# An Exploratory Study About Contamination Of Pens Of Finishing Pigs By Ubiquitous *Salmonella*.

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## Introduction

Salmonella is one of the most common food-borne pathogens transmitted to humans and human salmonellosis is primarily caused by contaminated food. Porcine products have been identified as important food vehicles in outbreaks of salmonellosis (1, 2, 3). In France, the majority of *Salmonella* infected pig herds are sub-clinically infected. *S.* Choleraesuis does not occur and only ubiquitous serotypes are isolated (4). Sub-clinically infection is characterized by intermittent shedding of small numbers of *Salmonella*. In these sub-clinically infected pig herds, an infection-contamination-infection cycle is maintained with mainly an endemic "house flora" of *Salmonella enterica* (5). When contaminated batches from these farms are processed on the slaughter line, slaughtering practices contribute to *Salmonella* dissemination and carcass contamination. Within batches, there is a strong correlation between the proportion of animals with *Salmonella* spp. in their feces and the proportion of contaminated carcasses at the end of the line (6, 7). As a result of subclinical *Salmonella* infection in pig herds, *Salmonella* contamination of pork carcasses constitutes a threat to human health. The influence of a wide range of factors on subclinical *Salmonella* contamination of pig farms is not well known. A good understanding of risk factors for *Salmonella* contamination of pig herds is an essential stake in order to avoid *Salmonella* spread within herds and between herds and slaughterhouses. Thus, the aim of this study was to investigate potential risk factors for the presence of ubiquitous *Salmonella* in the finishing sheds of farrow-to-finish farms in France.

## Materials and methods

### Study design and herds sampling

Sixty-nine farrow-to-finish pig farms with different levels of *Salmonella* contamination were investigated. The 69 finishing herds from 69 farrow-to-finish farms involved in our study were affiliated with five pig productions or feedstuffs companies. They were visited from April to August 1998. All farms were located in Brittany (France). They were selected for *Salmonella* contamination according to the knowledge of farm veterinarians and the willingness of the farmers to cooperate. The *Salmonella* status of the finishing sheds investigations were assessed by using environmental swabs.

### Bacteriological testing

*Environmental samples from housing pens*

In this survey, 2 rooms in which fattening pigs (the closest to market weight) were housed, were examined through an environmental bacteriological testing. The rooms varied in size from 8 to 12 pens. In each room, the slatted floor and the bottom of the walls and pen partitions soiled with fecal material were wiped with 7 environmental SODIBOX® swabs (Sodibox, La Forêt-Fouesnant, France) : 14 environmental swabs per herd were used. The SODIBOX ® environmental swabs are a sterile square piece of cotton cloth (32 cm ´ 32 cm) moistened with isotonic saline. After use the soiled environmental swabs were placed into a sterile plastic bag using a sterile glove and immediately brought to the laboratory.

*Microbiological investigations*

Environmental swabs were analysed for the presence of *Salmonella enterica*. 150 ml of buffered peptoned water (BPW) were poured directly into each plastic bag with the swab. The bag was closed and incubated overnight at 37°C. Following pre-enrichment, selective enrichments were performed both on Modified Semisolid Rappaport Vassiliadis agar (MSRV) and in Müller-Kauffmann tetrathionate broth (MKTB). The selective media were respectively incubated at 41,5°C and at 42°C for 18 to 20 hours. A delayed secondary enrichment of 3 days for MSRV plates was made in order to increase the sensitivity. Plating included streak inoculations from the migrated colonies of MSRV plates onto Rambach agar plates and from MKTB onto Xylose-Lysine-Tergitol-4 (XLT4) agar plates. Rambach and XLT4 were both incubated at 37°C for 24 hours. Two presumptive positive colonies on XLT4 or Rambach plates were verified by biochemical reactions. They were inoculated in Kligler-Hajna medium incubated at 37°C for 24 hours. The microbiological analysis was performed according to a protocol defined by the French National Center for Veterinary and Food Hygiene Research (8). All isolates were serotyped by agglutination according to the Kauffmann-White scheme (9).

### Definition of variables

A farm was considered as contaminated if only one or more environmental samples tested positive. If all the swabs tested negative, the farm was declared non-contaminated.

The dependant variable was thus dichotomous (contaminated finishing shed vs. non-contaminated finishing shed). A questionnaire was used to gather information on potential risk factors by interviewing systematically the farmers. The questions dealt with characteristics of the farm, type of management, type of feeding (dry or liquid), water quality, housing, health disorders during post-weaning and finishing phases, cleaning and disinfection procedure, rodent control and desinsectization. Information about diseases were based on veterinarians' clinical diagnostics or results of laboratory investigations if they were required by veterinarians. Data obtained during this survey were stored using EpiInfo 6 fr software (10).

### Statistical analysis

In a first step, quantitative independant variables were categorized and all independant variables described. The binary herd status with respect to *Salmonella* (contaminated vs. non-contaminated) was combined with the questionnaire data. A bivariate analysis was thus carried out using $c^2$ test or 2-tail Fisher's exact test in order to select a pool of independant variables significantly related to the outcome ($p<0.25$) (11). Multiple linear logistic regression (PROC LOGISTIC, SAS, 1989) was used to assess the association between the occurrence of *Salmonella* and the presence of potential risk factors in pig herds (12). The relationship between the dependant variable and selected independant variables were investigated by a backward stepwise logistic regression analysis to analyse explanatory variables simultaneously and thus to adjust for confounding factors. Interactions were not tested because of small sample size. Odds Ratios (OR), adjusted for other risk factors in the model, with accessory 90% confidence intervals (CI), were calculated to measure the strength of the associations.

Table 1 : Growth performance characteristics of the 69 finishing herds (Western France, 1998)

| Characteristics (between 25-105 kg live weight) | Minimum | Maximum | Median | Mean | SD |
|---|---|---|---|---|---|
| Mean daily weight gain (g/day) | 382 | 892 | 760 | 744.6 | 90.8 |
| Feed conversion ratio | 2.44 | 3.29 | 2.8 | 2.86 | 0.21 |

There was no significant difference in Mean Daily Weight Gain and Feed Conversion Ratio during the fattening period between the positive (745.33 g/day ; 2.83) and the negative (744.14 g/day ; 2.87) finishing herds.

## Results

### An overview on the farms sample

Table 1 gives the average growth performance characteristics between 25 and 105 kg weight of the participating finishing herds.

### Measurement of finishing unit contamination by ubiquitous Salmonella

Among the 69 finishing herds studied, 58 % were positive for *Salmonella*. The most prevalent isolated serovars were *S.* Typhimurium and *S.* Derby (Table 2).

Table 2 : Percentages of contaminated finishing sheds, according to the serovars of *Salmonella enterica* subsp. *enterica* (69 farrow-to-finish farms, Western France, 1998).

| Serotypes | Number | Percentage (%) |
|---|---|---|
| Agona | 1 | 1.4 |
| Anatum | 1 | 1.4 |
| Bredeney | 1 | 1.4 |
| Infantis | 1 | 1.4 |
| London | 1 | 1.4 |
| Montevideo | 1 | 1.4 |
| Brandenburg | 4 | 5.8 |
| Derby | 7 | 10.1 |
| Typhimurium | 12 | 17.4 |
| negative | 40 | 58.0 |
| | 69 | 100 |

**The search for risk factors using logistic regression**

In the bivariate analysis, the following 6 variables were associated with *Salmonella enterica* contamination of finishing pigs ($p < 0,25$) : diarrhea at the weaning, cleaning with a detergent, digestive disease (other than salmonellosis) during finishing phase, use of an unique feed from the mid-postweaning phase to slaughtering, type of feeding (dry or humid) during the finishing phase, duration of the period while the rooms were "empty and clean" between two successive batches for the finishing room. Since the type of feeding and the number of feeds used after the mid-postweaning phase were correlated ($p = 0.052$), it was decided to include only the type of feeding. Other correlations between variables that remained after the univariate step were considered low, and therefore all these variables were included in the multivariate analysis. No confounding was found. Table 3 presents results of the multivariate logistic regression.

Table 3 : Variables and variable categories in the final logistic regression model for risk factors for *Salmonella* contamination of finishing pig sheds (69 western France farrow-to-finish farms, 1998).

| Variables | Logistic Regression Model | |
|---|---|---|
| | OR | 90% CI |
| Digestive disorders during the finishing phase [a] | | |
| Yes | 3.6 | 1.3 - 9.9 |
| No | 1 | — |
| Type of feeding during finishing phase | | |
| Dry | 3.8 | 1.5 - 9.6 |
| Liquid | 1 | — |
| Duration of the period while the rooms were "empty and clean" between two successive batches (day) | | |
| $D \leq 1$ | 5.1 | 1.6 - 16.5 |
| $1 < D \leq 4$ | 1.7 | 0.52 - 5.3 |
| $D > 4$ | 1 | — |

[a] other than salmonellosis

Digestive disorders during the finishing phase was a potential risk factor of *Salmonella* shedding (OR = 3.6). Dry feeding during the same phase was significantly related to the *Salmonella* status (OR = 3.8). When the duration of the period while the rooms were "empty and clean" between two successive batches was short (<1 day), the risk of contamination was significantly increased (OR = 5.1).

## Discussion

In subclinically infected herds, slaughter pigs have a chronicle and silent infection with only intermittent excretion with a low level of *Salmonella* in the feces. So, examination of individual feces samples on a pig sample may constitute a poor-sensitive method. According to the literature, environmental isolations in contaminated pig herds occur in all kinds of material but is most often found out from slurry, fecal material pen samples (13). Since ubiquitous *Salmonella* are resistant in the environment of pigs, pen samples representing several individuals (between 15 to 20 pigs) appeared to be an appropriate way to assess microbiological status of herds. *Salmonella* is isolated from all sections within contaminated herds. Moreover, in low contaminated, subclinically infected herds, *Salmonella* tended to be more frequently isolated from the finisher unit than from other part of the herd (13). With respect to those results, a test on environmental samples seems to be an useful control point for monitoring the *Salmonella* status of pig herds.

As there is not an effective database about the *Salmonella* status of the pig farms available at the moment, a formal randomization couldn't be carried out. The required willingness of farmers might have led to the selection of farms with a relatively high-performance level. Nevertheless, technical characteristics as Mean Daily Weight Gain and Feed Conversion Ratio included in the present study were similar ($p < 0.05$) to the means calculated at the area level (Region of Brittany, France).

The most common serovars isolated in our study were *S.* Typhimurium and *S.* Derby. These serotypes were also the most frequent serovars isolated from pigs on farms in the UK in 1996 (14). In Denmark, *S.* Typhimurium and *S.* Derby were the most frequently isolated serotypes in the 1995 routine submissions with respectively 86.6% and 5.7% of the isolates (15). *S.* Derby and *S.* Typhimurium were also the most frequent serotypes isolated in faecal samples from finishing pigs from swine herds in North Carolina, USA (16).

The risk of *Salmonella* shedding is increased when diarrhea occurs during the finishing phase. The enteric health status is quoted by authors as a potential risk factor which have not been proven but is plausible based on field experience. Thus it is generally believed that enteric health problems caused by other pathogens (porcine intestinal adenomatosis, swine dysentery) predisposes for subclinical salmonellosis, because enteric health problems disturbs the ecological balance of the gut flora (17).

A duration of the period while the room is "empty and clean", between two successive batches, of less than 1 day increased the risk for a finishing shed to be contaminated. Strategic removal of pigs from pig herds infected with *Salmonella* Typhimurium was shown to be an efficient way of controlling *Salmonella* infection. This could indicate, that

*Salmonella* infection could be managed, if strict all in-all out procedures including cleaning and disinfection was applied (18).

The risk for a finishing shed to be *Salmonella* contaminated also increased when the distributed feeding is dry. This result confirms those of two Danish epidemiological studies, which showed that *Salmonella* infection was more prevalent in dry-fed herds than in those which received wet feed (19, 20). Automated liquid feeding of by-products significantly increases the odds of a negative Salmonella status (21). Explanations of this phenomenon are not so clear. It was demonstrated that high levels of undissociated volatile fatty acids inhibited the growth of salmonella in sterilized cæcal content (22). In most wet feeding systems, a natural fermentation process increases the growth of lactic-acid producing bacteria and yeasts (23). The low pH of between 4.0 and 4.5 and the presence of organic acids can explain this protective effect (24). This could explain the protective effect of wet feed.

That study was a preliminary screening for risk factors for *Salmonella*. The clarification of risk factors will require larger scale study, and/or prospective intervention studies to document the effect.

## References


1. Desenclos, J. C., I. Rebière, P. Bouvet, et coll. 1996. Bilan de 5 épidémies communautaires de salmonelloses, France, 1993-1994. Bull Epidemiol Hebd. 9 : 39-43.

2. Hald, T., K. Mølbak, D. L. Baggesen, J. Neimann. 1997. An outbreak of salmonellosis in Denmark caused by pork from a single slaughterhouse, p. 60. *In* Proceedings of The Second International Symposium on Epidemiology and Control of Salmonella in Pork. Copenhagen, Denmark, 20-22 August 1997.

3. Wegener, H. C., D. L. Baggesen. 1996. Investigation of an outbreak of human salmonellosis caused by *Salmonella enterica* ssp. *enterica* serovar Infantis by use of pulsed field gel electrophoresis. Int. J. of Food Microbiol. 32 (1-2) : 125-131.

4. Laval, A., H. Morvan, G. Deperez, B. Corbion. 1991. La salmonellose du porc. Rec. Med. Vet. 167 : 835-848.

5. Berends, B. R., H. A. P. Urlings, J. M. A. Snijders and F. V. Knapen. 1996. Identification and quantification of risk factors in animal management and transport regarding *Salmonella* spp. in pigs. Int. J. of Food Microb. 30 (1/2) : 37-53.

6. Berends, B. R., F. Van Knapen, J. M. A. Snijders and D. A. A. Mossel. 1997. Identification and quantification of risk factors regarding *Salmonella* spp. on pork carcasses. Int. J. of Food Microb. 36 (2/3) : 199-206.

7 Chau, F. Y., K. F. Shortridge and C. T. Huang. 1977. Salmonella in pig carcasses for human consumption in Hong Kong : a study on the mode of contamination. J. Hyg. Camb. 78 : 253-260.

8. Humbert, F. 1996. Texte français de référence BA 70/00 pour l'application du programme d'accréditation n° 116 du COFRAC. Maisons-Alfort, Centre National d'Etudes Vétérinaires et Alimentaires.

9. Popoff, M. Y., L. Le Minor.1992. Antigenic formulas of the *Salmonella* serovars. WHO collaborating centre for reference and research on *Salmonella*. Paris, Publ. Institut Pasteur.

10. Dean, A. G., J. A. Dean, D. Coulombier, K. A. Brendel, D. C. Smith, A. H. Burton, R. C. Dicker, K. Sullivan, R. F. Fagan, T. G. Arner. 1995. Epi Info, Version 6 : a word processing, database, and statistics program for public health on microcomputers. Atlanta, Georgian, USA, Centers for Disease Control and Prevention.

11. Hosmer, D.W. and S. Lemeshow. 1989. Applied Logistic Regression. John Whiley & Sons, New York : 82 - 134.

12. SAS Institute Inc., 1989. SAS/STAT User's Guide, Version 6, fourth edn. SAS Institute, Cary, NC.

13. Baggesen, D. L., J. Dahl, A. Wingstrand and B. Nielsen. 1996. Critical Control Point (CCP) in pig herds in relation to subclinical salmonella infection, p. 171. *In* Proceedings of the 14th International Pig Veterinary Society Congress. Bologna, Italy, 7-10 July 1996.

14. Alexander, T. 1998. Zoonoses, p. 167. *In* Proceedings of the 15th International Porcine Veterinary Society Congress. Birmingham, England, 5-9 July 1998.

15. Christensen, J. and D. L. Baggesen. 1998. The occurrence of serotypes of *Salmonella enterica* and phage types of *S.* Typhimurium in Danish swine herds, p. 170. *In* Proceedings of the 14th International Porcine Veterinary Society Congress. Bologna, Italy, 7-10 July 1996.

16. Davies, P. R., W. E. M. Morrow, F. T. Jones, J. Deen, P. J. Fedorka-Cray and I. T. Harris. 1997. Prevalence of salmonella in finishing swine raised in different production systems in North Carolina, USA. Epidemiol. Infect. 119 : 237-244.

17 Dahl, J. and A. Wingstrand. 1997. Reduction of subclinical *Salmonella*-infection in Danish pig herds. A summary of documented and plausible risk-factors and how this knowledge is implemented into a guide for reduction and control of *Salmonella*-infections, p. 631-635. *In* Proceedings of Salmonella and Salmonellosis'97 Congress. Ploufragan, France, 20-21-22 May 1997.

18 Dahl, A., A. Wingstrand, B. Nielsen, D.L. Baggesen. 1997. Elimination of *Salmonella* Typhimurium infection by strategic movement of pigs. The Veterinary Record. 140 : 679-681.

19. Dahl, J. 1997. Cross-sectional epidemiological analysis of the relations between different herd factors and *Salmonella* seropositivity. Epidemiologie et Sante Animale. N°31/32 : 04.23.1-04.23.3.

20. Stege, H., N. C. Feld, D. L. Baggesen, J. P. Nielsen and P. Willeberg. 1997. Subclinical *Salmonella* infection in Danish finishing pig herds : risk factors. Epidémiologie et Santé Animale. N°31/32 : 07.13.1-07.13.3.

21. van der Wolf, P. J., A. R. Elbers, W. B. Wolbers, J. M. C. C. Koppen, H. M. J. F. van der Heidjen, F. W. van Schie, W. A. Hunneman, M. J. M. Tielen. 1998. Risk factors for *Salmonella* in slaughter-pigs in the Netherlands, p. 68. *In* Proceedings of the 15th International Porcine Veterinary Society Congress. Birmingham, England, 5-9 July 1998.

22. Prohazska, B. M., B. M. Jayarao, A. Fabian, S. Kovacs. 1990. The Role of Intestinal Volatile Fatty Acids in the *Salmonella* Shedding of Pigs. Journal of Veterinary Medicine. B57 : 570-574.

23. Wingstrand, A., J. Dahl, L. K. Thomsen, L. Jorgensen, B. B. Jensen. 1997. Influence of dietary administration of organic acids and increased feed structure on *Salmonella* Typhimurium infection in pigs, p. 170. *In* Proceedings of the Second International Symposium on Epidemiology and Control of Salmonella in Pork. Copenhagen, Denmark, 20-22 August 1997.

24. van Winsen, R. L., H. A. P. Urlings, J. M. A. Snijders. 1997. Feed as a vehiculum of *Salmonella* in pigs, p. 157. *In* Proceedings of the Second International Symposium on Epidemiology and Control of *Salmonella* in Pork. Copenhagen, Denmark, 20-22 August 1997.