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Original article

Risk factors for *Salmonella* seroconversion of fattening pigs in farrow-to-finish herds

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Abstract – We did a prospective observational 9-month long study to quantify risk factors of managerial and hygiene practices, and pig-health status for *Salmonella* seroconversion of fattening pigs reared in subclinically infected French farrow-to-finish farms. During the fattening phase, 2 649 pigs belonging to the same batch of contemporary pigs, from 89 conventional farrow-to-finish farms were individually followed and regularly blood sampled on a monthly basis. Farm recruitment was based on the farmer's willingness to cooperate. Pig status was assessed using an indirect ELISA test. Evolution of the serological status was studied by means of survival analysis. A Cox proportional-hazards model, taking into account the clustering of animals at the farm level, was used to examine the effects of explanatory variables on the time to *Salmonella* seroconversion of pigs. Applying group level antibiotic treatment to the pigs during the fattening period (Hazard Ratio (HR) = 2.4; 95% CI: 1.7, 3.4) was identified as a risk factor for *Salmonella* seroconversion, as the presence of residual *Salmonella* contamination in the fattening pen before placing the pigs into the pens (HR = 1.9; 95% CI: 1.2, 2.9). Porcine reproductive and respiratory syndrome virus (PRRSV) seropositivity during the fattening period also indicated an increased hazard for seroconversion (HR = 1.6; 95% CI: 1.1, 2.5). The batch size was identified as a risk factor for *Salmonella* seroconversion: the higher the number of pigs was in the fattening room followed, the higher was the risk ($HR_{+10pigs}$ = 1.05 for a 10-pig increment; 95% CI: 1.03, 1.06). The biosecurity measures of wearing specific clothes before entering the facilities (HR = 0.5; 95% CI: 0.3, 0.9) and enclosing the pig farm facilities were protective (HR = 0.4; 95% CI: 0.2, 0.8).

***Salmonella* / pigs / seroconversion / risk factors / survival analysis**

1. INTRODUCTION

Salmonella infected pigs are recognised as an important source for human *Salmonella* infections and pose potential threats to consumers. Positive *Salmonella* status of finishing pigs assessed on the farm, either by serological or bacteriological examinations, increased the risk of asymptomatic intestinal carriage of *Salmonella* by market-age pigs at slaughter [2]. Whenever slaughter pigs are intestinal carriers of *Salmonella*, contamination of carcasses and pork products thereof may occur in the slaughter process [4].

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A reduction of the *Salmonella* intestinal-carriage prevalence of pigs at the herd level should reduce the contamination pressure at the slaughterhouse. Monitoring, prevention and control efforts at the pre-harvest level are important elements of food-safety assurance strategies to prevent or reduce the transmission of *Salmonella* at the harvest level of pork production.

Better knowledge of the epidemiology of *Salmonella* infections in pig herds is necessary to identify effective intervention and control measures prior to implementation of a control programme. Epidemiological studies reporting risk factors for *Salmonella* infection are based on dependent variables which described either the bacteriological or (more frequently) the serological status of finishing pigs. Serological response to *Salmonella* serotypes in pigs is assessed by Calibrated Optical Density (COD) results of indirect anti-lipopolysaccharide ELISA tests [21, 22]. The sero-epidemiologic studies were case-control [26] or retrospective cross-sectional [6, 14, 16, 18, 28] studies. The aims of those studies were to identify, mainly at the herd level, risk factors associated with the detection of antibodies against *Salmonella* in finishing pigs.

The main factors influencing *Salmonella* contamination of finishing pigs reported in those studies are related to (1) *hygiene*: washing hands [18]; (2) *herd management*: size of the herd [28], batch production system [18], and housing (type of pen partitions and wall separation) [18]; (3) *feeding practices*: groundness and pH of feed [18, 28] and type of feeding (wet versus dry) [7, 14]; (4) *health disorders*: parasite infestation [28], use of antibiotics [9, 16, 28] and health status of the herd [7]. Hypotheses of factors raised in the literature concern concurrent infections that might favour *Salmonella* infection, such as *Lawsonia intracellularis* [20] or porcine reproductive and respiratory syndrome virus (PRRSV) [31].

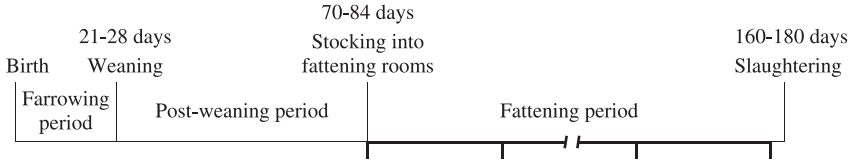
However, few epidemiological studies have been carried out to determine risk factors for *Salmonella* seroconversion during the rearing period with a design assuring the time sequence of the factor and the effect. The longitudinal time-course of serological response has been reported in detail in experimentally infected pigs [21, 25, 32] or naturally infected pigs in subclinically infected herds [1, 15]. Those observational studies report delayed onset of seroconversion during the second half of the fattening phase and also suggest that individual and collective factors may influence the time-course of infection of pigs.

On the basis of the main factors reported and the hypotheses raised in the literature, our aim was to identify and quantify the effects of certain farm characteristics, managerial and hygiene practices and pig-health status on the *Salmonella* seroconversion of fattening pigs reared in subclinically infected farrow-to-finish farms, by means of survival analysis.

2. MATERIALS AND METHODS

2.1. Study sample

The study involved 89 French farrow-to-finish family pig farms, located all over France, selected among those affiliated with 14 farmer organisations and 8 feed companies. The farms involved in the study had to be confined farrow-to-finish operations of the intensive type and managed according to the batch-farrowing system (weaning on the same day of a group of piglets born the same week and age-segregated rearing) and an all-in/all-out hygiene policy for farrowing, post-weaning and fattening sections. Farm selection was also based on the farmer's willingness to cooperate. No farm included in the study vaccinated breeding or growing pigs against *Salmonella*. Since recruitment was not based on random sampling, the general characteristics (herd size, reproductive



	First visit	Intermediate visit[a]	Intermediate visit[a]	Last visit
Type of questionnaire:	'General and retrospective'	'Follow-up'	'Follow-up'	'Follow-up'
Observations:	Measurements of pens	Sneezing, coughing, diarrhoea	Sneezing, coughing, diarrhoea	Sneezing, coughing, diarrhoea
Blood collection:	Identification of 30 random piglets Blood collection	Blood collection	Blood collection	Blood collection
Environmental samples:	Residual *Salmonella* contamination (swabs)	—	—	—

Figure 1. Prospective study design (2649 fattening pigs, 89 farrow-to-finish pig herds, France, 2000–2001). [a] 2 or 3 monthly visits were held between the first and last visits.

and growing performances, mortality rate in the different sectors) of the final sample of farms were compared retrospectively with the average values available in broad national and regional (Brittany) databases [12].

2.2. Data collection

2.2.1. Study design

A batch of contemporary growing pigs housed in the same fattening room was studied on each farm. The follow-up of the batch began on the day the batch left the post-weaning unit and lasted until the end of the fattening period. In each batch, 30 pigs were randomly selected (simple random sampling i.e. with no stratification per pen) on the day of entry into the fattening section. The random process was based on a table of random numbers. The selected pigs were individually identified by ear-notching.

The follow-up of the randomly selected pigs during the fattening period was performed by means of monthly visits (i.e. 4 or 5 times during the fattening period). It was carried out by investigators previously trained, belonging to the Afssa laboratory and the farmer organisation. The first visit was held just before transferring the growing pigs to the fattening unit and the last visit was held several days before slaughter. The randomly selected pigs were bled at each monthly visit. Individual sera were tested for *Salmonella* serological status at our laboratory by an indirect ELISA [22]. The follow-up procedure, previously detailed by Beloeil et al. [3], is illustrated in Figure 1. Information on potential risk factors related to *Salmonella* infection of the pigs was gathered by questionnaires, on-farm records, measurements and bacteriological laboratory investigations.

Residual *Salmonella* contamination of fattening rooms was checked by means

of environmental swabs after cleaning and disinfection before placing the pigs. The sampling scheme applied to the fattening rooms defined pens as sub-units of sampling so as to sample each pen of a same room separately with one swab per pen. A *Salmonella* positive swab therefore allowed the assessment of the residual contamination of a pen. The sample collection procedure and the microbiological investigations for *Salmonella* isolation and identification were described in detail elsewhere [3].

At each visit, a specific questionnaire was administered by an investigator to each farmer. Data concerning the general characteristics of the farm and the premises, biosecurity procedures, type of feeding and the rearing characteristics of the batch followed during the farrowing and post-weaning periods were collected with a "general and retrospective questionnaire" (Tab. I) administered at the first visit. The on-farm technical documents were checked for this purpose.

The rearing characteristics and sanitary events occurring during the fattening period were recorded by "follow-up" questionnaires administered at the monthly visits (Tab. I). Between monthly visits, the farmer was required to record any mortality, health disorders, medical treatments and change in the feed process, concerning the batch followed. All clinical signs (mortality, respiratory and digestive signs, such as coughing and diarrhoea, etc.) and treatments concerning specifically ear-notched pigs or sub-groups of the batch (pens) including ear-notched pigs were recorded at the individual and pen levels with the number of the pens and the ear-notch numbers of the pigs concerned as well as the date of the event. Special data-collection forms were designed for the purpose and investigators provided explanations at the first visit on how to use the forms. Accuracy and completeness of information written on the forms were verified and validated by an investigator at each visit.

2.2.2. Serological investigation

The *Salmonella* serostatus of the pigs was assessed using an indirect ELISA [22]. *Salmonella* IgG antibodies were detected in serum using a complete ELISA based on LPS from *S*. Typhimurium, Enteritidis, Anatum, Hadar and Infantis [22]. Optical densities were determined by a Dynatech MR5000 plate reader spectrophotometer using 490- and 630-nm filters as the test and reference filters, respectively. The COD were calculated as follows:

$$\mathrm{COD} = (\mathrm{OD}_{sample} - \mathrm{OD}_{negativecontrol}) / (\mathrm{OD}_{positivecontrol} - \mathrm{OD}_{negativecontrol})$$

Samples with COD $\geqslant 0.4$ were considered to be positive according to the previously defined cut-off value [22]. ELISA sensitivity and specificity were estimated at 97% and 94%, respectively by Proux et al. in 2000 [22].

The overall proportion of seropositive pigs among those followed was calculated with the Rogan-Gladen formula [24], taking into account the imperfect sensitivity and specificity of the ELISA [22].

2.3. Definition of the outcome variable

The unit of observation was the fattening pig followed from the day of entry into the fattening section. The event of interest was the *Salmonella* seroconversion of pigs. The failure time was defined as the number of days between birth and seroconversion. Due to periodic assessments of the serological status of pigs, the time to seroconversion cannot be observed exactly, but can only be determined to lie in an interval obtained from a sequence of visit times. Such observations are said to be interval-censored. Because of the interval-censoring, we used the midpoint data of the

Table I. Summary of factors included in the questionnaire and used to analyse risk factors for *Salmonella* seroconversion of fattening pigs in French farrow-to-finish herds (2 649 fattening pigs, 89 farrow-to-finish pig herds, France, 2000–2001).

General items related to the farm
***Farm characteristics* (*n* = 21)[a]**
– Farm staff characteristics, – Size, – Size of sections (farrowing, post-weaning, fattening), – Location, – Productivity, – Health level,
***Biosecurity* (*n* = 30)[a]**
– Access to facilities and surroundings, – Working procedures, – Hygiene procedures (dead pig disposal…), – Control of wildlife (rodents, insects), – Acclimatisation phase for replacement gilts (accommodation, duration…),
***Feeding* (*n* = 21)[a]**
– Type of feeding during farrowing, PW and fattening period, – Water quality, – Feeding and drinking practices, – Hygiene management of feed storage,
***Vaccination scheme in sows and growing pigs* (*n* = 6)[a]**
Retrospective questionnaire about the batch followed
***Post-weaning phase* (*n* = 33)[a]**
– Characteristics of the post-weaning facilities, – Health disorders in piglets, – Hygiene procedure, – Cleaning and disinfection procedure,
Fattening room housing the batch followed* (*n* = 46)[a] *(before placing the pigs)
– Characteristics of the fattening room, – Cleaning and disinfection procedures applied,
Follow-up questionnaire administered at intermediate and final visits (*n* = 15)[a]
Data recorded by the investigator on the day of the visit
– Number of pigs per pen, – Respiratory signs: coughing, sneezing, – Clinical signs,
Questions related to "between visit" periods
– Dung management, – Pest control, – Heating, – Sanitary events (e.g. respiratory and digestive signs[b]), – Health management (treatments[b], vaccination).

[a] The number of questions per subset is indicated in brackets.
[b] Recorded at the individual level for the ear-notched pigs.

interval (between the last negative visit and the first positive visit) as the date of seroconversion time [23]. The left-censored pigs (which tested seropositive at the first visit) were removed from the analysis. The left truncation situation was taken into account in the model by specifying the age of the first blood sampling in the study as the left truncation time. The pigs lost-to-follow-up due to early death or loss of ear notch were considered right-censored after their last serological testing, even if they died several days later at a known date. In the same way, the last blood sampling date was taken into account for seronegative pigs slaughtered after this date.

2.4. Putative risk factors – explanatory variables

Data collected by "follow-up" questionnaires and on farm records were treated as "yes/no", categorical as well as continuous variables, either at the individual or at the group level. In particular, the variables "Residual *Salmonella* contamination of the fattening pen before placing the pigs followed" and "Group level antibiotic treatment during the fattening period" were treated at the individual level. All antibiotic administrations consisted in metaphylactic or therapeutic treatments (i.e. the dosages applied corresponded to therapeutic dosages) given in feed or in water. In many cases, only certain pens in a room were treated and treatment was therefore only attributed to those pigs of the pen followed (sometimes a single pig). All the time-specific events occurring during the fattening period and recorded by the follow-up procedure were treated as time-dependent variables [13].

2.5. Statistical procedure

The Cox proportional-hazards model was used to examine the effects of collected variables on the outcome variable. Survival analysis was performed in two steps, using univariable analysis first for screening (variables were kept for the second step at $P < 0.20$) and then examining putative risk factors in a multivariable Cox proportional hazards model. We used the PHREG procedure in the SAS System Release 9 (SAS Institute, Cary, NC, USA) for the Cox regression. Since pigs from a same farm tend to be more alike than pigs on different farms, dependence among pigs from a same farm was taken into account using the robust sandwich estimate of the covariance matrix [17]. Since pigs were not followed from birth but from the day of stocking into the fattening section, late entry was taken into account. Tied survival times were handled using the exact method.

The Cox proportional hazards analysis requires the proportional hazards assumption to be met. This assumption was tested for all variables using interaction between time and variables. Interaction terms between time and the variables under consideration were assessed for the statistical significance (likelihood ratio test, $P < 0.05$) as part of the Cox model.

All bilateral relationships between the possible explanatory variables were checked. If two variables were highly collinear (likelihood ratio test, $P < 0.05$), the more closely related to the outcome variable of the two was included in the model. In the final models, biologically plausible one-way interaction terms were considered. A manual backward-stepwise selection was used for variable selection in the Cox model. The criterion for removal of the variables from the model was $P \geqslant 0.05$.

3. RESULTS

3.1. Study sample, follow-up and application of the protocol

The general characteristics of the 89 farms involved in this study were

Table II. Sample profile compared to a reference group of French pig farms (2000).

	Survey sample (n = 89)		Mean of reference groups[a]	
	Mean	S.D.	France (n = 3927)	Brittany (n = 1640)
Size of herds (number of sows)	182	118.8	148[b]	168
Prolificacy (piglets born alive/L, 1 year)	11.9	0.66	11.9	12
Piglets weaned/sow per year	24.6	3.7	25.2	25.9
Post-weaning mortality (% per year)	2.8	1.66	2.9	3.0
Average daily weight gain 7–25 kg (g per day)	442	40	433	431
Feed conversion ratio 7–25 kg	1.66	0.22	1.67	1.66
Fattening mortality (% per year)	5.30	2.5	4.9	5.4
Average daily weight gain 25–105kg (g per day)	746	57	756	753
Feed-conversion ratio 25–105 kg	2.87	0.21	2.82	2.82
Age at 105 kg live weight (day)	174	10	175	174

[a] Reference group source: ITP, Le porc par les chiffres, 2000 (ITP, France).
[b] Only this mean was significantly different between the sample and the "France" reference group ($P < 0.05$); no differences were detected between the sample and "Brittany".

similar to those of the groups taken as reference except for the average herd size, which was significantly higher than that of the national reference group (Tab. II).

The follow-up began when growing pigs left the post-weaning section (first blood collection) at the average age of 74.2 days (S.D. = 9). The average number of follow-up visits was 4.5 (S.D. = 0.8) with a mean interval between consecutive visits of 26 days (S.D. = 8). The average age of finishing pigs at the last visit was 171 days (S.D. = 13) and the mean age at slaughter was 176 days (S.D. = 10). One hundred and three of the pigs (3.9%) were lost-to-follow-up due to early death or loss of ear notch.

Among the 2 670 piglets identified and followed, 21 pigs (0.8%) were excluded from the analysis due to an early seropositivity, assessed at the first blood sample serological analysis. Two thousand six hundred and forty-nine pigs from 89 farms were therefore included in the analysis, with on average 29.8 pigs per farm (S.D. = 0.9). A seroconversion during the fattening phase was observed for 24.2% of the pigs included in the study (641/2649). The overall proportion of seropositive pigs among those followed, adjusted according to the sensitivity and the specificity of the ELISA, was 20%. The within-herd seroincidence, adjusted according to the sensitivity and the specificity of the ELISA, was 16% (interquartile range (IQR) = 27%). From 0 to 25 pigs per farm seroconverted (median = 5; IQR = 8). No pigs seroconverted in four farms. Less than 5% of the tested pigs (1 pig) seroconverted in 13 farms. The mean age at seroconversion was 126 days (S.D. = 28.3), median, first and third quartile were 126, 99 and 152 days, respectively.

Pigs were located in 672 pens, with 7.6 pens per farm on average (S.D. = 3.1). In each pen from 1 to 16 pigs were followed (median = 3; IQR = 3). Before placing the pigs into the fattening rooms, *Salmonella* spp. were recovered from 21 of the 89 fattening rooms and from 45 of the 672 pens tested, with 1 to 6 pens positive per positive room.

Table III. Distribution of explanatory variables selected after the screening analysis of *Salmonella* seroconversion of pigs during the fattening phase (2649 fattening pigs, 89 farrow-to-finish pig herds, France, 2000–2001).

Definition of variables	Sample pigs		Seropositive pigs		Results of the univariable analysis[a]	
	N	%	N	%	HR	*P*
Farm characteristics and herd size						
Other animal production on the farm						
– Yes	1029	38.8	334	52.1	1.55	0.05
– No	1620	61.2	307	47.9	ref.	–
Rearing density in the fattening room followed (1 pig/m² increments)[b]	NA[c]	NA	NA	NA	1.05	0.01
Batch size: number of pigs in the fattening room followed (10-pig increments)[b]	NA	NA	NA	NA	1.05	< 0.01
Hygienic characteristics and biosecurity rules						
Application of biosecurity measures: wearing of specific clothes before entering the facilities[b]						
– Yes	479	18.1	83	13	0.61	0.08
– No	2170	81.9	558	87	ref.	–
Application of biosecurity measures: a fence enclosed the pig farm facilities[b]						
– Yes	150	5.7	18	2.8	0.49	0.02
– No	2499	94.3	623	97.2	ref.	–
Order of circulation of the staff within the facilities during the working day						
– Correct[d]	1991	75.1	450	70.2	ref.	–
– Incorrect	658	24.9	191	29.8	1.39	0.21
Number of disinfections performed before placing the batch followed						
– One	2524	95.3	622	97.1	ref.	–
– > 1	125	4.7	19	2.9	0.5	0.14
Number of visits in the fattening room between the last disinfection and the placing the batch followed						
– None	1143	43.1	198	30.8	0.56	0.006
– 1 or more	1506	56.9	443	69.2	ref.	–
Residual *Salmonella* contamination of the fattening pen before placing the batch followed[b, e]						
– Yes	182	6.9	70	10.9	1.97	< 0.04
– No	2467	93.1	571	89.1	ref.	–
The cleaning and disinfection procedure has begun by soaking faecal materials immediately after the pigs leaving to the slaughterhouse[b]						
– Yes	2468	93.2	572	89.2	ref.	–
– No	181	6.8	69	10.8	1.81	0.09
Sanitary characteristics						
PRRSV serological status of the batch followed at the end of the fattening period[b]						
– Seropositive	1010	38.1	302	47.1	1.40	0.13
– Seronegative	1639	61.9	339	52.9	ref.	–

Table III. Continued.

Definition of variables	Sample pigs		Seropositive pigs		Results of the univariable analysis[a]	
	N	%	N	%	HR	*P*
Group level antibiotic treatment during the fattening period (time-dependant variable)[b, e]						
– Yes	2469	93.2	609	95.0	4.2	< 0.01
– No	180	6.8	32	5.0	ref.	–
Feeding practices and characteristics						
Groundness of feed fed during the second phase of the post-weaning period						
– Pellets	1823	68.8	479	74.7	1.47	0.12
– Crumbs	826	31.2	162	25.3	ref.	–
Events during the fattening phase						
Thinning of the batch during the finishing period (time-dependent variable/pen level)[b]						
– Yes	1784	67.4	451	70.4	1.53	0.10
– No	865	32.7	190	29.6	ref.	–

[a] $n = 2\,649$ (641 events).
[b] Variable retained and offered to the multivariate model.
[c] Not applicable.
[d] From the less contaminated section (farrowing unit) to the most potentially contaminated section (fattening unit).
[e] Variable defined at the individual level in the model.

3.2. Cox proportional hazards modelling

Variables retained after the first univariable step are listed in Table III. The variables "Other animal production on the farm", "Order of circulation of the staff within the facilities during the working day", "Number of disinfections performed before the entry of the batch followed" and "Number of visits in the fattening room between the last disinfection and the entry of the batch followed" were discarded from the multivariate analysis because of a strong association with the variable "Wearing of specific clothes before entering the facilities", which was also the most associated with the outcome variable.

The final model presented no obvious violation of the proportional hazards assumption. The factors associated with *Salmonella* seroconversion of pigs during the fattening period in the final Cox proportional hazards model are presented in Table IV. The six variables retained in the final model were mostly related to good hygiene practices during the rearing period of the pigs, biosecurity measures, health status of the herd and antibiotic treatment of pigs during the fattening period.

4. DISCUSSION

The previous analytical studies carried out to determine risk factors for *Salmonella* infection of market-age pigs were cross-sectional [26] or case-control studies [6, 14, 18, 28]. Such studies are very efficient, inexpensive and quick [27] but time sequence between the risk factors and the outcome is in such study design often difficult to assess, especially when exposure factors are not time-invariant [8]. Therefore, risk factor assumptions provided by those surveys should be further

Table IV. The final Cox proportional hazards model for risk factors for *Salmonella* seroconversion of French fattening pigs (2 649 fattening pigs, 89 farrow-to-finish pig herds, France, 2000–2001).

Explanatory variables	Cox proportional hazards model[a]				
	Regression coefficient	SE	*P*	HR	95% CI
Batch size: number of pigs in the fattening room followed (10-pig increments)	0.05	0.001	< 0.01	1.05	1.03, 1.06
Application of biosecurity measures: wearing of specific clothes before entering the facilities: Yes (vs. No)	–0.63	0.28	0.02	0.5	0.3, 0.9
Application of biosecurity measures: a fence enclosed the pig farm facilities: Yes (vs. No)	–0.89	0.34	< 0.01	0.4	0.2, 0.8
Residual *Salmonella* contamination of the fattening pen before placing the pigs followed: Yes (vs. No)	0.66	0.23	< 0.01	1.9	1.2, 2.9
PRRSV serological status of the herd: seropositive (vs. seronegative)	0.49	0.22	0.03	1.6	1.1, 2.5
Group level antibiotic treatment during the fattening period: Yes (vs. No) (time-dependant variable)	0.87	0.18	< 0.01	2.4	1.7, 3.4

[a] n = 2 649 (641 events), model deviance = 7630.28 (χ^2 = 189.2, d.f. = 6, $P < 0.0001$), R^2 = 6.9%.

investigated through cohort studies or experiments. In this study, the longitudinal design of the data collection and the survival analysis of the data gave the opportunity of better observing the time sequence between risk factors and the outcome.

The pigs were bled monthly. Considering that *Salmonella* seroconversion has been estimated to occur within 10–14 days post-infection (inoculation) in the laboratory [21, 29] and after a longer period in naturally occurring infections [15], a one month interval between two successive blood samples might be considered as appropriate, to allow early detection of seroconversion. Moreover, logistical constraints, inherent to the large number of farms included in the study, did not permit to visit and sample each farm more frequently than once a month. Misclassification of the outcome might have been limited. The realisation of serological analyses by a single laboratory was privileged. ELISA results were interpreted qualitatively at the individual level by comparison with a previously defined cut-off value (COD $\geqslant$ 0.4, [22]).

Because the validity of the data is of paramount importance in observational studies, special attention was paid to the design of the study. Investigator training was designed to minimise investigator bias [3]. Thorough follow-up, by combination of on-farm data recording performed by the farmers and "follow-up" questionnaires administered by the investigators, allowed recording of all sanitary or zootechnical events occurring during the four months of the fattening period with limited memory bias. Moreover, on-farm data recording allowed recording of the exact date

of occurrence of sanitary or zootechnical events (such as thinning of the batches, antibiotic treatment administration, manure spreading…) at the individual and group level. An information bias might have been limited by the complementary use of these on-farm records and the questionnaires that were administered monthly by investigators belonging to the production technical staff of the farm organisation and therefore having a preliminary good knowledge of the farm. To limit the follow-up bias, farmers were asked not to modify their practices at the beginning of the study and no partial results were given until the end of the follow-up. It was verified that the number of pigs lost-to-follow-up due to early death or loss of ear-notch was coherent with the average mortality during the fattening phase (4.9%) calculated on the national level [12].

The overall proportion of followed pigs that seroconverted during the follow-up was 20%. The within-herd seroincidence was 16% (IQR = 27%). The high variability of the within herd seroincidence observed in this study was similar to that of the seroprevalence assessed at the end of the fattening period reported by Lo Fo Wong et al. in 2004 [18]. Nevertheless, comparison of the *Salmonella* infection observed with those reported by the literature is difficult due to the fact that no other studies were based on the ELISA test we used. The average age of seroconversion was situated close to and between those observed by precedent studies [1, 15]. Among the 2 670 piglets identified and followed, 21 pigs (0.8%) seroconverted before the beginning of the follow-up. They were probably infected during the post-weaning phase. Such an early and rare seroconversion has already been reported [1, 15].

The assumption of independent censoring appeared to be reasonable considering that animals were censored mainly due to slaughtering, death or ear-notch loss during the follow-up, all events were a priori independent from seroconversion. In each herd, great attention was paid to perform a blood sample a few days before animals were slaughtered (6 days on average) in order to know their *Salmonella* status at slaughter time. This could not be performed for animals that were slaughtered before the planed date. However, it has been previously assessed that daily weight gain and mortality are independent of seroconversion and *Salmonella* status [1]. Subclinically *Salmonella*-infected pigs are characterised by asymptomatic intestinal carriage [30]. As a consequence, seropositive pigs were not likely more at risk of (early or late) death or culling and animals censored could not be thought to be systematically more or less at risk of seroconversion.

A risk factor associated with pig seroconversion was the previous administration of antibiotics as a group level treatment. Association between seroconversion and antibiotic exposure was also reported in The Netherlands [28], Greece [16] and Canada [9]. Studies were of retrospective cross-sectional design type, and Farzan et al. in 2006 [9] could not distinguish between a risk or a confounding factor. The prospective design of our study allowed us to take into account antimicrobial exposure at the pig level as a time-dependent variable. Our results sustain the hypothesis of a role of antimicrobial exposure on pig seroconversion. Antibiotics are thought to have a damaging effect on the indigenous Gram-positive flora of the intestine, resulting in a decreased colonisation resistance [16, 28]. Infection in pigs may therefore be facilitated by antibiotic treatments.

Identified risk factors were in a great part related to the management of the farm in the respect of biosecurity and hygienic rules. Residual environmental *Salmonella* contamination of the fattening room increased the risk of individual *Salmonella* infection during the fattening period. The importance of stringent implementation of

the all-in/all-out hygiene procedure in the farrowing section including cleaning and disinfection was reported as a protective factor for *Salmonella* seroconversion in European market-age pigs by Lo Fo Wong et al. in 2004 [18]. The presence of residual *Salmonella* was found to be a source of contamination for incoming pigs in an experimental study [11] and in an observational survey [3]. In the latter study, residual environmental *Salmonella* contamination of the fattening room before placing the batch was found to increase the risk of *Salmonella* shedding of the finishing batch [3]. However, in this cross-sectional study, precedence between the factor and the outcome could not be assessed and residual environmental *Salmonella* contamination could either be a risk factor or a marker of the presence of *Salmonella* in the facilities. The prospective design of the present study, performed in field conditions on a large scale, allowed the control of the time sequence between the factor and the *Salmonella* status of the pigs. The effect of the "Residual environmental contamination of the fattening pen", considered as a time-dependent variable, was studied at the pig level. It was therefore observed that pen *Salmonella* contamination before placing seronegative pigs was a risk factor for their seroconversion.

We found two risk factors for *Salmonella* infection during the fattening period related to the application of biosecurity measures. The fact that the staff of the farm did not wear specific clothes before entering the facilities increased the risk of infection during the fattening period. This measure is thought to prevent the introduction of pathogens into the herd. In the same way, the presence of a fence enclosing the pig farm facilities was found to be associated with a lower risk of infection during the fattening period. All these identified factors may reflect the pig producer awareness of and attitude towards hygiene, a general aspect highlighted in other studies based on serological examinations [18] or bacteriological ones [10]. Wearing non-specific clothes before entering the facilities and not enclosing the pig farm facilities by a fence as well as *Salmonella* residual contamination of the fattening pens before placing a new batch indicate risk of *Salmonella* exposure and reveal possibilities for direct intervention. These factors are identified as points of special interest of a *Salmonella* risk management programme that must be devised and applied at the batch or farm level. In this respect, implementation of biosecurity measures and effective cleaning and disinfection of fattening pens between successive batches should be recommended and measurements should be performed to assess the efficacy of hygiene practices [19]. However, these recommended control measures should be considered more as guidelines for good manufacturing hygiene practices to be applied as standard practice, rather than control measures specifically concerning *Salmonella*.

The PRRSV serological status of the herd was found to be associated with a higher risk of seroconversion. This was previously found studying risk factors for *Salmonella* shedding by market-age pigs [3]. Respiratory viruses, such as PRSSV, could induce immunodepression, which could facilitate *Salmonella* contamination and multiplication. A synergism between PRRS virus and *Salmonella* Choleraesuis was observed experimentally by Wills et al. in 2000 [31].

The increase in size of the fattening batch was found to be associated with a slightly higher risk of seroconversion. The influence of the size of the herd on the within herd seroprevalence was reported in Denmark [5]. The doubling of the size of the herd is a moderate risk factor for seropositivity in market-age pig batches [6, 14].

The results of the study confirmed by the literature invite to improve biosecurity and hygiene measures on farms and to maintain pig herds at a high level of health to control *Salmonella* infection. Good Agricultural Practices should be presented in a specific guide to help farmers to control *Salmonella* on farms.

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