Results of a longitudinal study of Salmonella enterica infections in 5 sero-positive and 5 sero-negative finishing swine herds in The Netherlands. (SALINPORK workpackage 1 task 5)

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Introduction
In order to monitor the course of Salmonella-infections in finishing pig herds, repeated sampling of consecutive batches of finishing pigs is necessary. This study followed 5 sero-negative and 5 sero-positive herds during 7 sampling rounds with 3 months between samplings.

Material and Methods

Herd selection
In this study, 5 sero-negative and 5 sero-positive herds were randomly selected from a database containing a little over 400 herds. From these 400 herds blood samples of slaughter pigs had been collected in two slaughterhouses (van der Wolf et al., 1997). To establish the sero-prevalence for Salmonella of these herds, at least forty bloodsamples from three different batches of slaughter pigs had to be sampled. The sero-negative herds were selected from those herds in which a maximum of 2 blood samples out of at least fifty with an OD% over 10 were found. The sero-positive herds were selected from those herds in which at least 10 blood samples out of 50 (20%) with an OD% over 40 were found.

Sampling
The selected herds were followed by 7 subsequent sampling rounds. Sampling was done 3 months apart, so that each herd is followed for 2 years. During each visit 25 blood samples were collected from growers after transfer to the finishing unit with a minimum live weight of 20 kg and 25 blood samples from fattening pigs as close to slaughter weight as possible. Only one compartment with growers and one compartment with finishing pigs were sampled each round. The compartment with growers was used in the next round to sample as finishing pigs, however, not exactly the same animals were sampled twice but just the same pens and compartments. For blood sampling Monovette tubes (plastic, sterile, plunger, gel, 9 ml) (Sarstedt, Nümbrecht, Germany) were used. Per visit, 10 faecal samples consisting of a minimum of five droppings of 5 gram each (total of 25 g) were collected. Five pen samples were collected from growers and 5 from finishing pigs. The samples were forwarded to the laboratory as soon as possible for microbiological examination.

Serological examination
The Dutch ELISA is a mix-ELISA containing the O antigens 1,4,5,6,7 and 12 extracted from S. typhimurium (serotype B: O 1,4,5,12) and S. livingstone (serotype C1: O 6,7) at 2 micrograms of LPS per serotype per microtiter well (Microwell®, Life Technologies B. V., Breda, the Netherlands). Serum is diluted 1:16. Per plate 4 positive control samples, 2 blanks and 2 intermediate samples are tested as controls (Boleij, 1996; van der Heijden et al, in preparation). Colour change as a result of a positive reaction is measured by photometer (SLT-reader, Spectra) at 450 nm. The result is calculated as:

\[
\%OD = \left( \frac{\text{sample OD} - \text{mean OD blanks}}{\text{mean OD positives} - \text{mean OD blanks}} \right) \times 100 / 2.4
\]

The result is divided by 2.4 to make comparison with Danish results possible. Samples with an OD% over 10 are considered positive.

Bacteriological examination
Data collection was performed according to the Microbiological Standard Operating Procedures (MSOP)-Final Version (Copenhagen, 16 October 1996) as defined for the SALINPORK-project. In short, 4 steps are required to detect Salmonella most efficiently: pre-enrichment in BPW (1:10 at 37°C for 18-24 hrs), enrichment in RV broth (1:10 at 42°C for 18-24 hrs), plating out on BGA (at 37°C for 18-24 hrs) and conformation. Salmonella isolates were stored and forwarded to the European Salmonella Reference Laboratory (RIVM, Bilthoven, The Netherlands) for determination of serotypes of Salmonella and the phagetyp distribution within S. typhimurium (Guinee et al, 1974).
Results

Due to the Classical Swine Fever (CSF) outbreak in The Netherlands from February 1997 to April 1998 we have missed one or more sampling rounds at most of the participating farms. To compensate for this loss of data we included two extra positive herds. Figure 1 shows the slaughterhouse (S) results and the results of the serological testing rounds (1-7) of the follow-up in sero-positive finishing pig herds. Grey spaces between the bars are missing sampling round or the separation between herds. Round 7 of herd 4 has a prevalence of zero.

Figure 2 shows the results of the serological testing rounds of the follow-up in initially sero-negative herds. Due to governmental decisions to restructure the Dutch pig industry, three farmers were forced to quit and could not finish the follow-up study (N1, N2 and N5).

Faecal sampling

Table 1 shows the Salmonella serotypes found, phagetypes and sampling rounds. In total 5 serotypes of Salmonella were found. These included S. typhimurium (4 different phagetypes, one a-typical and one no reaction), S. panama, S. londons, S. infantis and S subgen. I, O:21, nm. In P5 no Salmonella was isolated from faecal samples taken in the follow up study but S. typhimurium was isolated from a post mortem on a 5 months old pig from a clinical outbreaks.

Correlation between serum from growers and finishers

Due to the CSF outbreak sampling precisely every three months was hardly possible. Because of that only in a few herds groups of animals were sampled as grower and 3 months later as finisher. Figures 3, 4 and 5 shows the results of herds P1, P4 and N1 respectively, of growers which were sampled later as finishers. The arrows with similar points indicate the same groups of animals as grower and as finisher.

Figure 1. Sero-prevalence of finishers in sero-positive finishing herds over a period of 2 years.

Figure 2. Sero-prevalence of finishers in initially sero-negative finishing pig herds.
Table 1. Serotypes of Salmonella found in the follow-up study

<table>
<thead>
<tr>
<th>herd</th>
<th>sero-positive (%)</th>
<th>number of pos. faecal samples</th>
<th>round</th>
<th>serotype</th>
<th>sero-group</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>92</td>
<td>1</td>
<td>4</td>
<td>S. typhimurium (no reaction)</td>
<td>B</td>
</tr>
<tr>
<td>N4</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>S. infantis</td>
<td>C1</td>
</tr>
<tr>
<td>P1</td>
<td>100</td>
<td>1</td>
<td>4</td>
<td>S. typhimurium (a-typical)</td>
<td>B</td>
</tr>
<tr>
<td>P1</td>
<td>100</td>
<td>2</td>
<td>5</td>
<td>S. panama</td>
<td>D1</td>
</tr>
<tr>
<td>P2</td>
<td>30</td>
<td>1</td>
<td>1</td>
<td>S. london</td>
<td>E1</td>
</tr>
<tr>
<td>P2</td>
<td>28</td>
<td>1</td>
<td>5</td>
<td>S. typhimurium (phagetype 506)</td>
<td>B</td>
</tr>
<tr>
<td>P4</td>
<td>20</td>
<td>1</td>
<td>4</td>
<td>S. typhimurium (phagetype 296)</td>
<td>B</td>
</tr>
<tr>
<td>P4</td>
<td>14</td>
<td>1</td>
<td>6</td>
<td>S. typhimurium (phagetype 301)</td>
<td>B</td>
</tr>
<tr>
<td>P5*</td>
<td>36 post mortem</td>
<td></td>
<td>4</td>
<td>S. typhimurium (phagetype 510)</td>
<td>B</td>
</tr>
<tr>
<td>P6</td>
<td>78</td>
<td>2</td>
<td>1</td>
<td>S. london</td>
<td>E1</td>
</tr>
<tr>
<td>P6</td>
<td>78</td>
<td>1</td>
<td>1</td>
<td>S. subgen. I, O:21, nm</td>
<td>L</td>
</tr>
<tr>
<td>P7</td>
<td>100</td>
<td>1</td>
<td>1</td>
<td>S. typhimurium (phagetype 61)</td>
<td>B</td>
</tr>
</tbody>
</table>

*this is a post mortem result after a clinical outbreak

**Discussion and Conclusion**

Looking at the positive herds in figure 1 it is clear that all herds stayed positive during the follow up study. Apparently, if one establishes the seroprevalence of a herd based on a proper random sampling and the resulting seroprevalence is high, this is an indication for a long term problem. These herds have a permanent influx of infected animals (figure 3) and/or a continuous spreading Salmonella infection (figure 4). Also noticeable is the fact that only part of the animals become sero-positive. Even in herds with clinical outbreaks (P4 and P5) this is the case. Combined with earlier experiences we conclude from this that there can be a large difference in sero-prevalence between pens, between pens on either side of an aisle within a compartment and between compartments.

Looking at the negative herds in figure 2 it becomes clear that it is possible for a fattening herd to be free from Salmonella for a longer period. Both herd 3 and 4 feed fermented liquid feed (FLF) to their fattening pigs (25 kg to finishing). FLF contains acidified or fermented by-products from the human food industry. Probably as a result of the high concentration of lactic acid and also acetic acid in those feeds, which have a strong negative effect on Salmonella, these herds stay free from Salmonella infection.

Figure 3: Comparison of sero-prevalence of growers and 3 months later as finishers in herd P1.
Looking at the other three herds in figure 2 one could make two sets of complimentary herd infection incident classifications. Firstly, minor and major incidents, and secondly, incidents with within herd and outside origin. A minor incident is where only one or two pigs get / are infected. The slaughterhouse result of N3 and the isolation of S. infantis in N4 are probably such examples. Introduction of Salmonella apparently does not lead to major outbreaks and the permanent loss of the Salmonella free status. N1, N2 and N5 suffer major incidents. However, N1 is probably an example of an incident with an outside origin, demonstrated by the fact that animals that arrive clearly positive, become less positive during their stay and the herd does not become infected itself (figure 5). Herd N1 was a multi-site farrow-to-finish herd. During the CSF-outbreak the farmer was not allowed to transport the ready-to-shed feeders to the finishing location. The resulting overcrowding at the sow location caused a outbreak of Salmonella. Later, these infected ‘heavy’ ready-to-shed-feeders were moved to the finishing location, and caused a major increase in sero-prevalence. During this period 88% of the sampled animals were sero-positive and S. typhimurium was found in one faecal sample; three months later around 24% and another 3 months later only 2% of the sampled animals were sero-positive. For some unknown reason Salmonella is not able to establish itself in that particular pig house, even when animals are shedding.

N2 and probably N5 are examples of major incidents where Salmonella infection is introduced into the herd and the herd becomes infected and the infection is passed on from compartment to compartment, demonstrated by the fact that several rounds of sampling are positive. One could see this as a within herd infection incident because, after one introduction from outside, the herd maintains its own infection cycle.

Conclusions

* If a positive Salmonella-status is based on a proper random sampling of the herd, than this status is an indication of a long term problem.

* Even in sero-positive herds only part of the animals are sero-positive / infected and not all. Large differences can exist between animals and different clusters of animals.

* Clinical problems are not directly related to sero-prevalence.

* Herds can remain Salmonella negative over a longer period, probably as a result of feeding FLF.

* It is possible to deliver finishers, which were sero-positive as growers, Salmonella negative to the slaughterhouse, in other words, an immune response can disappear within the time span of a fattening period.

* Different classes of infection incidents can be distinguished: minor - major and outside - within.
References


Acknowledgements

First of all we thank the participating farmers and slaughterhouses for their kind co-operation despite the extremely difficult situation during the CSF-outbreak. Without their help this project would not have been a success. We also thank the involved employees of the Animal Health Service for their assistance in the project, especially during the CSF-outbreak. Finally we thank the EU commission (SALINPORK contractnr FAIR1-CT95-400), Product Boards for Livestock, Meat and Eggs, the Ministry of Agriculture, Nature Management and Fisheries and the Animal Health Service for funding this project.