Quantitative determination of the challenge strain content of the ileum, caecum and ileocaecal lymph nodes following oral challenge of swine with \textit{S. typhimurium} PT 104

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Abstract

A \textit{S. typhimurium} challenge model for swine was developed which takes into consideration both clinical changes and the colonization of various organs. Young swine were infected, using bait, with various doses of \textit{S. typhimurium} DT 104 resistant to nalidixic acid. After infection, the presence of the challenge strain in the ileum, caecum and ileocaecal lymph nodes was determined quantitatively. The level of antibodies in the serum to \textit{S. typhimurium} was established pre-challenge and 6 days after challenge using ELISA.

Challenge 5x by the oral route yielded results with the highest degree of reproducibility and appears to be a suitable method for the investigation of vaccines and other measures to combat the disease.

Introduction

The aim of the study was to develop an oral infection model for young swine in which both the clinical course of disease is monitored and the presence of the challenge strain is quantified in selected organs.

Oral infection models have been described for chickens in which latent or subclinical infections are reproduced (Methner et al. 1994, Cooper et al. 1994). These comprise oral infection with a virulent labelled strain and quantitative determination of the challenge strain in various organs some days later. \textit{S. typhimurium} infections of swine run a subclinical course in most animals, although cases with clinical signs have been described for young swine.

Materials and Methods

Bacteria

The challenge organism was a nalidixic acid-resistant \textit{S. typhimurium} strain (BgVV No. 958/96). The strain was phage type DT 104 and was cultured in Strm 6/83 Medium. After washing it was taken up in buffered physiological saline and stored at -80 °C.

Swine

Male and female hybrid swine (Landrace x German “Edelschwein”) weighing 30 - 35 kg were used. The animals were sourced from a population monitored for Salmonella infections and were subjected to bacteriological tests for Salmonella and serological tests for antibodies to \textit{S. typhimurium} and \textit{S. choleraesuis}. All animals were given a commercial complete feed and water \textit{ad libitum}. Feed was withdrawn from the animals 22 - 24 hours before challenge. Animals were again allowed free access to feed after challenge.

Methods

The 4 animals in Group 1 were anaesthetized prior to challenge (0.1 mg / kg acepromazine i.m. and 10 mg / kg thiopental sodium i.v.). The challenge dose was $1 \times 10^{16}$ CFU \textit{S. typhimurium} DT 104, NaF, in 50 ml buffered physiological saline, given by the intragastric route using a nasal stomach tube. The 4 animals in each of groups 2 and 3 were challenged 4 and 5 times respectively using bait (jam doughnuts) on 2 successive days (Table 1).
Table 1: Dose, mode of administration and days post-challenge at which the organs were investigated for presence of bacteria

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (cumulative)</th>
<th>Mode of administration</th>
<th>Isolation of <em>S. typhimurium</em> from the ileum, caecum and ileocaecal lymph nodes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 x 10^{10} CFU</td>
<td>Intragastric (0h)</td>
<td>7 d</td>
</tr>
<tr>
<td>2</td>
<td>4 x 10^{10} CFU</td>
<td>Oral (0h, 7h, 24h, 31h)</td>
<td>7 d</td>
</tr>
<tr>
<td>3</td>
<td>5 x 10^{10} CFU</td>
<td>Oral (0h, 4h, 7h, 24h, 31h)</td>
<td>6 d</td>
</tr>
</tbody>
</table>

* Days after last administration of the challenge strain

The general well-being of the animals, their feeding habits, incidence of diarrhoea and vomiting and rectal temperature were recorded before and after challenge. Blood samples were taken pre-challenge and after the last challenge and the sera tested for the presence of antibodies to *S. typhimurium*. A commercial test, the SALMOTYPE™ Meat Juice-ELISA (Labor Diagnostik GmbH, Leipzig), was used. This ELISA recognises antibodies to Salmonella O antigens 1, 4, 5, 6 and 7 and can be used for sera and meat juices.

The animals in all 3 groups were sacrificed on days 6/7 after the last administration of the challenge strain and the presence of *S. typhimurium* was quantitatively determined per gram tissue of the ileum, caecal mucosa and ileocaecal lymph nodes. At the same time spleen and liver cells were transferred to RVM enrichment culture (Merck 07700.0500).

The micro-organism count was determined using the Koch plating method. Two Leifson deoxycholate agar plates (SIFIN TN 1121), containing 50 mg nalidixic acid (Sigma N 8878) per mL, were used for each dilution. Samples which failed to grow when plated out directly were enriched in RVM and then plated out on the Leifson agar containing nalidixic acid and on Rambach agar (Merck 7500.0003). The results were then converted to values per 1 g organ weight and the micro-organism counts were expressed logarithmically. The mean values and standard deviation were derived. Samples from which the challenge strain could only be isolated after further culture were assigned the value 1 and those which were negative were given the value 0.

To establish which mode of infection yielded the most reproducible results the coefficient of variation (ratio of mean values to their standard deviations) was calculated and the significance of differences between the variances in micro-organism numbers was established using the F- test for each organ and each group.

**Results**

The animals in Group 1 did not show any disturbance in their general well-being or feeding habits during the study. All animals exhibited a short-term rise in body temperature in the subfebrile range. One animal in Group 2 and 2 animals in Group 3 developed diarrhoea and a slight disturbance to their well-being and feeding habits approximately 24 - 48 hours after the last challenge. The body temperature of the animals increased to subfebrile values for a short period after challenge, with the diseased animals exhibiting the highest values. All animals were free of clinical signs of disease by 3 days after the last administration of the challenge strain.

At necropsy all animals were found to have a reddening and thickening of the ileal and caecal mucosa and in some cases bleeding in the region of the caecum.

The results of quantification of *S. typhimurium* in the organs are presented in Table 2.

Table 2: Values of *S. typhimurium* in the organs after challenge (Studies 1 - 3)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of challenge strain organisms (x ± SD) in log CFU / g tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ileum</td>
</tr>
<tr>
<td>1</td>
<td>4.28 ± 0.38</td>
</tr>
<tr>
<td>2</td>
<td>2.96 ± 0.91</td>
</tr>
<tr>
<td>3</td>
<td>4.16 ± 0.14</td>
</tr>
</tbody>
</table>
The animal in Group 3 which exhibited the most marked clinical changes was also found to have the challenge strain in the liver following enrichment culture.

The coefficients of variation for the individual organs in Groups 1 - 3 are presented in Table 3.

<table>
<thead>
<tr>
<th>Group</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ileum</td>
</tr>
<tr>
<td>1</td>
<td>8.89</td>
</tr>
<tr>
<td>2</td>
<td>30.74</td>
</tr>
<tr>
<td>3</td>
<td>3.36</td>
</tr>
</tbody>
</table>

The F-test revealed significant differences for the ileum and caecum (p < 0.05) both between groups 1 and 3 and between groups 2 and 3.

Figure 1 below shows the optical density (as an indicator of sera antibody values) pre-challenge and 6 days post-challenge (Group 3).

![Optical density graph](image)

Figure 1: The presence of antibodies to *S. typhimurium* in meat juice ELISA

**Discussion**

In contrast to the results of Wingstrand et al. 1996, administration of even a very high dose of *S. typhimurium* by the intragastric route failed to elicit clinical changes. One possible reason for this is that a sodium bicarbonate solution, which is thought to offer the challenge strain optimal conditions for replication in the stomach, was not administered before challenge. Another possible reason is that both the challenge strain and the breed of swine can exert an influence on the course of infection.

In agreement with the results of Blaha et al. 1997, oral challenge with a bait was found to be a very elegant method which is very close to infection conditions in nature. Only relatively mild clinical signs were evident for a short period in a few animals after repeated oral challenge.

Marked numbers of the challenge organisms were found in the organs investigated in all 3 studies.

The low variance in micro-organism counts for the ileum and caecum of animals in Group 3 - which differed considerably from those in Groups 1 and 2 - has shown that the most reproducible results are achieved following 5 inoculations of the challenge strain. The animals in Group 3 exhibited a marked rise in antibody titre on day 6 after challenge, indicating a reaction between the host and the pathogen.

This infection model is thus a very good approximation of conditions encountered in practice and lends itself to the testing of vaccines and other measures to combat disease.
References


