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Sources for *Salmonella* Contamination During Pig Production in Eastern Spain

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Abstract

Salmonella spp. is one of the important foodborne pathogen and pose potential threats to consumers. The aims of this study are to determine the main sources of *Salmonella* contamination in pig production to assess the main risk factors for *Salmonella* contamination of pig herds at the end of the rearing period and determine the main serovars involved in pig production. Over two years, 47 commercial pig farms from the Valencian Region were intensively sampled. Each farm was sampled at different times during the fattening period. First, when the previous herd was taken to the slaughterhouse, *Salmonella* status of the house was assessed before and after cleaning and disinfection (C&D). During rearing, each farm was visited four times (day 1, 45, 90 and 135) taking samples of feces, dust, pen surfaces, corridor surfaces, water and farmers' boots. In addition, fly and rodent traps were set up insisted the house. All samples taken were analyzed according to ISO 6579:2002 (Annex D) and positive samples were serotyped using Kauffman-White-Le-Minor technique. A total of 2,226 samples were taken and the total prevalence of positive samples was 19.6%. The results showed that 72.3% and 65.9% of the houses were positive for *Salmonella* before and after C&D, respectively. At the end of rearing period, houses were contaminated with feces (63.6%), corridor surfaces (54.5%), dust (40.5%), farmers' boots (34.1%), water from drinkers (11.4%), tank water (9.1%) and pen surfaces (6.8%). The main risk factors for *Salmonella* contamination of pig herds at the end of the rearing period are *Salmonella* status of the house before C&D, *Salmonella* status of corridors and water from drinkers. The most prevalent serovars isolated from broiler production were *S. Typhimurim* (32.3%), followed by *S. Rissen* (23.6%) and *S. Derby* (19.7%). In this context, prevention of *Salmonella* contamination in swine products requires detailed control throughout the production chain to eradicate the bacteria from the primary production stage.

Keywords

Food Safety, Risk Factors, Public Health, Serovars

1. Introduction

Salmonella has long been recognized as an important zoonotic pathogen of economic significance in animals and humans [1]. Eggs and poultry currently remain the main sources for these human infections. However, in recent years pork has also been acknowledged as a significant source of human salmonellosis. The EFSA [1] reported that around 10-20% of food-borne *Salmonella* infections in the European Union (EU) may be attributable to consumption of pork meat and pork products. As *Salmonella* is identified in all stages of porcine production, the EU have promoted a farm to fork approach including stronger regulations throughout the production chain. The food safety system must include *Salmonella* control programs at pre-harvest level, intended to identify the bacteria using on-farm intervention strategies to reduce the prevalence of *Salmonella* infection [2], [3].

Several authors reported the wide variety of routes by which *Salmonella* can be disseminated within porcine farms from different countries [4], [5], [6]. In poultry production, it has been suggested that that two of the most important risk factors are the *Salmonella* status of the previous herd and day-old chick herds [7], [8]. Similar results were obtained in

pig production, where different studies demonstrated that infection in piglets could be vertical from infected breeding sows [9], [10] or horizontally transmitted at farm level, from the house environment [10], [11], [12], [13].

Contamination of the resident environment of animal housing has been implicated in many studies as a source of *Salmonella* infection. These bacteria are able to survive 6 years or more in the environment and the challenges of cleansing and disinfecting animal housing are well documented [10], [13]. Moreover, the risk of *Salmonella* infection was also increased when removing rodents and insects took place during rearing period [11]. These authors also reported that the presence of contaminated carriers was involved in entrance, transmission and recontamination of houses by *Salmonella* after cleaning and disinfection.

In addition, several studies showed that feed and water in pig houses are risk factors significantly related to the herd status. The role of feed and water in the spread of *Salmonella* throughout the pig industry has received a great deal of attention in recent years [14], [15], [16]. Other factors, such as farmer management, number of visits, herd size, farm housing facilities, and use of antibiotics or intercurrent diseases have been reported as possible risks of *Salmonella* contamination [5], [6], [14], [17].

The main objectives of this study are: (i) to determine the main sources for *Salmonella* contamination in pig production, (ii) to assess the main risk factors for *Salmonella* contamination of pig herds at the end of the rearing period and (iii) o determine the main serovars involved in pig production systems in Eastern Spain.

2. Materials and Methods

2.1. Study Sample

For two years, 47 commercial pig farms from the Valencian Region (East Spain) were sampled. The number of farms analyzed provides this study with a 95% confidence level and a power of 80% (Win episcope® 2.0). Only one herd was studied on each farm. These farms belong to eight different companies, which have the major part of slaughtered pigs in the Valencian Region. In order to take part in the study, the farms had to be commercial fattening units with all-in all-out system. Farm buildings were rectangular shaped, with a central corridor or two lateral corridors depending on farm size. They were divided into pens with an area of approximately 10 m^2. Moreover, all the farms were provided with natural air flow and had some windows on the walls. The farm locations and day of placing the piglets were provided by the companies. All the farm owners were willing to cooperate during the life span of the swine herd.

2.2. Sample Collection

Each farm was visited and sampled at 7 different times during the rearing period. The first visit occurred when the previous herd left for the slaughterhouse (before cleaning and disinfection, C&D). The second visit was after C&D. Then, the farm was visited a third time, just before placing piglets (when animals were approximately 2 months old). During the rearing period, each farm was visited three more times after the piglets were placed: after one month and a half, after three months and on slaughter day. Apart from these visits, the farms were also visited for pest captures (flies and rodents).

2.2.1. Before Cleaning and Disinfection

To assess the *Salmonella* status of the previous herd, three feces samples were taken from the pens with three pairs of swab-socks. One pair of swab-socks was used in each sample. Feces were taken by walking over three different pens and each pair of swab-socks with fecal material fixed was analyzed as an individual sample. In addition, two water samples were taken (500 mL): one from the tank and another from three different drinking troughs. Water samples were homogenized at the laboratory and 25 mL was analyzed from each source. Then, two feed samples were taken (500 g): one from the bins and another from three different hoppers. The feed samples were homogenized in the laboratory and 25 g were analyzed. Moreover, 100 g of dust was collected from different points of the house, the sample was homogenized in the laboratory and a 25 g sample was analyzed. Then, farmers' boots were swabbed with sterile wet gauze pads (AES laboratories®, Bruz Cedex, France). Finally, two surface samples were taken: one from the pen's walls and another from the corridor floor. These samples were taken using sterile wet gauze pads (AES laboratories®, Bruz Cedex, France). Pig houses were declared contaminated if at least one of the samples taken tested positive for *Salmonella*.

2.2.2. After Cleaning and Disinfection

Salmonella status of the house was assessed taking samples of dust, pen surfaces, corridor surfaces, under-slat feces, feed from bins and hoppers, water from tank and drinkers, and farmers' boots as described above. In addition, three surface samples were taken from hoppers, drinkers and farm tools using sterile wet gauze pads (AES laboratories®, Bruz Cedex, France). If possible, one sample was taken from remainder feces in pens. Pig houses were declared contaminated if at least one of the samples taken tested positive for *Salmonella*.

2.2.3. First Day of Rearing

To determine the *Salmonella* status of the piglet herds, three feces samples were taken directly from the rectum. Pig herds were declared contaminated if at least one of the samples taken tested positive for *Salmonella*.

2.2.4. During the Rearing Period

In each visit, *Salmonella* status of the house was assessed taking samples of dust, pen surfaces, corridor surfaces, feed (bins and hoppers), water (tank and drinkers), and farmers' boots as described above. Moreover, three samples of feces were taken using swab-socks as described before.

2.2.5. At the End of Rearing Period

Salmonella status of the house was assessed taking samples

of dust, pen surfaces, corridor surfaces, feed (bins and hoppers), water (tank and drinkers) and farmers' boots as described above. Likewise, three samples of feces were taken using swab-socks as described above. Finally, sticky strips (Fly-Kol®, Kollant) were installed inside the house to trap flies. In addition, rodent traps (Cage All®, Tom cat® and T-Rex® by Bell, USA) were set on the house floor.

2.2.6. One Week After Installing the Traps

Flies sticky strips and rodents traps were collected. Flies were analyzed as a pool. Liver, spleen and intestines of rodent carcasses were removed aseptically for culture.

2.3. *Salmonella* Isolation

Samples were collected directly into 500 mL sterile sample jars and analyzed according to ISO 6579:2002 (Annex D) [18]. First, the samples were pre-enriched in 1:10 vol/vol Buffered Peptone Water 2.5 % (BPW, Scharlau®, Barcelona, Spain) and then incubated at 37±1 ºC for 18±2 h. The pre-enriched samples were transferred onto Semi-Solid Modification Rappaport Vassiliadis (MSRV, Difco®, Valencia, Spain) agar plate (0.1 mL) and incubated at 41.5±1 ºC for 24-48 h. The culture obtained in MSRV was inoculated onto Xylose-Lysine-Desoxycholate (XLD, Liofilchem®, Valencia, Spain) and Xylose-Lysine-Tergitol-4 (XLT4, Biokar Diagnostics®, Pantin Cedex, France) and incubated at 37±1 ºC for 24-48 h. After incubation, 5 colonies that showed the expected colony characteristics of *Salmonella* were streaked onto the surface of pre-dried nutrient agar plates (Scharlab®, Barcelona, Spain) 37±1 ºC for 24±3 h. Then, a biochemical test API (API-20®, bioMerieux, Madrid, Spain) was done to confirm *Salmonella spp.* Moreover, *Salmonella* strains isolated were serotyped by the Ministry of Environment and Rural and Marine Affairs Reference Laboratory (Algete, Madrid, Spain) in accordance with Kauffman-White-Le-Minor technique.

2.4. Statistical Analysis

The prevalence of *Salmonella* contamination according to the type of sample collected and the moment of sampling (previous herd leaving, first day of rearing, during rearing and at the end of rearing) were compared by a Chi-square Test. On the other hand, a two-stage procedure was used to assess the relationship between samples collected and *Salmonella* status of the herd at the end of the rearing period. The unit of observation was the herd. A herd was declared contaminated by *Salmonella* if one or more samples taken from the house at the end of rearing period tested positive. The outcome variable was thus dichotomous (contaminated herd versus non-contaminated herd). Logistic regression analysis was used according to the method described by Rose et al. [7]. In the first stage, a univariable analysis was performed to relate *Salmonella* contamination of the herd to each sample. Only factors associated with *Salmonella* contamination of the herd were considered for the next analysis (Chi-square Test, $P<0.25$). The second stage involved a logistic multiple-regression model which included all factors that passed the first screening test. The contribution of each factor to the model was tested using a Chi-squared Test. The variable with the highest P was removed and the logistic regression was rerun. This process was continued until a model was obtained with all factors significant at $P<0.05$. Statistical analyses were performed using a commercially available statistics package (Statgraphics Plus, Version 5.1, STSC Inc., Rockville, MD, USA).

3. Results

A total of 2,226 samples were taken at different times of the rearing period and the total prevalence of positive samples was 19.6 %.

3.1. Environmental *Salmonella* Contamination of Pig Houses

When the previous herd left for the slaughterhouse, Salmonella prevalence of the samples according to the moment of sampling (before and after C&D) was statistically different ($P<0.05$). The results showed that 72.3% and 65.9% of the houses were positive for Salmonella before and after C&D, respectively. Before C&D, houses were contaminated (from highest to lowest) with feces from the previous herd (61.7%), corridor surfaces (40.4 %), pen surfaces (23.4%), farmers' hands and boots (21.3%), dust (14.9%), water from drinkers (8.5%) and tank water (2.1%, Table 2). Significant differences were found between Salmonella contamination and the type of sample collected before C&D ($P<0.05$). After C&D, 65.9 % of the houses remained positive for Salmonella in all samples collected, except in tank water samples (Table 1). Residual fecal material was observed on feeder, drinkers and pen surfaces. Also, if the slats were open, 34.0% of samples collected were positive for Salmonella contamination.

Table 1. *Percentage of Salmonella-positive houses before and after cleaning and disinfection.*

Samples	Cleaning and disinfection	
	Before	After
Tank water	2.1[a]	0.0[a]
Drinker water	8.5[a]	2.1[a]
Faeces	61.7[c]	53.2[c]
Boots	21.3[b]	13.0[ab]
Dust	14.9[ab]	13.6[ab]
Pen surfaces	23.4[b]	23.4[b]
Corridor surfaces	40.4[bc]	27.7[b]

a,b,c,d: Data in the same column with uncommon letters are different ($p < 0.05$).

When pigs (two months old) arrived from the fattening farm, 53.2% of the herds were determined positive for Salmonella.

After one and a half month of fattening, 53.3% of the houses assessed were environmentally contaminated with Salmonella. Significant differences were found between Salmonella contamination and the type of sample collected ($P<0.05$). Houses were contaminated (from highest to lowest) in feces (44.4%), farmer boots (15.9 %), dust (13.6%), pens

and corridor surfaces (8.8%), water from drinkers (8.8%) and water from the tank (2.2%) (Table 2). After three months of rearing, 63.8% of the houses assessed were environmentally contaminated with Salmonella. Significant differences were found between Salmonella contamination and the type of sample collected (P<0.05). Houses were contaminated (from highest to lowest) in feces (46.8%), dust (26.1%), corridor surfaces (25.5%), farmer boots surfaces (20.0%), pens surfaces (12.8%), water from drinkers and water from the tank (4.2 and 4.3%, respectively) (Table 3). No significant differences were found between Salmonella contamination of feed samples collected from the bins (5.0 %) and those collected from feeders (7.1 %).

At the end of rearing period, 77.3% of pig houses assessed were environmentally contaminated with Salmonella. Significant differences were found between Salmonella contamination and the type of sample collected (P<0.05). Houses were contaminated (from highest to lowest) in feces (63.6%), corridor surfaces (54.5%), dust (40.9 %), farmer boots (34.1%), water from drinkers (11.4%), water from the tank (9.1%) and pens surfaces (6.8%) (Table 2).

Finally, the total prevalence of positive carriers trapped was 29.7%. No significant differences were found between Salmonella contamination and the carrier trapped. The prevalence was 31.5% and 26.3% for flies and rodents respectively.

Table 2. *Percentage of Salmonella-positive houses by samples collected during rearing.*

Samples	Rearing period (days)			
	1	45	90	135
Tank water	0.0^{a}	2.2^{a}	4.4^{a}	9.1^{a}
Drinker water	2.1^{a}	8.9^{ab}	4.3^{a}	11.4^{a}
Faeces	53.2^{c}	44.4^{c}	46.8^{c}	63.6^{c}
Boots	13.0^{ab}	15.9^{b}	20.0^{b}	34.1^{b}
Dust	13.6^{ab}	13.6^{b}	26.1^{b}	40.5^{b}
Pen surfaces	23.4^{b}	8.9^{ab}	12.8^{ab}	6.8^{a}
Corridor surfaces	27.7^{b}	8.9^{ab}	25.5^{b}	54.5^{bc}

a,b,c,d: Data in the same column with uncommon letters are different ($p < 0.05$).

3.2. Main Risk Factors for *Salmonella* Contamination in Pig Herds at the End of the Rearing Period

The results of this study suggested that Salmonella status of the house when the previous herd left for the slaughterhouse (before C&D), corridor surfaces and drinkers water were the main risk factors related to Salmonella contamination of the herd at the end of the rearing period (P=0.0135, Table 3). In contrast, factors such as dust, pens surfaces, traces of feces, status of the pigs entering the house and feed do not seem to be related to Salmonella contamination of the herd at the end of the rearing period (Table 3).

Table 3. *Relationship between the house status before cleaning and disinfection and the samples collected after cleaning and disinfection in Salmonella status of the flock at the end of rearing period.*

Samples	n	P-value
House status b/C&D	47	0.0431
Drinker water a/C&D	47	0.0403
Dust a/C&D	44	0.2841
Pen surfaces a/C&D	47	0.2584
Corridor surfaces a/C&D	47	0.0073
Under-slat faeces a/C&D	47	0.8535
Feed from hoppers a/C&D	43	0.9999

n: number of farms. b/C&D: Before cleaning and disinfection. a/C&D: After cleaning and disinfection. Logistic-regression model: Model P-value=0.0135; Model deviance=19.3 %. Percentage of deviance explained by the model=38.6 %.

3.3. Serotypes Isolated in Pig Production Related Samples

During this study, a total of 492 Salmonella strains were isolated and 29 different serotypes were determined. The most prevalent serotype isolated was S. Typhimurium (32.3%), followed by S. Rissen (23.6%), S. Derby (19.7%), S. enterica enterica 4,12:i:- (7.1%), S. Goldcoast (3.9%), S. Anatum (2.4%) and S. Wien (1.2%) (Figure 3). The rest of the serotypes isolated (9.8 % of the total) were in decreasing order: S. Bovismorbificans (1.0%), S. London (0.8%), S. Bredeney (0.6%), S. Lisboa (0.6%), S. Toulon (0.6%), S. Amsterdam (0.4%), S. Salamae (0.4%), S. Mbandaka (0.4%), S. Infantis (0.4%), S. Enteritidis (0.4%), S. Senftenberg (0.2%), S. Rubislaw (0.2%), S. Orion (0.2%), S. Ohio (0.2%), S. Livingstone (0.2%), S. Kapemba (0.2%), S. Idikan (0.2%), S. Hadar (0.2%), S. Fresno (0.2%), S. Brikama (0.2%), S. Altona (0.2%) and S. Agona (0.2%).

4. Discussion

Salmonella infected pork and pork products are recognized as an important source for human *Salmonella* infections and pose potential threats to consumers [1]. Positive *Salmonella* status of finishing pigs assessed on the farm increased the risk of asymptomatic intestinal carriage of *Salmonella* during slaughter [19]. In Spain, around 11.0% of *Salmonella* human outbreaks are pork-related. The Hygiene Package and Regulation EC-2160/2003 require information flow from farm to slaughterhouse to enhance European consumer protection in a 'farm to fork' approach. This obligation especially concerns food-borne zoonotic hazards transmitted to humans through pork consumption [20]. In this context, prevention of *Salmonella* contamination in products of pig origin requires detailed knowledge of the most important risk factors associated with its presence in the production system [21].

The results obtained from this study showed that C&D procedures applied by farmers in Eastern Spain were unable to eradicate *Salmonella* from pig houses, in line with previous studies [13], [22]. Consequently, the status of the house before

C&D has been determined as an important risk factor related with contamination of the herd at the end of the fattening period. Little is known about the effectiveness of cleaning and disinfection methods used on commercial pig farms worldwide [12]. Several hypotheses are related with the high persistence of *Salmonella* after C&D in the agricultural sector [20], [23], [24], such as the lack of scientific literature on disinfection in farms [22], absence of official methods for testing disinfectants [25], incorrect hardness and temperature of cleaning water [26], [27] and absence of detergent during C&D procedures, which might significantly reduce the efficacy of disinfection [22], residual contamination of pig facilities [28] and biofilm development of *Salmonella* strains [29]. Consequently, it is important for C&D to be supervised properly to ensure that procedural errors do not take place [30].

In agreement with several authors, our results showed that remain of feces, contaminated surfaces, corridors and dust are an important *Salmonella* reservoir between herds [19], [22]. As a consequence of inaccurate C&D procedures, contaminated corridors on first day of fattening were determined as an important risk factor for *Salmonella* contamination of the herd at the end of rearing period. This could be explained because all pigs from the herd have to cross the corridors to reach their pens, thus disseminating the bacteria though the house. The high rate of *Salmonella* contamination of the house before swine arrival was further evidenced by the fact that 21.3 % of the farmers' boots were contaminated at this stage. Cardinale et al. [13] reported that farmers are able to spread the bacteria with their boots or tools between consecutive herds. So, when growing started, contaminated environment and farmer management could infect feeders, carriers, ventilation systems and finally the fattening herd [22].

This study revealed that there are particular problems with the cleaning of feeders and drinkers. In fact, there is a significant relation between the status of the herd at the end of the fattening period and contaminated water collected from drinkers. In line with these results, Mannion et al. [12] detected *Salmonella* in drinkers and in troughs, despite the water tanks and feed bins being negative. Because the water and feed in the pens were contaminated, the pigs could have been a reservoir of infection inside pens [12]. This may be due to the power-washing of the pen floors having caused splashing of contaminated material onto feeders and drinkers and it is possible that lowering the pressure may limit the contamination spread [12]. It is well known that residual environmental *Salmonella* contamination of the fattening house increases the risk of individual *Salmonella* infection during the fattening period [19]. Otherwise, the results of this study showed that samples protected from environmental cross contamination such as water tank or feed from bins have a lower *Salmonella* prevalence and do not seem to be linked to *Salmonella* contamination of the houses at the end of the rearing period.

The role of pests in *Salmonella* persistence in fattening houses has been reported in several studies [13], [22], [23]. In line with these previous studies, our results suggested that nearly 30% of the houses were pest contaminated with *Salmonella*. So, C&D and pest control management must be implemented effectively between herds, to minimize the chance of herd infection [11].

At the end of the rearing period, the spread of *Salmonella* was confirmed, around 50.0 % of the houses studied remained contaminated [11], [13]. Moreover, the results of this study demonstrate that environmental contamination with *Salmonella* increases throughout the growth period, being maximum at the end of fattening period in all samples collected. As a consequence of the high levels of house environment contamination, the bacteria could contaminate the animal skin and facilitate cross-contamination between carcasses and equipment during processing, increasing the contamination status of the final food products [21], [31].

The main serovar isolated from in this study (91.2 % of cases) were *S. Typhimurium, S. Rissen* and *S. Derby*, in agreement with European reports [21]. Thus, contamination with *S. Typhimurium* is an important threat to food safety, being the second serovar involved in human salmonellosis outbreaks in Europe [1].

5. Conclusion

In conclusion, the most contaminated samples related with pig production throughout the rearing period are feces, surfaces, farming boots, dust and vectors. Moreover, the main risk factors for *Salmonella* contamination of pig herds at the end of the rearing period are *Salmonella* status of the house before C&D, *Salmonella* status of corridors and water from drinkers. The most prevalent serovar isolated from broiler production in the Valencian Region is *S. Typhimurim*, followed by *S. Rissen* and *S. Derby*.

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References

[1] EFSA (European Food Safety Authority). The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. The EFSA Journal. 2011;9:2090.

[2] EC (European Commission). Commission Regulation No 2160/2003 of the European Parliament and of the Council of 17 November 2003 on the control of Salmonella and other specified food-borne zoonotic agents. Official Journal of the European Union 2003;L 325/1:12.12.2003.

[3] EC (European Commission). Commission Regulation No 646/2007 of the European Parliament and of the Council of 12 June 2007 implementing Regulation (EC) No 2160/2003 as regards a Community target for the reduction of the prevalence of Salmonella enteritidis and Salmonella typhimurium in broilers and repealing Regulation (EC) No 1091/2005. Official Journal of the European Union 2003;L151/21:16.06.2007.

[4] Nollet N, Houf K, Dewulf J, De Kruif A, De Zutter DL, Maes D. Salmonella in sows: a longitudinal study in farrow-to-finish pig herds. Vet. Res. 2005;36:645-656.

[5] Hotes S, Kemper N, Traulsen I, Rave G, Krieter J. Risk factors for Salmonella infection in fattening pigs - An evaluation of blood and meat juice samples. Zoonoses Public Health. 2010;57:30-38.

[6] Smith RP, Clough HE, Cook AJC. Analysis of meat juice ELISA results and questionnaire data to investigate farm-level risk factors for Salmonella infection in UK pigs. Zoonoses Public Health. 2010;57:39-48

[7] Rose N, Beaudeau F, Drouin P, Toux JY, Rose V, Colin P. Risk factors for Salmonella enterica subsp. enterica contamination in French broiler-chicken herds at the end of the raring period. Prev. Vet. Med. 1999;39:265-277.

[8] Marin C, Balasch S, Vega S, Lainez M. Sources of Salmonella contamination during broiler production in Eastern Spain. Prev. Vet. Med. 2011;98:39-45.

[9] Funk JA, Davies PR, Nichols MA. Longitudinal study of Salmonella enterica in growing pigs reared in multiple-site swine production systems. Vet. Microbiol. 2001;83:45-60.

[10] Beloeil PA, Fravalo P, Fablet C, Jolly JP, Eveno E, Hascoet Y, Chauvin C, Salvat G, Madec F. Risk factors for Salmonella enterica subsp. enterica shedding by market-age pigs in French farrow-to-finish herds. Prev. Vet. Med. 2004;63:103-120.

[11] Lo fo Wong DMA, Hald T, Van der Wolf PJ, Swanenburg M. Epidemiology and control measures for Salmonella in pigs and pork. Livest. Prod. Sci. 2002;76:215-222.

[12] Mannion C, Lynch PB, Egan J, Leonard FC. Efficacy of cleaning and disinfection on pig farms in Ireland. Vet. Rec. 2007;161:371-375.

[13] Cardinale E, Maedder S, Porphyre V, Debin M. Salmonella in fattening pigs in Reunion Island: Herd prevalence and risk factors for infection. Prev. Vet. Med. 2010;96:281-285

[14] Oliveira CJ, Carvalho LF, Domingues FJ Jr, Menezes CC, Fernandes SA, Tavechio AT. Dunging gutters filled with fresh water in finishing barns had no effect on the prevalence of Salmonella enterica on Brazilian swine farms. Prev Vet Med. 2002;55:173-8.

[15] Zheng DM, Bonde M, Sorensen JT. Associations between the proportion seropositive slaughter pigs and the presence of herd level risk factors for introduction and transmission of Salmonella in 34 Danish organic, outdoor (non-organic) and indoor finishing-pig farms. Livest. Prod. Sci. 2007;106:189-199.

[16] Binter C, Straver JM, Häggblom P, Bruggeman, G, Lindqvist P, Zentek J, Andersson MG. Transmission and control of Salmonella in the pig feed chain: A conceptual model. Int. J. Food Microbiol. 2001;145:7-17.

[17] Benschop J, Spencer S, Alban L, Stevenson M, French N. Bayesian zero-inflated predictive modeling of herd-level Salmonella prevalence for risk-based surveillance. Zoonoses Public Health. 2010;57:60-70.

[18] ISO 6579:2002 (Annex D). Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Salmonella spp. International Organization for Standardization. Geneva, Switzerland. 2002.

[19] Beloeil PA, Chauvin C, Proux K, Fablet C, Madec F, Alioum A. Risk factors for Salmonella seroconversion of fattening pigs in farrow-to-finish herds. Vet. Res. 2007;38:835-848.

[20] Fosse J, Seegers H, Magras C. Prevalence and risk factors for bacterial food-borne zoonotic hazards in slaughter pigs: A review. Zoonoses Public Health. 2009;56:429-454.

[21] EFSA (European Food Safety Authority). Report of the Task Force on Zoonoses Data Collection on the analysis of the baseline survey on the prevalence of Salmonella in slaughter pigs, in the EU, 2006-2007. The EFSA Journal. 2008;135:1-111.

[22] Baptista FM, Alban L, Nielsen LR, Domingos I, Pomba C, Almeida V. Use of herd information for predicting Salmonella status in pig herds. Zoonoses Public Health. 2010;57:49-59.

[23] Funk J, Gebreyes WA. Risk factors associated with Salmonella prevalence on swine farms. J. Swine Health Prod. 2004;12:246-251.

[24] Lo fo Wong DMA, Dahl J, Stege H, Van der Wolf PJ, Leontides L, Von Altrock A, Thorberg BM. Herd-level risk factors for subclinical Salmonella infection in European finishing-pig herds. Prev. Vet. Med. 2004;62:253-266.

[25] Marin C, Hernandiz A, Lainez M. Biofilm Development Capacity of Salmonella Strains Isolated in Poultry Risk Factors and their Resistance against Disinfectants. Poult. Sci. 2009;88:424–431.

[26] Leriche V, Carpentier B. Viable but nonculturable Salmonella typhimurium in single and binary-species biofilms in response to chlorine treatment. J. Food. Prot. 1995;58: 1186-1191.

[27] Taylor JH, Holah JT. A comparative evaluation with respect to the bacterial cleanability of a range of wall and floor surface materials used in the food industry. J. Appl. Bacterial. 1996;81:262-266.

[28] Funk JA, Davies PR, Gebreyes W. Risk factors associated with Salmonella enterica prevalence in three-site swine production systems in North Carolina, USA. Berl. Munch. Tierarztl. Wochenschr. 2001;114:335-338.

[29] Marin C, Lainez M. Salmonella detection in feces during broiler rearing and after live transport to the slaughterhouse. Poult. Sci. 2009;88:1999-2005.

[30] Davies RH, Breslin M, Corry JEL, Hudson W, Allen VM. Observations on the distribution and control of Salmonella species in two integrated broiler companies. Vet. Rec. 2001;149:227-232.

[31] McCrea B, Tonooka K, Van Worth C, Boggs C, Atwill E, Schrander J. Prevalence of Campylobacter and Salmonella species on farm, after transport, and at processing in specialty market poultry. Poult. Sci. 2006;85:136-143.