Longitudinal study describing time to Salmonella seroconversion in piglets on three farrow-to-finish farms

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ABSTRACT

Background Pigs are frequently colonised with Salmonella enterica, and this constitutes a major risk for human salmonellosis. The infection can be assessed by the serological response of pigs to S enterica. A longitudinal study was undertaken on-farm to correctly describe this serological response and investigate factors associated with age at Salmonella seroconversion.

Methods Three pig farms and in each farm three successive batches were considered. Per batch, 40 piglets were selected at random from 10 sows (four piglets per sow). Blood was sampled from sows one week after farrowing and from piglets at weeks 1, 6, 10, 14, 18 and 22 and at the slaughterhouse. Salmonella antibodies were detected in serum using a commercial ELISA test. Factors related to farm characteristics, batch management system, porcine reproductive and respiratory syndrome infection, and sows’ Salmonella serological status were recorded to assess their effect on age at seroconversion.

Results At week 1 after farrowing, 96.5 per cent of the sows had antibodies against Salmonella. The serological results of piglets at weeks 1 and 6 only were positively correlated with those of the sows. The average age at Salmonella seroconversion was 137±2.2 days (confidence interval at 95 per cent). The first seroconversions occurred from weeks 10 to 14, but most of the pigs (54.6 per cent) were seropositive at the end of the fattening period, with variations between farms and batches (28.9–75.7 per cent). Herd/farm was significantly associated with age at seroconversion.

Conclusion This longitudinal study allowed the authors to follow precisely the evolution of Salmonella seroconversion from maternity to slaughterhouse and confirm the relationship between the seroconversion of sows and serology of their piglets. Moreover, factors related to farm practices and management as a whole are more influential than individual factors (at the pig level) on age at Salmonella seroconversion.

INTRODUCTION

Salmonella enterica subspecies enterica is a zoonotic Gram-negative bacterium producing foodborne disease in human beings worldwide through contaminated food of animal origin. This foodborne pathogen was the second-ranking bacterial agent responsible for gastroenteritis in the EU in 2015. The incidence of human salmonellosis cases was recently estimated at 307 per 100,000 population, or 192,450 cases per year in France.

Pigs are a recognised reservoir of Salmonella, and the prevalence of Salmonella in the French pig production industry is high: 38.7 per cent of farms, and 17.6 per cent of pork carcases are positive for Salmonella. Between 10 per cent and 20 per cent of human Salmonella infections in the EU are attributed to pork, and pork ranks third among food categories associated with human salmonellosis outbreaks.

Infections of pigs by Salmonella are usually asymptomatic and relatively well known. Pigs generally become infected on the farm by oral-faecal transmission, although infection by nose-to-nose contact is also possible.

Several factors influencing Salmonella prevalence on pig farms have been reported, including biosecurity measures, hygiene, viral coinfections such as porcine reproductive and respiratory syndrome virus (PRRSV) infection, antibiotic treatment, movement of animals, and contact with other animals.

Diagnostic tools based on bacteriological or serological tests can help identify the age at which pigs become infected. Moreover, within-herd seroprevalence data can be used to categorise pig herds and may, therefore, have an impact on food business operators’ decisions on the use of meat from high-risk herds for raw pork products.

Sero logical tests, such as an ELISA, are used to detect antibodies to Salmonella in the meat juice and serum of pigs and are advantageous because they are both quick and relatively inexpensive. They give an indication of previous exposure to Salmonella and are not necessarily correlated with Salmonella shedding by pigs at the time of testing. However, serology is considered to be one of the better alternatives for establishing the level of a herd’s Salmonella exposure and may help to predict the risk of Salmonella shedding at

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slaughtering. To follow the dynamics of *Salmonella* infection in pig herds over time, longitudinal studies on the bacteriological and serological status of pigs need to be performed. However, because these studies are tedious and expensive, few longitudinal studies describing the dynamics of *Salmonella* infection and serological response in pig herds have been conducted. Moreover, farm management practices can vary over time and space and this may change the risk factor patterns. The purpose of this study was (1) to establish the age at *Salmonella* seroconversion (as days of age of piglets) on three farrow-to-finish farms by monitoring three batches of pigs on each farm and (2) to identify factors that may be associated with age at *Salmonella* seroconversion.

**Materials and Methods**

**Study design**

The study was carried out on three farrow-to-finish farms located in Brittany, France, named F3, F5 and F6. On each farm, three successive batches of pigs coded B1, B2 and B3, respectively, were followed from one week of age to slaughter. Each batch was composed of 40 piglets from 10 sows, all of which were randomly selected. Each piglet was ear-tagged to make sure that the same pigs were monitored from the beginning to the end of the experiment. A total of 90 sows (30 per farm) and 360 pigs (120 per farm) were therefore included in this study.

Each farm was visited every four to five weeks (eight visits per batch) and a visit was also performed at the slaughterhouse. Blood samples were taken from sows one week after farrowing and from piglets at weeks 1, 6, 10, 14, 18 and 22 (coded W1 to W22) and at the slaughterhouse (26±2 weeks) (WSlaugh). Blood samples were collected by jugular vein puncture into evacuated tubes (Vacuette, Dutscher SAS, Brumath, France) without additives. Serum was obtained by centrifugation of the blood for 10 minutes at 3500 g and stored at −20°C until analysis.

**On-farm data collection**

Data related to the general characteristics of the farms, type of feeding, housing, ages when pigs were moved to the different production stages, vaccination and antibiotic treatments were collected during the longitudinal follow-up (Table 1).

**Antibody response to *Salmonella***

Serum samples (n=2463) were analysed to detect the presence of antibodies against *Salmonella* with the IDEXX Swine *Salmonella* Ab Test (IDEXX, Montpellier, France), which has a sensitivity of 99.1 per cent and a specificity of 99.4 per cent when used as recommended by the manufacturer. The presence or absence of antibodies to *Salmonella* in the sample was determined by calculating the sample to positive control (S/P) ratio corresponding to the absorbance value at 650 nm of the sample (S) over the mean absorbance value of the positive control (P). The results were expressed as a percentage of optical density (OD%), and samples with OD% values equal to or higher than 15 per cent were considered positive.

**Antibody response to PRRSV**

The presence of PRRSV-IgG antibodies was assessed using the PRRS X3 Ab ELISA (IDEXX Laboratory, Eragny sur Oise, France) according to the manufacturer’s instructions. Results were expressed as S/P optical density ratios. A sample was considered positive when the S/P ratio was equal to or higher than 0.4.

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**Table 1** List of variables collected on farms and used in the univariate and multivariate analyses to identify factors associated with age at *Salmonella* seroconversion (three farms, three batches per farm, 357 piglets from 90 sows)

| Type of variable       | Variable names                                                                 |
|------------------------|--------------------------------------------------------------------------------|
| **Sows**               | *Salmonella* serological status of dams one week after parturition. Parity.     |
| **Salmonella status of piglets** | Detection of maternal antibodies/*Salmonella* in piglets at week 1. |
| **PRRSV infection**    | PRRSV seroconversion (yes/no). PRRSV seroconversion before 12 weeks of life (yes/no). PRRSV seroconversion before 16 weeks of life (yes/no). |
| **Characteristics of piglets** | Sex (male, female). |
| **Housing and feeding types** | Feeding (dry/wet). Floor type (slatted or not). Weaning age (in days). Age of entry into fattening system (in days). Movements during the nursery step (room changes, pen changes). Movements during the fattening period (room changes, pen changes). |
| **Antibiotic treatment** | Antibiotic treatment in nursery. Oral antibiotic treatment postweaning. |

PRRSV, porcine reproductive and respiratory syndrome virus.
Statistical analysis

Correlation between Salmonella serological results

Spearman’s correlation tests were performed to assess the correlation between the serological status of sows one week after parturition and their piglets at each sampling time. The correlations between the serological results of the piglets over time were also tested (R V.3.2.4 software).

Analysis related to age at Salmonella seroconversion in infected herds

Definition of the outcome

The event of interest was the age at which Salmonella seroconversion occurred, and this was assessed at the individual piglet level. The serological results (expressed as OD%) were used to estimate the supposed time interval during which seroconversion was expected to occur. The age at seroconversion was estimated taking into account the presence of maternally derived antibodies at four weeks of age. If no seropositive pig was present in the four-week-old batch, the midpoint between the individual pig’s age on observation of the first OD% higher than 15 was retained as the time to event. If seropositive pigs were present in the four-week-old batch and the OD% then decreased to under 15 per cent, the midpoint age between the seronegative status and a positive status was selected as the time to seroconversion. When the OD% remained higher than 15 per cent, the midpoint between the first measurement that showed an increase and the preceding one was considered to be the age at seroconversion.

Survival analysis

Survival analysis was used to identify the explanatory variables (collected by the questionnaire and related to PRRSV infection) which were associated with age at Salmonella seroconversion (table 1). The first step involved testing the difference in survival distributions between levels of each explanatory variable using the log-rank test (PROC LIFETEST, SAS V.9.1, SAS Institute, Cary, USA; P<0.25). Given that the assumption of linearity in the continuous variables did not hold, all the independent variables were grouped into categories for further analysis. A Cox model (TPHREG procedure, SAS V.9.1) was then used to relate each variable where P<0.25 (univariable log-rank test) to the age at seroconversion. The proportional hazards assumption for the Cox proportional hazards model was checked by examining the log-negative-log plot of the Kaplan-Meier survival function estimates and the Schoenfeld residuals versus log of time and time, respectively. All the selected explanatory variables were checked for multicollinearity (chi-squared test, P<0.05), and those most strongly associated with the outcome variable and having biological relevance were retained. The last step involved the multivariable Cox proportional hazards model, which included all the factors that had passed the first screening step (TPHREG procedure, SAS V.9.1). Since pigs from the same litter could not be considered as independent, a sow cluster effect was used (generalised estimating equations for population-averaged model) and the farm/ herd effect was taken as fixed effect. A backward elimination procedure was used by progressively removing variables that were not significant (P>0.05) by the likelihood ratio chi-squared test. Variables considered to be potential confounders were retained in the final model if they changed the estimates of the hazard ratios by greater than 30 per cent. All two-way interactions for variables in the final model were checked for significance.

RESULTS

IgG antibodies against Salmonella in sows and piglets

Overall 96.5 per cent of the sows were seropositive to Salmonella one week after farrowing. The average OD% varied between batches from 30.4 per cent to 54.1 per cent (figure 1). At W1, maternally derived antibodies were detected in every piglet (figure 2). The OD% of the sows one week after parturition was significantly correlated with the OD% of the piglets at one and six weeks of age, with Spearman’s correlation coefficients of 0.84 and 0.78, respectively (P<0.0001 and P<0.0001).

The analysis of piglets was carried out on 40 pigs per batch except in F6-B1 and F6-B3, in which two pigs and one pig died, respectively, before W1. The OD% values for pigs decreased at W6 and then increased again at W10 or W14, depending on the herd and batch. Thereafter, it remained high until slaughter (figure 2). The frequency of seropositive pigs increased during these weeks (figure 3). On farm F6, the pigs from the three batches became positive from W14, while on farm F3 seroconversion occurred at a different time with a batch effect, either at W14 (F3-B3) or W18 (F3-B2), or only at the slaughterhouse (F3-B1). On farm F5, batch B3 remained negative from W6 (mean <15 per cent) up to slaughter,
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Figure 2 Dynamics of Salmonella antibody levels as shown by optical density per cent (OD%) of ELISA (IDEXX Swine Salmonella Ab Test) from one week to 26 weeks of age (slaughtering, S) (three batches from three farms: B, batch; F, farm). The analysis was carried out on 40 pigs per batch except for F6-B1 and F6-B3, where 38 pigs and 39 pigs, respectively, were analysed from week 1 on due to the death of two pigs and one pig from each batch.

while batches B1 and B2 contained a high proportion of pigs that were seropositive only at the slaughterhouse; the corresponding OD% value stayed low in all three batches and reached a mean of only 17.1 per cent, 17.4 per cent and 14.5 per cent, respectively, between W22 and WSlaugh. The highest frequency of seroconverted pigs from all the farms (75.1 per cent) was found at the slaughterhouse (figure 3).

The correlation between pigs’ serological results over time is presented in table 2. Significant positive correlations (P<0.001) were found between W1–W6, W14–W18, W18–W22 and W22–WSlaugh.

Factors associated with age at seroconversion
The mean age at seroconversion was 137 days (±2.2 days) (figure 4). In the univariable analysis, apart from the farm effect, six variables were significantly associated with age at seroconversion (table 3). The final statistical analysis showed that only one factor related to a herd/farm effect remained significantly associated with age at Salmonella seroconversion. Pigs from farm F6 seroconverted earlier (mean age at seroconversion: 117.9±3.0 days) than pigs from the other farms (P<0.001). Indeed, the mean age at seroconversion for farms F3 and F5 were 142.4±3.9 days and 146.6±3.4 days, respectively (figure 5).

DISCUSSION
This study was performed in Brittany, which is the largest pig production area in France, producing 56 per cent of French pigs being reared in this region (13.3 million heads). All the farms involved in this study were farrow-to-finish farms, which in France gather 85 per cent of the sows and 60 per cent of the growing and finishing pigs. In the current study, all sows had antibodies against Salmonella one week after farrowing and pigs from all herds seroconverted before slaughter, indicating exposure to the bacteria. This is a common situation in pig herds. Even though these farms were typical of French production, they were selected on a voluntary basis, so the authors cannot necessarily
extend the results to other farm contexts. However, the piglets were selected at random so they could be considered as representative of the batches.

Serology was used in the present study to assess age at seroconversion and thus to deduce previous exposure to *Salmonella*. Controversies between manufacturers and scientists related to the sensitivity and specificity of these tests have been published. However, the commercial IDEXX ELISA kit that the authors used is both highly sensitive and specific. Detection of antibodies against *Salmonella* is not the best indicator of infection at the pig level because neither the infection of lymph nodes nor the shedding of *Salmonella* organism in faeces is necessarily related to the presence of antibodies in pigs. Serology at the farm level is considered as a good solution to categorise livestock. For this reason, the authors did not analyse the results using an individual approach.

All three farms showed a high OD% for sows and one-week-old piglets. The presence of antibodies against *Salmonella* was detected in piglets up to 10 weeks of age. There was a stronger correlation between the serological response of piglets and sows at one week and six weeks of age. The persistence of maternal antibodies up to eight weeks of age has already been described in the literature. The composition and duration of maternal immunity acquired through the colostrum and milk intake have a large influence on the dynamics of *Salmonella* transmission on a pig farm. In the present study, the results of univariable analysis did not reveal any effect of maternal antibodies on the seroconversion age. Indeed, the serological statuses of the sows one week after farrowing were not associated with age at seroconversion. However, other studies have attributed the relatively high frequency of infection but the low frequency of disease in suckling pigs to the protective effects of maternal antibodies.

The authors noticed that the OD% levels increased mainly during the fattening period and remained high until slaughter. Wales and others described the degree of serological response in fattening pigs tending to increase after around 10 weeks of age. This is probably due to an active immune response to the natural infection produced at the beginning of the fattening stage. A positive correlation was found between piglets’ serological status at the end of the fattening phase from the 18th week up to slaughter. This is probably because the antibodies of pigs infected for the first time during the fattening period gradually increase over time since the bacteria continue to circulate within the herd and contaminate the animals again. This hypothesis could be validated by conducting bacteriological analyses together with serological analyses.

The mean age at *Salmonella* seroconversion was estimated at 137 days. This period corresponds to the second half of the fattening phase. The onset of an immune

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Table 2 Correlation coefficient of the serological results for the sows and their piglets and for the piglets alone over time, for the three farms and the three batches per farm (357 piglets from 90 sows)

| OD sows | W1  | W6  | W10 | W14 | W18 | W22 | WSlough |
|---------|-----|-----|-----|-----|-----|-----|---------|
| OD sows | 1   |     |     |     |     |     |         |
| W1      | 0.84| 1   |     |     |     |     |         |
| W6      | 0.78| 0.86| 1   |     |     |     |         |
| W10     | 0.25| 0.30| 0.37| 1   |     |     |         |
| W14     | 0.40| 0.08| 0.10| 0.35| 1   |     |         |
| W18     | 0.52| 0.07| 0.10| 0.26| 0.62| 1   |         |
| W22     | 0.58| 0.03| 0.09| 0.11| 0.48| 0.60| 1       |
| WSlough | 0.06| 0.11| 0.12| 0.15| 0.33| 0.48| 0.69    |

In bold: Spearman’s coefficient with P<0.001.

OD, optical density; W1, one week after farrowing; W6, pigs at six weeks of age; W10, pigs at 10 weeks of age; W14, pigs at 14 weeks of age; W18, pigs at 18 weeks of age; W22, pigs at 22 weeks of age; WSlough, slaughtered pigs.
Table 3  Variables significantly associated with age at seroconversion using univariable analysis

| Type of variable                      | Variable names                                  | HR  | 95% CI            | P value |
|---------------------------------------|-------------------------------------------------|-----|-------------------|---------|
| Housing and feeding types             | Feeding system                                  |     |                   |         |
|                                       | Dry                                             | 0.40| 0.28 to 0.58      | <0.0001 |
|                                       | Wet                                             | 1   | –                 |         |
| Floor type in fattening step          | Slatted floor                                   | 0.40| 0.28 to 0.58      | <0.0001 |
|                                       | Straw-bedded                                    | 1   | –                 |         |
| Rearing practices                     | Room or pen change of weak or ill pigs during fattening step |     |                   | 0.1     |
|                                       | No                                              | 1   | –                 |         |
|                                       | Yes                                             | 0.68| 0.43 to 1.07      |         |
| Room or pen change of weak or ill pigs during the second month of fattening | No                                              | 1   | –                 | 0.06    |
|                                       | Yes                                             | 0.60| 0.35 to 1.03      |         |
| Room or pen changes during the fattening step | No                                              | 1   | –                 | 0.15    |
|                                       | During the first month of the fattening period  | 0.77| 0.47 to 1.26      |         |
|                                       | During the second month of the fattening period | 0.55| 0.29 to 1.03      |         |
| Antibiotic treatments                 | Antibiotic treatment during the fattening step  |     |                   | <0.0001 |
|                                       | No                                              | 1   | –                 |         |
|                                       | Yes                                             | 0.40| 0.27 to 0.58      | <0.0001 |
| Farm effect                           | Farm                                            |     |                   |         |
|                                       | 3                                               | 0.40| 0.34 to 0.68      | <0.0001 |
|                                       | 5                                               | 0.33| 0.25 to 0.46      |         |
|                                       | 6                                               | 1   | –                 |         |

Factors significantly associated with the outcome after a backward selection process are highlighted in bold. 95% CI, confidence interval at 95%; HR, hazard ratio.

Figure 5  Survival distribution function (Kaplan-Meier estimate) for each farm showing the farm effect. The mean age at Salmonella seroconversion on farm F6 (117.9±days) was earlier (P<0.001) than on farms F3 (142.4±3.9 days) and F5 (146.6±3.4 days). Age: age of pigs in days.

response has been described between seven and 30 days after natural infection.17 Thus, the authors could consider that pigs could be exposed to Salmonella during the first half of the fattening period. Another study has described an active immune response against natural infection at the beginning of the fattening period.18 Factors influencing Salmonella infection in the fattening period have been described in the literature.27 They are related to hygiene and biosecurity measures, such as the cleaning of feed tubes, wearing protective clothing when entering the farms and controlling the proliferation of rodents. Farm practices in the farrowing section—such as allowing pigs from adjacent pens to have snout contact and purchasing pigs from more than one supplier—were associated with an increased odds of seropositivity for Salmonella.28

In the present study, a farm effect was found to significantly influence the age at seroconversion. This farm effect included a set of practices and biosecurity measures such as weaning age, room changes, pen changes, age of entry into the fattening system, cleaning and disinfection, movement and mixing of animals, and so on. Other studies have shown a farm effect for farm contamination with microorganisms such as Campylobacter but not Salmonella.29

Unlike previously published studies,9 the authors did not find any other factor associated with age at seroconversion. This may be due to the present study’s limited sample size (three farms and 120 pigs per farm). There may, therefore, have been a lack of statistical power to differentiate
the farm factors influencing seroconversion. Unlike other works, the authors did not observe a link between Salmonella seroconversion and PRRSV serological status.

In conclusion, maternal antibodies were detected in piglets up to 10 weeks of life. Seroconversion on the different farms occurred during the fattening period. However, the seroconversion age happened earlier on some farms, a fact that may be related to their own characteristics. The current study's limited sample size means that further studies are required to confirm these observations on a larger scale. Furthermore, to clarify the dynamics of Salmonella infection, it is also essential to include bacteriological analysis of faecal samples.

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