DOSE DEPENDENT ESTABLISHMENT OF SUBCLINICAL S. TYPHIMURIUM INFECTION IN PIGS AND PROTECTION AGAINST HOMOLOGOUS RE-CHALLENGE

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The fact that the majority of *Salmonella* infected pig herds are subclinically infected is accepted worldwide. In subclinically infected herds shedding of *Salmonella* from infected pigs and the contamination of the environment is markedly below the level known from herds having experienced a clinical outbreak (Baggesen et al., 1996).

Reduction of the infection level in the environment by all in-all out management including cleaning and disinfection of the units between batches of pigs is one part of the effort to control *Salmonella* infections in Danish pig herds. In combination with correction of feed, control of diarrhea and acidification of feed or drinking water, reduction of contamination level is an effective tool, but when used alone to control *Salmonella* in practice it tends to be insufficient.

This observation led us to investigate the dependence of infection dose on establishment of *S. Typhimurium* infection.

MATERIALS AND METHODS

Experimental design:

*Pigs:* 36 pigs (25-30 kg mean bw) were obtained from a herd, in which no pigs were tested seropositive to *Salmonella* at monthly serological examination of 20 pigs (40-70 kg bw) for more than a year. Cross sectional examination of the herd for *Salmonella* was negative except for few seropositive sows. Pigs for the experiment were tested seronegative and culture negative (faecal pools) prior to the experiment.

*Housing:* Pigs were removed to experimental facilities 7 days prior to challenge and randomly allocated to experimental groups in pens with fully slatted floor and no faecal passage between pens. Precautions were taken to avoid transfer of faecal material between pens. A separate unit with separate clothing and equipment was used for the uninfected control group. Prior to the experiment the units were thoroughly cleaned, slurry pits were emptied and alkalinized and units were disinfected (fumigation with formalin). Pigs were fed *ad libitum*, compound feed (pelleted, heat treated at 81°C) as dry feed. Main constituents were: barley (40%), wheat (32%), soy bean meal (21%), animal fat (2%), sugar beet molasses (1%), vitamins, minerals and amino acids. No antibiotic growth promoters were added. Protein content: 17.5%. The pigs had free access to water.

*Challenge:* *S. Typhimurium* PT12 Rif⁶ (O5+), lymph node isolate from porcine salmonellosis. Challenge dose was prepared by 2-step incubation in nutrient broth, spinning and resuspension in 5ml physiol. saline (doses in table 1). The feed was withheld for 21-23 hours prior to each

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challenge (incl. uninfected controls). Pigs were challenged individually. The challenge dose was poured onto approx. 25g of feed which was offered the pig. The pigs had free access to feed shortly after challenge. First challenge was given day 0, 2nd challenge day 21. Individual faecal samples were collected on day -1, 2, 4, 14, 20, 23, 25, 28, 35, 42 and 49. Blood samples day -1, 7, 14, 20, 28, 35, 42 and 49.

Table 1. Challenge doses for experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. pigs</th>
<th>First challenge dose (day 0)</th>
<th>Second challenge dose (day 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>Uninfected controls</td>
<td>5x10^6</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>5x10^2</td>
<td>5x10^6</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>5x10^4</td>
<td>5x10^6</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>5x10^6</td>
<td>5x10^6</td>
</tr>
</tbody>
</table>

Examinations:

Bacteriological examination for Salmonella: 10-fold dilutions of faeces were submitted to bacteriological examination for Salmonella, detection level: culture dilution 10^2. Analyses for Salmonella were performed according to standard methods and included non-selective pre-enrichment, selective enrichment biochemical verification of Salmonella and identification of the challenge strain by polyclonal O%-serotyping and analysis for rifampicin resistance.

Serological examination: blood samples were analysed for antibodies to Salmonella in a mix-ELISA (antigen: O-types 1, 4, 5, 6, 7 and 12) (Nielsen et al., 1995). The results were presented as OD% with an OD% of 10 as scientific cut-off for seropositivity.

RESULTS

No. culture positive pigs/group

![Graph showing the number of culture positive pigs per group over time, with a peak at day 0 and a drop to baseline by day 21.](image)

Figure 1. No. of pigs per group shedding the challenge strain. (N=9).
Figure 2. No. of seropositive pigs per group (N=9)

After first challenge no shedding was detected in pigs inoculated with 500 CFU. One pig inoculated with 50,000 CFU excreted low numbers of the challenge strain day 4. 6 pigs inoculated with 5x10^6 CFU excreted low to moderate numbers of the challenge strain. No shedding or seroconversion was detected in the control group.

After second challenge (all 5x10^6 CFU) shedding of the challenge strain was reduced in groups challenged first time with 5x10^6 CFU or 50,000 CFU but not with 500 CFU.

All pigs seroconverted after first challenge with 5x10^6 CFU. Five pigs seroconverted after first challenge with 50,000 CFU, and only one pig seroconverted (low seroreaction: 32 OD% day 20) after 500 CFU. After second challenge all pigs had seroconverted at least at one sampling. The seroreaction after second challenge was lowest in pigs challenged first time with 50,000 CFU (data not shown).

DISCUSSION AND CONCLUSION

A clear dose-dependent shedding and seroreaction was observed after oral S. Typhimurium challenge and dose dependent protection against homologous re-challenge was obtained (higher dose gives higher degree of protection). The results indicate that, at least under certain circumstances, pigs are able to resist establishment of enteral infection after exposure to low doses (500 CFU/pig) of S. Typhimurium.

REFERENCES
