FACTORS ASSOCIATED WITH *SALMONELLA ENTERICA* SHEDDING BY FINISHING PIGS. AN EPIDEMIOLOGICAL SURVEY IN FRENCH PIG FARMS

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Abstract

An analytic epidemiological survey was carried out in France to better assess the circumstances for subclinical *Salmonella* contamination of fattening pigs. A cohort study was carried out from November 2000 to October 2001. 105 French farrow-to-finish pig farms were included in this study. In each herd, a batch of contemporary pigs was followed from the end of the post-weaning phase up to slaughter, through several visits. *Salmonella* shedding was tested with a piece of sterile tissue used to wipe fecal material on the slatted floor of the pens. A poor hygiene during farrowing and post-weaning phase increases significantly the risk of *Salmonella* excretion at the end of the finishing period. *Salmonella* contamination of the finishing pens before loading was associated with *Salmonella* excretion. Seroconversion regarding Porcine Respiratory Coronavirus and *Lawsonia intracellularis* was significantly associated with *Salmonella* shedding. Wet feeding during the fattening period tended to reduce shedding at the end of the finishing phase. Our study demonstrated the importance of animal health and of proper implementation of hygiene procedures regarding *Salmonella* excretion.

1. INTRODUCTION

Human salmonellosis is recognized as an important zoonotic disease of worldwide importance and is primarily caused by contaminated food. Several studies in different industrialised countries indicate that on average 15 % of human cases of salmonellosis could be attributable to the consumption of pork and pork products (Hald et al., 1999). Contamination of pork products is related to asymptomatic intestinal carriage of *Salmonella* by pigs (Borch et al., 1996). Living pigs arriving at the slaughterhouse are the major source of contamination of the process line (Berends et al., 1997). To reduce the contamination pressure at the slaughterhouse, a reduction of *Salmonella* carriage at the herd level is needed. A preliminary step of the control of *Salmonella* contamination of the finishing pig, is the identification of risk factors. In France, the traditional pig farms are of farrow-to-finish type. They are indoor units and the herds are managed according to the batch farrowing system coupled to an age-segregated rearing. The influence of this type of rearing system on *Salmonella* carriage is not well known. Therefore, the aim of the present study was to investigate potential factors associated with *Salmonella* excretion by pigs at the end of the finishing period.

2. MATERIAL AND METHODS

2.1 Study sample:

Our study involved 105 French farrow-to-finish pig farms and was carried out from November 2000 to October 2001. The selected farms had to be of confinement type and farrow-

to-finish. All farmers and farm organisations were selected on volontary basis (n=14 organisations and 8 feed companies). Since this type of recruitment is not a random sampling, the sample of farms was retrospectively compared to a broad data base. 34 investigators took part in the survey. In order to standardize data collection, the investigators were trained by us on how to carry out the sampling of material for the laboratory, the measurements and the questionnaires. They were also given a handbook concerning the data collection procedures.

2.2 Data collection:

In each herd, a batch of contemporary grower pigs housed in the same room was observed. This batch of pigs constituted the epidemiological unit and was followed from the end of the post weaning phase (around 25 kg liveweight on average) to slaughter through several visits. The first visit occurred just before moving grower pigs into finishing facilities and the last one a few days before the finishing pigs left for the slaughterhouse. For each farm, 4 to 6 visits took place during the survey. Data were collected about the characteristics of the farms, biosecurity measures including cleaning and disinfection procedures, type of feeding and housing. Health disorders were recorded in the followed pigs throughout their life. At the first visit, a group of 15 piglets was selected at random. The pigs were individually identified. Serum samples were obtained twice from this 15 randomized pigs : at 115 days old (second visit) and at the last visit (just before shipping).

The *Salmonella* status of the pens in the finishing room was assessed using sterile gauze swabs (Sodibox, La Forêt Fouesnant, France) after that the hygiene routines were realized i.e. just before the pigs entered the room. The Sodibox swabs are sterile square pieces of 32cm×32cm cotton cloth moistened with isotonic saline solution. In each pen, one swab was used to wipe the bottom of the walls and the pen partitions and 1m² of the slatted floor of the pen. A room was considered *Salmonella* - residually contaminated as soon as one sample tested positive for *Salmonella*. At the last visit, occurring a few days before slaughter, the excretion was evaluated on the slatted floor soiled with faecal matter with Pedichifs, i.e. sterile pairs of gauze socks (Sodibox, La Forêt Fouesnant, France). For each pen, the sampling method consisted in walking on the floor wearing the Pedichifs. These sterile piece of cotton cloth was pulled on sterile plastic over-boots. In the case of large pens, 2 or 3 Sodibox swabs (first visit) and 2 or 3 Pedichifs (last visit) were used in order to wipe approximately the same surface per swab or per Pedichif. After use, the Sodibox soiled swabs and Pedichifs were placed into a sterile bag and brought to our laboratory on the day they were collected.

The *Salmonella* detection protocol involved four steps. Environmental swabs and Pedichifs were incubated 20 hours at 37 °C in respectively 150 mL and 300mL of buffered peptonned water (neutralised for the post cleaning and disinfection swabs) (AES Laboratoire, Combourg, France). Following the pre-enrichment step, two selective media were used : Müller -Kauffman Tetrathionate Broth (MKTB) and Modified Semi-Solid Rappaport Vassiliadis agar (MSRV), incubated respectively 24 hours at 42°C and 48 hours at 41,5°C. The migrated colonies of MSRV were isolated on Rambach agar plates and each MKTB on Xylose-Lysine-Tergitol4 (XLT4) agar plates. Both media were incubated 24 hours at 37 °C. The presumptive colonies were biochemically confirmed on Kligler-Hajna medium (AES Laboratoires, Combourg, France). All isolates were serotyped by agglutination following the Kauffman-White scheme using *Salmonella* polyvalent O and H antisera (Diagnostics Pasteur, Paris, France) (Popoff and Le Minor, 1992).

The serum samples obtained were submitted to antibody detection. The following infections were looked at : *Lawsonia intracellularis* (Knittel et al., 1998), Swine influenza, PRRS and PRCV.

2.3 Statistical process:

Salmonella shedding was considered as soon as one of the Pedichifs, taken at the last visit, tested *Salmonella* positive. The outcome variable was dichotomous (excretion *vs.* no excretion). The binary herd status with respect to *Salmonella* was combined with the questionnaires, the *Salmonella* status of the room prior to the room loading (residual contamination *vs.* no residual contamination) and serological examination results. A two stage procedure was used to assess the relationship between explanatory variables and the *Salmonella* status at the end of the rearing period. A bivariate analysis was performed using a χ^2 test or Fisher's exact test for qualitative variables and t Student test or Kruskall-Wallis test for quantitative variables (depending whether the variables were normally distributed or not). The factors statistically associated with the outcome variable were retained ($p \leq 0{,}20$) and were offered to multivariate models. The second stage involved a logistic regression model (proc logistic; SAS Institute Inc., 2001). A model was built (backward elimination, likelihood ratio χ^2 test, $p = 0{,}10$) according to the method described by Hosmer and Lemeshow (1989). The odds ratios were converted into relative risks (Beaudeau and Fourichon, 1998).

3. RESULTS AND DISCUSSION

3.1 Results:

The retrospective comparison of our sample of farms to national reference data set did not show any significant difference except for the size of the farms (188 sows/farm in our sample *vs.* 148 sows/farm national, $p<0.05$). Sow productivity was identical 11.94 *vs.* 11.90 ($p>0.05$) as well as average daily gain (750 *vs.* 756 g/day from 25-105 kg, $p>0.05$) for our sample and the group of reference respectively.

The average pen surface wiped per Pedichif did not significantly differ between pens found positive or negative (12.3 m²; SD=8.1, *vs.* 10.0 m2; SD=3.5; $p>0.05$).

A high residual contamination was observed since 33 % of the rooms (35/105 rooms) were found *Salmonella* positive before the piglets entered the facilities. At the end of the rearing phase, 36.2 % of the rooms (soiled floor) tested positive for *Salmonella* (38/105). *Salmonella* Derby and *Salmonella* Typhimurium were the most prevalent isolated serotypes prior to pig loading and at the end of the finishing phase (Table 1).

Table 1. *Distribution of Salmonella serotypes isolated in positive samples in the fattening room prior to the loading and at the end of the finishing phase (105 farrow-to-finish pig farms, France, 2000-2001)*

	Serotype												
	S. Derby	*S. Typhimurium*	*S. Brandenburg*	*S. Bredeney*	*S. Anatum*	*S. Infantis*	*S. Kedougou*	*S. O: 13,22,23 Hi*	*S. Agona*	*S. Heidelberg*	*S. Newport*	*Others*	***Total***
Before loading	37.50	35.80	2,3	-	-	2.90	1.20	3.40	13.10	2.30	0.60	0.9	**100**
Before leaving	50	23.9	6.4	5.6	4.9	4.3	1.4	0.7	0.7	-	-	2.1	**100**

Out of 14 variables tested in the screening analysis, only seven were significantly associated with the *Salmonella* shedding status of the followed batch ($p<0.10$). Table 2 indicates these factors.

Table 2. *The risk factors of Salmonella excretion by finishing pigs a few days before leaving to the slaughterhouse (n = 105 farms, November 2000 - October 2001)*

	Logistic regression model[1]			
	OR	90% CI	RR [2]	90% CI
Emptying the pit below the slatted floor between two successive batches of sows in the farrowing room				
Yes	1,0	-	1,0	-
No	2,7	1,1-6,6	1,9	1,1-3,9
Frequency of sows' faeces removal in the farrowing room [3]				
≥ 2/day	1,0	-	1,0	-
<2 /day	3.7	1.5-8.9	2.3	1.3-4.3
Duration of the period while the post weaning rooms are "empty and clean" before loading of the followed pigs[3]				
> 7 days	1.0	-	1.0	-
≤ 7 days	3.2	1.3-8.2	2.1	1.2-3.3
Salmonella contamination of the finishing room prior to the introduction of the followed batch of piglets[3]				
No	1.0	-	1.0	-
Yes	3.4	1.4-8.3	2.1	1.2-3.3
Type of feeding during the finishing phase[3]				
Wet	1.0	-	1.0	-
Dry	3.6	1.5-8.4	1.9	1.1-3.1
Serological status of the followed batch regarding PRCV at the end of the survey[3]				
Seronegative	1.0	-	1.0	-
Seropositive	6.9	2.2-21.6	4.3	1.7-12
Seroconversion against *Lawsonia intracellularis* in the second half of the fattening phase				
No	1.0	-	1.0	-
Yes	3.0	1.2-7.6	1.9	1.1-3.1

1 : Goodness of fit, Hosmer et Lemeshow ($p = 0.88$)
2 : Relative risks according to Beaudeau and Fourichon (1998)
3 : significant also at $p< 0.05$ (likelihood ratio χ^2 test)

3.2 Discussion:

Due to the study design and the absence of a database about *Salmonella* status of pig farms, a randomization could not be carried out. Therefore the technical profile of the studied herds was compared to national data. *Salmonella* enterica infection in pigs is silent most of the time and intermittent shedding of *Salmonella* occurs in the faeces (Schwartz, 1999). Since *Salmonella* are resistant in pig environment, we decided to swab the environment of the pigs in order to test excretion at the batch level. In our study, *Salmonella* Derby and *Salmonella* Typhimurium were the most common serotypes isolated. Similar findings were reported in other countries (Rajic et al., 2002 ; Davies et al., 1997).

In our study, dry feeding during the finishing phase was found to be a risk factor of *Salmonella* shedding. This result was also previously reported in the literature (Kranker et al., 2001 ; Dahl, 1997). However the explanations of the protective effect of wet feeding is not yet well known. The chemical and microbiological properties of wet feed and the gut microflora of the pigs are two major fields for investigations. On the one hand, wet feed has a lower pH than dry feed and this can be explained by natural fermentation involving the lactic acid bacteria and yeast largely present in this type of feed (Wingstrand et al., 1997). This low pH (around 4,2) inhibits the growth of Enterobacteriaceae such as *Salmonella* (Van Winsen et al., 2001). On their side Van Winsen et al. (1997) demonstrated that the presence of organic acids and a low pH inhibit *Salmonella* growth in the gut of pigs.

The health of the pigs appeared to be another protective factor regarding *Salmonella* excretion. The risk of *Salmonella* shedding is increased when the pigs seroconverted against *Lawsonia intracellularis* at the end of the fattening period. Enteric health problems caused by pathogens are reported in the literature as possible risk factors (Dahl and Wingstrand, 1997). Thus, the disturbance of the microflora balance within the gut could favour *Salmonella* growth in the gut (Dahl and Wingstrand, 1997). PRCV infection during the finishing phase is another risk factor retained in the final model. In our study, PRCV seroconversion was found to be strongly correlated with influenza and PRRS viruses infection. It should therefore be viewed as a general indicator of respiratory disease. It seems plausible that those "influenza-like outbreaks" modify transiently feed intake and the general health status of the pigs during the acute phase. These conditions might subsequently favour *Salmonella* growth in the gut and shedding.

Finally, in our study four of the seven risk factors identified are related to hygiene level at the different sections of the farm and two of them were found to take place early in the farrowing section. This result could be explained by an early contamination of the piglets by a frequent and repeated exposure to the faeces of shedding sows. Davies et al (1998), previously described sows excretion. Later on, a poor hygiene in post weaning section and fattening unit, in particular a residual *Salmonella* contamination of the pens, increased the risk of *Salmonella* shedding at the end of the finishing phase. This study reinforces the importance of a strict all-in/all-out procedures as far as the methods which are implemented are really effective especially for cleaning and disinfection. This point is often mentioned in the literature as a key factor to prevent *Salmonella* contamination in pig farms (Fedorka-Cray et al., 1997 ; Tielen et al., 1997).

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