 2, farms from integrators B and F showed a higher proportion of positive PFS. A wide variety of serovars, but not Typhimurium, were identified in these samples (Table 2). All the isolates were pansusceptible (6/11) or only resistant to streptomycin (5/11) alone or combined with tetracycline (Table 2).

Salmonella MLN infection in sows

As shown in Table 1, *Salmonella* spp. was found in 16/264 (6.1%) MLN of sows, which were in 6/15 (40%) breeding

farms. Within positive farms, the mean prevalence of the pathogen was 14.5%, but 80% of the farms showed $\leq 10\%$ animals infected, displaying a marked left-biased distribution of the infection (Fig. 1).

A total of 6 serovars from 4 different serogroups were detected in sows' MLN (Table 2). The most common serovar was Typhimurium (43.7%) followed by Derby (18.7%), Enteritidis (12.5%) and Montevideo (12.5%) (Table 2). A total of 43.7% (7/16) of MLN isolates were pansusceptible and found distributed in all positive farms except one (i.e., Farm 4) (Table 2). The remaining 56.3% (9/16) MLN isolates (serovars Typhimurium or Derby) showed multiresistance to three (SSuT) or more (ACSSuT and ASSuT-Nx-Cfx) antimicrobial families. These three different multidrug-resistance patterns were found in three different farms (Table 2). The SSuT Derby isolates were common to Farms 2 and 4, and the two different multidrug-resistant Typhimurium were restricted to one origin each (i.e., Farms 1 or 4). In fact, the five Typhimurium isolates found in Farm 1 showed the typical pentaresistant profile (ACSSuT) associated to DT104 phage type, and the DT195 found in Farm 4 showed a particular ASSuT-Nx-Cfx multidrug-resistant profile. Overall, the distribution of the *Salmonella* phenotypes suggested a different origin of infection for each farm.

Seroprevalence

All farms studied had at least one seropositive sow, even at the maximum 40% O.D. ELISA cutoff. The mean seroprevalence was 41.8% (Table 1) varying from 15% to 80% between farms (Table 2), but all farms showed $>10\%$ seroprevalence (Fig. 1). These results indicated that seroprevalence was higher than MLN infection. The discrepancy between both diagnostic techniques was evidenced by (1) the 8 farms that showed 25–80% of seropositive sows but none of them infected in MLN (Table 2); and (2) the high proportion (86/99) of sows negative by bacteriology but positive in ELISA (Table 3). Accordingly, no concordance between serology and microbiology was statistically confirmed by a $k=0.15$ Kappa index.

Risk factor analysis of Salmonella MLN infection in sows

The risk factor questionnaire was accurately completed by 13/15 of the farms and two farms were excluded because their

TABLE 1. PREVALENCE OF *SALMONELLA* IN MESENTERIC LYMPH NODES AND SEROLOGY (ENZYME-LINKED IMMUNOSORBENT ASSAY) OF SOWS SAMPLED AT ABATTOIR AND IN POOLED FECAL SAMPLES OBTAINED AT THE FARMS WHERE THE SOWS WERE KEPT

	MLN (mean %; CI _{95%})	Serology ^a (mean %; CI _{95%})	PFS (mean %; CI _{95%})
No. of positive ^b /total no. of samples	16/264 (6.1; 3.7–9.6)	99/237 (41.8; 35.7–48.1)	13/120 (10.8; 6.4–17.6)
No. of positive ^b /total no. of farms	6/15 (40; 19.8–64.2)	14/14 (100; 78.5–100)	6/12 (50.0; 25.3–74.6)
No. of positive pigs ^b /total no. of pigs analyzed in positive farms	16/110 (14.5; 9.1–22.3)	99/237 (41.8; 35.7–48.1)	13/60 (21.6; 13.1–33.6)
No. (%) of farms with $\leq 10\%$ prevalence/total no. of farms	12/15 (80.0)	0/14 (0)	7/12 (58.3)

^a40% O.D. cutoff.

^bPigs or farms where at least 1 colony-forming unit of *Salmonella* spp. was isolated.

CI_{95%}, 95% confidence interval; MLN, mesenteric lymph node; O.D., optical density; PFS, pooled fecal samples.

TABLE 2. SEROVARS, PHAGETYPES, AND ANTIMICROBIAL RESISTANCE OF *SALMONELLAE* ISOLATED IN MESENTERIC LYMPH NODES AND POOLED FECAL SAMPLES

Farm (integrator) codes	MLN			PFS			Serology No. (%) positive ^c / total samples
	No. (%) positive/total samples	Serovar/phagetype ^a (no. strains)	AR ^b profile (no. strains)	No. (%) positive/total samples	Serovar/phagetype ^a (no. strains)	AR ^b profile (no. strains)	
1 (A)	6/20 (30%)	Typhimurium DT104B (5) Rissen (1)	ACSSuT (5) Susceptible (1)	Negative	N.A.	N.A.	16/20 (80%)
2 (B)	3/15 (20%)	Derby (2) Enteritidis (1)	SSuT (2) Susceptible (1)	1/10 (10)	Lexington (1)	Susceptible (1)	4/15 (26.6%)
3 (C)	3/20 (15%)	Montevideo (2) Muenchen (1)	Susceptible (2) Susceptible (1)	Negative	N.A.	N.A.	N.D.
4 (D)	2/20 (10%)	Typhimurium DT195 (1) Derby (1)	ASSuT-Nx-Cfx (1) SSuT (1)	2/10 (20)	Derby (2)	SSuT (2)	11/20 (55%)
5 (E)	1/20 (5%)	Typhimurium DT193 (1)	Susceptible (1)	Negative	N.A.	N.A.	3/13 (23.1%)
6 (B)	1/15 (6.7%)	Enteritidis (1)	Susceptible (1)	2/10 (20)	Bovismorbificans (1); Mishmarhaemek (1)	S (1); AST (1)	7/15 (46.6%)
11 (F)	Negative	N.A.	N.A.	4/10 (40)	London (3); Derby (1)	Susceptible (4)	12/15 (80%)
12 (F)	Negative	N.A.	N.A.	2/10 (20)	London (1); Anatum (1)	ST (1); S (1)	5/20 (25%)
13 (G)	Negative	N.A.	N.A.	2/10 (20)	Derby (2)	Susceptible (1); S (1)	3/20 (15%)
7 (A), 8 (D), 10 (E)	Negative	N.A.	N.A.	Negative	N.A.	N.A.	15/54 (27.8%)
9 (F), 14, 15 (G)	Negative	N.A.	N.A.	N.D.	N.D.	N.D.	23/45 (51.1%)
Total: 15 (7)	16/264 (6.1%)	6 serovars (16)	3 AR profiles; Susceptible (7)	13/120 (10.8%)	6 serovars (13)	3 AR profiles; Susceptible (6)	99/237 (41.8%)

Serology (positive/total samples) found in the breeding sows of this study.

^aOnly for Typhimurium strains.^bA: ampicillin and/or amoxicillin/clavulanic acid; C: chloramphenicol; S: streptomycin; Su: sulfisoxazole, trimethoprim, and/or trimethoprim/sulfamethoxazole; T: tetracycline; Nx: nalidixic acid; Cfx: cefotaxime.^c40% O.D. cutoff.

AR, antimicrobial resistance; MLN, mesenteric lymph node; N.A., no applicable; N.D., not determined; O.D., optical density; PFS, pooled fecal samples.

FIG. 1. Distribution of *Salmonella* in breeding sows, expressed as the percentage of farms (ordinates) showing ≤10%, 11–20%, or >20% individual prevalence (abscissas). MLN (white bars) and blood serum (grey bars) were analyzed by the standard ISO 6579 and by the IDEXX® ELISA test at 40% O.D. cutoff, respectively. MLN, mesenteric lymph node; O.D., optical density.

data were inconsistent. Since these two farms showed all sows free from *Salmonella* infection, the eventual risk factor analysis was carried out with data from 6 farms showing 5–30% of *Salmonella*-infected sows and 7 farms showing all sows free from *Salmonella* infection.

In the univariate scrutiny, a total of 12/70 variables were initially associated with *Salmonella* MLN infection. From them, only two variables remained significant in the subsequent multivariable logistic regression model (Table 4), indicating that (1) administration of dry food (as compared with food mixed with water); and (2) lack of shed/barn/building renovations in the last 5 years were significant risk factors.

Discussion

All previous studies of salmonellosis in sows have been performed with fecal samples. However, the presence of *Salmonella* in feces could be due not only to excretion from

TABLE 3. DISTRIBUTION OF THE SEROLOGICAL AND MICROBIOLOGICAL SALMONELLOSIS RESULTS IN PAIRED SAMPLES OF SOWS ($N=237$)

No. of sows	MLN		Total samples
	Positive	Negative	
Serology			
Positive	13	86	99
Negative	0	138	138
Total samples	13	224	237

Sera and MLN samples were obtained from each sow at abattoir, and analyzed by the IDEXX® ELISA test at 40% O.D. cutoff and the standard ISO 6579 method, respectively.

MLN, mesenteric lymph node; O.D., optical density.

TABLE 4. EPIDEMIOLOGICAL VARIABLES SIGNIFICANTLY ASSOCIATED WITH *SALMONELLA* PREVALENCE IN MESENTERIC LYMPH NODE OF SOWS

Explanatory variable	Logistic regression parameters	
	p	OR (95% CI)
Food administration		
Mixed with water ^a		1
Dry	0.005	9 (1.9–39.4)
Shed/barn/building renovations		
Yes ^a		1
No	0.042	3.01 (1.04–8.73)
Constant	0.000	0.09 (0.04–0.21)

Determined by multivariable random effects logistic regression analysis after clustering pigs by farm of origin and considering MLN culture positive as the outcome variable.

^aReference category assigned as OR=1 for statistical purposes.

CI, confidence interval; MLN, mesenteric lymph node; OR, odds ratio.

MLN but also to cross-contamination or passive ingestion and survival of the pathogen in the intestinal tract of the animal. Thus, *Salmonella* isolation in MLN is the most reliable way to demonstrate asymptomatic infection in swine (DOUE, 2006). Also, MLN infection is considered as a reservoir and a source of intermittent excretion and dissemination of the pathogen to piglets (EFSA, 2011). However, to our knowledge, only one previous study has been published about *Salmonella* MLN infection in breeding sows (Keteran *et al.*, 1982). The prevalence found there (58.2%) was higher than that found in our conditions (6.1%), however, both studies are not exactly comparable, since animals of the former were maintained for 10 d in lairage, while in our study all sows were slaughtered within 2 h after arrival. Supporting our results, the level of infection found in sow MLN (6.1%) was similar to that observed in fattening pigs MLN (7.2%) of the same region (San Román *et al.*, 2018).

Herein, we present a novel study of *Salmonella* infection in sows MLN and its concordance analysis with serology in 237 sows paired samples. Overall, moderate–low prevalence of *Salmonella* was observed in sows' MLN in the Navarra vertically integrated production system. This finding is in agreement with the moderate–low prevalence observed in fattening pigs in this production system of Navarra (San Román *et al.*, 2018).

A large discrepancy between *Salmonella* infection and seropositivity (41.7%) was observed. It was higher than the results previously reported for fattening pigs (San Román *et al.*, 2018). Our observation of no concordance ($k=0.15$) could be attributed to endemicity of infection in breeding holdings, higher possibility of reinfections, or antigenic contacts in breeding sows than in young pigs (Vico *et al.*, 2010, 2011; Meroc *et al.*, 2012). Additionally, a longer persistence of humoral immune response than infection itself could also be an explanation (Scherer *et al.*, 2008). Other hypotheses such as lack of ISO 6579 sensitivity (Mainar-Jaime *et al.*, 2013), lack of ELISA specificity (Vico *et al.*, 2011), or absence of exotic serogroup antigens in the ELISA plate coating (Van Winsen *et al.*, 2001) could also contribute. However, serology has been applied in large studies for

salmonellosis control in Denmark, Germany, and Belgium, where infection and serology are considered highly correlated in finishing pigs (Mousing *et al.*, 1997; Merle *et al.*, 2011; Meroc *et al.*, 2012). Our results indicate that the serological diagnosis is of very limited efficacy (if any) to control salmonellosis in sows, at least in our epidemiological scenario.

In the EU baseline study, more than 50 different *Salmonella* serovars were found in feces of breeding sows, being Derby (23.9%) and Typhimurium (17.9%) the most frequent (EFSA, 2009). Contrarily, in our study, *Salmonella* Typhimurium was not detected in feces but was the serovar most frequently identified in MLN. Since this serovar is the most commonly reported in human infections in 2014 (EFSA-ECDC, 2016), future works could be focused on the study of sow offal (including internal organs and lymph nodes) as a possible source of human infections.

Besides the foodborne hazard, the inappropriate use of antimicrobial agents in humans (ECDC, 2018) and animals (EMA-ESVM, 2018) has led to a quick emergence of multidrug-resistant *Salmonella* of special epidemiological surveillance (DOUE, 2003). At the EU, 48.7% of sow feces isolates were multidrug resistant (EFSA-ECDC, 2019), whereas only 18.2% were found in our framework. In contrast to PFS, we found high proportion (56.2%) of multidrug-resistant *Salmonella* invading sow MLN, highlighting the phenotypic differences between both biological niches. Notably, one *Salmonella* Typhimurium DT195 was resistant to antimicrobials of six different families, including third-generation cephalosporins. This finding represents a proportion of cefotaxime-resistant isolates (0.4%) lower to that observed in EU (1.2%) and Spain (1.1%) (EFSA-ECDC, 2019). This particular clone should be monitored to avoid the expansion of emerging genetic mobile elements carrying resistance to multiple antibiotics of interest in human treatments.

Previous information indicated that a main risk factor associated to *Salmonella* fecal shedding in sows was a high replacement rate by external gilts (Davies *et al.*, 2000). In Navarra, the intensive production of breeding sows was based on a closed self-replacement with gilts born in the herd, which could contribute positively to the moderate-low prevalence observed in our study. In this study, the main risk factors associated to MLN infection were administration of dry food (instead of food mixed with water) and maintenance of sows in nonrenovated farms. The former could be associated to a lower persistence of *Salmonella* in feed water after acidic fermentation (Van Winsen *et al.*, 2000; Missotten *et al.*, 2015); and the latter, to an inefficient disinfection of old materials and/or the presence of *Salmonella* vectors, such as lizards, birds, or rodents (Andrés-Barranco *et al.*, 2014). Besides strategies directed to correct these risk factors, other measures could be implemented to reduce MLN infections in sows, such as nutritional programs, including the addition of organic acids or prebiotics (Andrés-Barranco *et al.*, 2015). Since some studies suggest a vertical dissemination of *Salmonella* from breeding to fattening pigs (EFSA, 2011), the application of all these control measures in sows would contribute to improve the control of this important zoonosis at farm level, to minimize the risk of pork food contamination from farm to fork and to preserve the competitiveness of this economical sector.

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