

## LOOKING FOR A RELIABLE METHOD TO FOLLOW THE *SALMONELLA* STATUS OF FINISHING PIGS

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A study was undertaken to answer questions about the epidemiology of *Salmonella* in pig production. Does the *Salmonella* excretion from a group of pigs (on the same farm) remain constant during time or is there variations ? Where, and how many samples do we have to take to follow these variations, if they exist ? Are the techniques to analyse samples (bacteriological and serological) practicable ? How is the relationship between serological and bacteriological methods ?

Firstly, 4 farms, 2 giving maximum positive results (from a preliminary study conducted at the slaughter house) and 2 giving maximum negative results were selected among 30 groups of 20 pigs from 30 pre-selected unrelated farms.

Each month, each one of these 4 farms is controlled by sampling feces and swabs from the group of finishing pigs leaving the farm for the abattoir in the same week.

In a further study, these results will be exploited and minimum sampling and best serological and bacteriological methods will be chosen to conduct a risk factors analysis study on *Salmonella* contamination in growing and finishing pigs farms.

### MATERIAL AND METHODS

This preliminary study was divided in two steps : 30 groups of pre-selected finishing pigs from unrelated farms were tested at time of slaughter. Then 4 selected farms were included in a long term study.

#### 1- Study on 30 groups of finishing pigs at the abattoir

From each farm, 20 pigs were bacteriologically and serologically analysed for *Salmonella* : approximately 10g of feces and one lymph node from the digestive tract per pig and the sera were considered. For analysis, feces were pooled by group of 5 pigs but lymph node and sera were individually processed.

The serological method was based on *S. Typhimurium* lipopolysaccharid antigens. Control sera were provided by the Danish Veterinary Laboratory. Sera were diluted 1/400, the conjugate was peroxidase conjugated rabbit anti-chicken IgG antibody, the substrate was the ortho-phenylene-diamine and reaction was stopped with 0,5M sulfuric acid. Coloration was read with a Dynatech MR 5000 spectrophotometer and two filters were used a 490nm test filter and a 630nm reference filter. Optical density was corrected with the positive and negative reference controls and the positive threshold value was 0,300.

For the bacteriological analysis, samples were diluted in peptone buffer water (1/5 to 1/10 dilution) incubated 16 to 20 hours at 37°C. The enrichment step used semi-solid Rappaport Vassiliadis medium incubated at 41.5°C and presumptive positive results (migration) were

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isolated on Rambach agar. Two isolated colonies were confirmed biochemically and serotyped.

### 2- Study on the 4 selected farms

During a first visit, in each of the 4 selected farms, 50 animals were analysed in order to validate the classification obtained from results at the abattoir. For 10 animals in each of the 5 following groups: pregnant sows, farrowing sows, 8 weeks old pigs, growing pigs of 40 kg and finishing pigs of 70 kg, sera and rectal swabs were tested. Furthermore, for 5 animals per group, rectal swabs and individual feces were compared for ability to recover *Salmonella*.

Once a month, 15 pigs, ready to go to the abattoir, were tested before leaving the farm. From each of these 15 pigs, rectal swabs were tested and from 10 of them, individual feces were also analysed. Then, 7 environmental swabs (5 on the walls, 1 on the machine to make the soup and 1 on the augers) were also analysed.

The same 15 pigs were followed at the abattoir and sampling consisted of feces, intestinal lymph node and sera individually analysed for each pig.

All samples were analysed following the methods already described in section 1.

## RESULTS

### 1- Study on 30 groups of finishing pigs at the abattoir

The correlation coefficient between serology and lymph node was 0,23 but only 0,12 between serology and fecal samples. On the other hand this coefficient was higher between lymph node and fecal samples ( $r=0,62$ ). Four farms among these 30 groups were selected because two gave a maximum positive results and two because they presented a maximum negative results.

### 2- Study on the 4 selected farms

During the first visit in the farm (Table 1), correlation coefficients were higher between serological and bacteriological methods than the ones obtained on finishing pigs ( $r$  between serological and fecal samples =0,62). But rectal swabs and serological samples did not present a good coefficient correlation ( $r=0,41$ ). These coefficients will be reestimated at the end of the study.

Table 1. Percentage of positive results in the 4 farms

ANIMALS	SEROLOGY	FECES	RECTAL SWABS
farrowing sows	68	53	14
pregnant sows	76	33	3
70 kg pigs	36	20	0
40 kg pigs	0	0	0
post-weaning	0	0	3

In the slaughterhouse, the correlation coefficient between serology and fecal samples are high ( $r=0,81$ ) as well as between serology and lymph node ( $r=0,85$ ).

Before and after slaughter bacteriological results were compared (Table 2). In a negative farm finishing pigs were negative (0 % positive results on two groups) before going to the abattoir and they became positive on fecal samples (23,1 and 30 % positive results) following transport. When results from all groups were compiled the positive percentage on fecal samples increased from 20% before slaughter to 30% after slaughter.

Table 2. Percentage of positive on pre-selected finishing pigs before and at slaughter time

	FECES	FECAL SWABS	ENV. SWABS « a »	LYMPH NODS
At farm	17,5	0	29	-
At slaughter	28	-	-	14

« a » Env. swabs : Environmental swabs

### 3- *Salmonella* serovars isolated in this study

*Salmonella* Typhimurium was the most common serovar isolated in our study followed by *S. Anatum*, Derby, Bredeney, London, Tennessee and Infantis.

Even if Typhimurium lipopolysaccharid antigens were used, it was possible to detect some animals positive by ELISA in farm where only serotypes which belong to the same group than *S. Typhimurium* (Derby and Bredeney) or even *S. Anatum* (E1 group) were identified by bacteriology.

## DISCUSSION AND CONCLUSION

Our study on the 4 selected farms is still going on but we already have made some choices concerning samples and methods for our further study on the risk factors associated with *Salmonella* in pigs.

Rectal swabs will not be selected because they are not sensitive enough.

In a farm most positive results are obtained on sows and 70 kg pigs perhaps because the young pigs are still not contaminated enough.

Moreover, the relationship between serological and bacteriological analysis (on fecal sample and lymph node) seems to be proven. Our first conclusion at this point is that it would be possible to use the serological method for screening and to confirm positive results with a bacteriological method because the ELISA has a lack of specificity (cross-reaction with other *Salmonella* serovars belonging to the group B like *S. Typhimurium*). These cross-reactions are not a problem because in our analysis of the risk factors we want to look for all the *Salmonella* and not only for *S. Typhimurium*.

Transport of swine induces fecal excretion of germs because of the stress and this is a critical point possibly responsible for increasing the risk of human *Salmonella* infection.