Experimental swine dysentery: comparison between infection models

Magdalena Jacobson,1 Claes Fellström,1 Ronny Lindberg,2 Per Wallgren1,3 and Marianne Jensen-Waern1

12Department of Large Animal Clinical Sciences1 and Department of Pathology2, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden
3National Veterinary Institute, Uppsala, Sweden

The aim of the present study was to develop a reproducible porcine infection model with Brachyspira hyodysenteriae. The influence of different factors was evaluated, namely, age, a diet containing large quantities of soybean meal, housing and administration of cortisol or antacids. Furthermore, the synergistic effect of additional bacteria (Escherichia coli O141, Bacteroides vulgatus or a mixture of Bacteroides fragilis, a field isolate of Bacteroides and Fusobacterium necrophorum) was studied. Experimental infection resulted in an increase in the serum concentrations of the acute-phase proteins serum amyloid A and haptoglobin and the percentages of neutrophils and monocytes. These alterations were specifically related to haemorrhagic diarrhoea. Inoculation combined with feeding of large quantities of soybean meal and group-housing induced swine dysentery in all experimental animals. If the pigs were fed soybean meal, kept in single pens and circulated between the pens, five out of nine developed disease.

INTRODUCTION

Swine dysentery is a severe, mucohaemorrhagic enteric disease in pigs. The disease was first described in 1921 (Whiting et al., 1937) and the aetiology was elucidated in 1971, when the causative spirochaete, Brachyspira hyodysenteriae, was cultured and Koch’s postulates were fulfilled by experimental inoculations in conventional pigs (Taylor & Alexander, 1971). However, in many studies, attempts to reproduce the disease in gnotobiotic pigs consistently failed, unless the pigs were inoculated with colonic scrapings (Meyer et al., 1974b). A synergistic action of other bacteria such as Bacteroides spp., Escherichia coli, Fusobacterium spp., Clostridium spp., Lactobacillus spp., Listeria spp. or Vibrio spp. has therefore been suggested (Meyer et al., 1974b, 1975; Harris et al., 1978; Whipp et al., 1979).

Furthermore, different experimental infection models have been applied. Stress has been suggested as a factor that facilitates the infection and can be induced experimentally by administration of corticosteroid drugs or withdrawal of feed (Eriksen & Andersen, 1970; Moreng et al., 1980). The occurrence of infection might also be influenced by: acid secretion in the stomach (Savage, 1980); the dose of the infectious agent (Wilcock & Olander, 1979); age (Olson, 1974); different bacterial strains and administration routes (Kinyon et al., 1977; Raynaud et al., 1980) and the use of mucosal scrapings versus bacterial cultures in broth or on agar plates as inoculum (Moreng et al., 1980). Recently, much interest has been focused on the influence of feed. Diets that are major substrates for microbial fermentation in the large intestine have been compared with diets that are mainly digested in the small intestine. A diet based on cooked rice and animal protein has been found to be highly protective against colonization with Brachyspira hyodysenteriae (Siba et al., 1996; Durmic et al., 1998). In contrast, soya-based diets have been shown to predispose to diarrhoea (Nabuurs, 1986; Dewey, 1993; Neef et al., 1994b).

Studies on the immune response to swine dysentery have mainly dealt with antibody reactions (Rees et al., 1989; Wright et al., 1989; Galvin et al., 1997), and information on the innate response, such as that of the acute-phase protein reactants (APPs), is sparse. APPs are used extensively as markers of inflammation and detectable concentrations can be found in the blood within hours of exposure to stimuli. The APP response varies between species and with different extents of injury (Gruys et al., 1994). In pigs, most studies have focused on the response to respiratory diseases, and four major APPs have been described: serum amyloid A (SAA), haptoglobin, C-reactive protein (CRP) and pig major acute phase protein (MAP) (Heegaard et al., 1998). In a previous study, SAA and haptoglobin were found to be useful...
markers of inflammation caused by abdominal surgery; SAA peaked 1 day after surgery and disappeared 4 days post-surgery, whereas haptoglobin peaked 2 days after surgery and then decreased slowly over a 2-week period (Jacobson et al., 2001).

The aim of the present study was to develop a reproducible porcine infection model for Brachyspira hyodysenteriae. In addition, the APP response to this infection was evaluated.

METHODS

Experimental design. The experimental design was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden. The study was carried out in three consecutive steps. First, the influences of age, route of administration and bacterial strains were investigated. Second, the influences of a provocative feeding regime, administration of corticosteroids and administration of antacids were evaluated. This step was repeated and the effect of different sources of animals was added. Third, the effect of individual housing and the possible synergistic actions of other bacteria were analysed.

Animals and accommodation. The study comprised a total of 47 pigs, consisting of 41 growers weighing ~20 kg, two slaughter pigs (~115 kg) and four 6-week-old weaners. Some pigs were included more than once in different parts of the study: four grower pigs from the first part were also included in the second part of the study, and four pigs were inoculated twice in the third part of the study. The slaughter pigs originated from an SPF (specific-pathogen-free) herd and three growers were purchased from the University research farm. The remaining pigs (n = 42) were purchased from a conventional gilts-producing herd. All three herds were monitored regularly and were found free from infection with Brachyspira hyodysenteriae, and no Brachyspira species had been isolated at the University farm during the last 10 years. The animals were housed either individually or two to four pigs per pen. All pigs were fed ad libitum with a commercial finisher diet containing 1–2% soybean meal and no growth promoters (Singel Veg SPF), and had free access to water. In 27 pigs, the commercial diet was replaced by pure soybean meal on every second feeding occasion for 7 days (see below). The soybean meal was solvent-extracted, heat-treated, non-GM with soybean meal on every second feeding occasion for 7 days (see below).

Influence of age, Brachyspira hyodysenteriae strain and route of administration. Ten animals (two slaughter pigs, four growers and four weaners) were included in this part of the study. All animals were fed the commercial finisher diet. The slaughter pigs were housed individually and inoculated with Brachyspira hyodysenteriae strain B204 (see below) through a caecal cannula (Jacobson et al., 2001). The growers and the weaners were group-housed according to age. One grower and one weaner were given strain B204, and one grower and one weaner were given a field isolate (see below) by gavage. One grower and one weaner were given strain B204 orally with a syringe, and one grower and one weaner were given a field isolate in the same way. All pigs were inoculated on 2 consecutive days.

Influence of a provocative feeding regime, administration of corticosteroids and administration of antacids. This part of the study comprised 27 grower pigs. Four of these had previously been included in the part of the study described above and were now kept two pigs in each pen, fed the experimental diet and inoculated a second time 40 days later. One pig in each pen was given strain B204 and one pig per pen was given the field isolate as previously described.

Eleven growers from the conventional herd were divided into four groups and inoculated with strain B204. One group (n = 3) was fed the experimental diet until the last day of challenge. The other groups were fed the commercial diet. One group (n = 3) was given an antacid orally (2200 mg, alumin. hydroxid. magnesii carb. gel) 15 min prior to inoculation and one group (n = 3) was given dexamethasone i.m. (0-15 mg kg⁻¹, Dexafort vet) 30 min before inoculation. The fourth group (n = 2) served as non-inoculated controls and were given sterile broth. All pigs were inoculated per os by a syringe on 3 consecutive days and, except those given antacids, were fasted in the morning prior to inoculation at 10:00.

The study was repeated in 12 additional growers, divided into four groups with three pigs in each. One group was fed the experimental diet as described above. The control group was replaced by three pigs from the University farm, given the experimental diet. Two groups were fed the commercial diet. One group was given dexamethasone on 5 consecutive days, starting 2 days prior to inoculation, and one group was given antacids before inoculation, as described above. All pigs were challenged orally as described above. Four pigs had profuse watery diarrhoea on arrival but no causative organism was identified and three of them had recovered at inoculation 5 days later.

Effects of housing and administration of other bacteria. Fourteen growers were housed individually, fed the experimental diet and challenged per os on 3 consecutive days with Brachyspira hyodysenteriae strain B204. Four of these were inoculated a second time 3 weeks later, with a reisolate of Brachyspira hyodysenteriae from a pig previously inoculated with strain B204. On this second occasion, the four pigs were circulated between the pens four times daily together with one pig that was given Bacteroides vulgatus (see below). In addition to the challenge with Brachyspira hyodysenteriae, three pigs were exposed to broth containing E. coli O141, which was poured on the pen floor (Melin et al., 2003) 2 days prior to inoculation. Three pigs were given 40 ml of a mixture of a field strain of Bacteroides species, Bacteroides fragilis and Fusobacterium necrophorum, partly given orally and partly poured on the floor. Four pigs were given 30 ml Bacteroides vulgatus orally through a syringe the day before inoculation, and one of these pigs was circulated between the pens (see above).

Bacterial inocula. The strains used were: Bacteroides fragilis ATCC 25285; Bacteroides vulgatus CCUG 36807; an untyped field isolate of Bacteroides spp.; Brachyspira hyodysenteriae B204 (GenBank accession no. U14932); Brachyspira hyodysenteriae field isolate AN 2857:99; E. coli O141 field isolate Bd 2027/75 (K85, STB, VT2); and F. necrophorum CCUG 9994. The infective doses of Brachyspira hyodysenteriae given to each pig ranged from 10³ to 10⁴ organisms ml⁻¹, and the dose of E. coli was 2 x 10⁶ cells cm⁻² floor area (Melin et al., 2003). Brachyspira hyodysenteriae strain B204 was grown on fastidious anaerobe agar for 2 days and in brain heart infusion broth at 37 °C for 2 days to exponential phase. The strain had been passaged two to four times before inoculation. Each culture was checked for motility before inoculation. On each occasion, the spirochaetes were spiral-shaped with active motility. Bacteroides spp., F. necrophorum and E. coli were grown in brain heart infusion broth at 37 °C for 18 h to 2 days. All cultures were analysed by phase-contrast microscopy for purity and growth prior to inoculation.

Analyses and observations. A clinical health examination was performed daily. The pigs’ general condition and appetite and the consistency and colour of their faeces were recorded. Dysentery was defined as the occurrence of mucous or haemorrhagic diarrhoea connected with shedding of Brachyspira hyodysenteriae.

Faecal samples were collected prior to the start of the study and daily from the first day of inoculation, and were analysed for the presence of Brachyspira spp. (Fellström & Gunnarsson, 1995). Faecal samples were
also collected once a week and analysed for the diversity of the coliform flora (Kühn et al., 1993). In addition, faecal samples (~50 g) from nine pigs were analysed for microflora-associated characteristics (MACs) as described by Midtvedt (1999). Eight of these pigs had developed swine dysentery; six pigs were penned in groups and fed soybean meal and two pigs were individually penned and exposed to an additional bacterial flora. One pig given an additional bacterial flora had not developed dysentery. Samples were collected prior to the start of the study and at euthanasia, and an additional sample was taken prior to inoculation in four of these pigs. The MACs included the microbial production of short-chain fatty acids, inactivation of trypsin, conversion of cholesterol to coprostanol, degradation of mucin and conversion of bilirubin to urobilinogen.

Blood samples were collected by jugular vein puncture into vacuum tubes without additives. In 19 pigs (four weaners and four growers in the first part of the study and the first 11 growers in the second part of the study), the samples were collected prior to the start of the study and on days 0, 1, 2, 3, 4, 5, 7, 10 and 14 after inoculation. The control pigs were sampled on the same occasions. On the basis of the results of the analyses of these samples, the sampling schedule was modified in a further 18 pigs (the remaining pigs from the second part of the study and in six pigs from the third part). In the latter pigs, blood was collected at the first sign of diarrhoea, 12 h later, when haemorrhagic diarrhoea occurred and once daily thereafter until the day of euthanasia. In pigs that remained healthy, samples were taken before challenge and at euthanasia. Sera were stored at −20 °C prior to analyses.

Serum samples were analysed for SAA and haptoglobin (Tridelta Phase range SAA kit), and for cortisol (Coat-A-Count; Diagnostic Products), as described previously (Jacobson et al., 2001).

Blood samples for total and differential leucocyte counts were collected into vacuum tubes with EDTA from the pigs according to the modified sampling schedule (see above). Samples were collected before challenge, in two cases also during the disease and at euthanasia. The samples were analysed immediately according to standard protocols (Cell-Dyn 3500).

Necropsy. Depending on the clinical signs and paying consideration to animal welfare aspects, dysenteric pigs (n = 22) were euthanized 1–22 days after the first sign of diarrhoea. Healthy pigs (n = 25) were euthanized 30 days after inoculation. Immediately after euthanasia, necropsy was performed on 32 inoculated pigs and two non-inoculated controls and the gastrointestinal tract was examined histopathologically. In the remaining healthy pigs, the carcasses and intestines were examined macroscopically.

Statistics. Statistical analyses were performed by the Mann–Whitney rank sum test (SigmaStat statistical software; SPSS Science). Statistical significance was considered if $P < 0.05$.

RESULTS

Influence of age, Brachyspira hyodysenteriae strain and route of administration

Irrespective of the bacterial strain, all four weaners started to shed Brachyspira hyodysenteriae 2 days after inoculation and developed mucous diarrhoea 5–10 days after inoculation. The older animals (growers and slaughter pigs) all remained healthy irrespective of the bacterial strain or route of administration used (Table 1).

<table>
<thead>
<tr>
<th>Factors tested</th>
<th>Incidence of dysentery (disease/total)</th>
<th>Culture-positive (positive/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/weaners (6 weeks old)</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>Age/growers (10 weeks old)</td>
<td>0/4</td>
<td>2/4</td>
</tr>
<tr>
<td>Age/slaughter pigs* (20 weeks old)</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Additional bacterial flora, growers*</td>
<td>3/10</td>
<td>4/10</td>
</tr>
<tr>
<td>Antacids, growers</td>
<td>1/6</td>
<td>4/6</td>
</tr>
<tr>
<td>Dexamethasone, growers</td>
<td>1/6</td>
<td>2/6</td>
</tr>
<tr>
<td>Soya, group-housed pigs, growers</td>
<td>9/9</td>
<td>9/9</td>
</tr>
<tr>
<td>Soya, limited contact, growers†</td>
<td>5/9</td>
<td>7/9</td>
</tr>
<tr>
<td>Soya, single pen, growers*</td>
<td>0/4</td>
<td>0/4</td>
</tr>
</tbody>
</table>

*Pigs were housed in single pens.
†Four pigs had previously been included in the group ‘age/growers’ and four pigs had previously been included in the group ‘soya, single pen, growers’, without developing clinical signs of the disease or shedding Brachyspira hyodysenteriae. One pig was also included in the group ‘additional bacterial flora, growers’.

Table 1. Impact of various factors on the incidence of dysentery, following the experimental inoculation of 45 pigs with Brachyspira hyodysenteriae

Unless otherwise indicated, all pigs were kept in groups of three or four per pen, and the growers were 12–15 weeks old. Additional bacterial floras consisted of either Bacteroides vulgatus, a mixture of Bacteroides species, Bacteroides fragilis and F. necrophorum or E. coli. Control pigs are not shown.
Influence of a provocative feeding regime, administration of corticosteroids and administration of antacids

All nine group-housed pigs that were inoculated the first time and fed soybean meal, developed dysentery (Table 1). In addition, two of four pigs (one pig in each pen, one inoculated with B204 and one with the field isolate) developed dysentery when they were fed soybean meal and inoculated a second time. The first sign of disease was noted 5–15 days after inoculation and, in nine of 13 pigs (one of the growing pigs inoculated a second time, one pig given antacids and seven pigs fed soybean meal), the diarrhoea became haemorrhagic 0–6 days later. The general condition was only slightly altered. One pig given dexamethasone for 5 days developed non-haemorrhagic diarrhoea 26 days after challenge with \textit{Brachyspira hyodysenteriae}. One pig from the first group given antacids developed haemorrhagic diarrhoea 12 days after inoculation. The other pigs remained healthy throughout the study.

Effect of housing and administration of other bacteria

None of the 14 pigs housed individually and given soybean meal developed dysentery unless other measures were taken (Table 1). When five still healthy pigs were circulated between the pens, three of these developed dysentery. Of ten pigs given additional bacteria, three pigs developed dysentery, one of which had received \textit{E. coli}, one the \textit{Bacteroides–Fusobacterium} mixture and one \textit{Bacteroides vulgatus} (Table 1).

Analyses and observations

Prior to the start of the study, all rectal swabs were negative with respect to \textit{Brachyspira hyodysenteriae}, but \textit{Brachyspira innocens} or \textit{Brachyspira murochii} was isolated in nine of 47 pigs. After inoculation, \textit{Brachyspira hyodysenteriae} was isolated in all animals with clinical signs of dysentery (Table 1). The first isolation was made 1–26 days post-inoculation, corresponding to the period from 14 days before to 2 days after the onset of diarrhoea. In 12 of 53 inoculations, the bacterium was isolated within 2 days of administration, and 8 of these 12 inoculations resulted in dysentery. In addition, the bacterium was repeatedly isolated in four pigs that never developed diarrhoea (one pig fed soybean meal and inoculated a second time, one pig given dexamethasone and two pigs given antacids). One pig that had diarrhoea on arrival was treated with a single dose of tylosine i.m. (Tylan vet) 12 days later, i.e. 5 days after the experimental inoculation. The pig had firm faeces the next day but started to shed \textit{Brachyspira hyodysenteriae} 2 days after treatment.

At the initial faecal sampling, the mean diversity of the coliform flora was 0.45 ± 0.29 (range 0.08–0.93). In the pigs with haemorrhagic dysentery, the mean diversity was 0.39 ± 0.31 (range 0.02–0.78). The corresponding value for the healthy pigs was 0.46 ± 0.30 (range 0.09–0.90). There were no statistically significant differences between the groups.

The mean microbial production of short-chain fatty acids was 175 ± 54 mg (kg faeces)$^{-1}$ at the initial sampling. After 4 days of feeding with soya, the corresponding value was 143 ± 42 mg (kg faeces)$^{-1}$ and, on the day of necropsy, it was 164 ± 50 mg (kg faeces)$^{-1}$. These differences were not significant. The mean microbial conversion of bilirubin to urobilinogen was 0.08 ± 0.03 mmol kg$^{-1}$ at the initial sampling and 0.04 ± 0.01 mmol kg$^{-1}$ at euthanasia 1–5 days after the development of dysentery ($P < 0.001$). The mean tryptic activity was 8.4 ± 11.6 mg kg$^{-1}$ at the initial sampling and 4.5 ± 8.5 mg kg$^{-1}$ at euthanasia ($P < 0.05$). Microbial conversion of cholesterol was 60.7 ± 5.0 % at the initial sampling and 63.5 ± 8.8 % after feeding with soybean meal ($P < 0.05$). No other statistically significant differences were found.

The patterns of SAA and haptoglobin are illustrated in Figs 1 and 2. At the initial sampling, in the clinically healthy pigs, the SAA values ranged from 0 to 20 mg l$^{-1}$ and the haptoglobin values from 0.27 to 3.9 g l$^{-1}$. A significant increase compared with pre-challenge values in both SAA and haptoglobin was noted when haemorrhagic diarrhoea occurred, ranging from 0 to 5000 mg l$^{-1}$ ($P < 0.01$) and from 1·9 to 6.2 g l$^{-1}$ ($P < 0.001$), respectively. On arrival, two of four pigs with profuse, watery diarrhoea had elevated APP levels (mean 459 mg SAA l$^{-1}$ and 4.2 g haptoglobin l$^{-1}$) at the

![Fig. 1. Serum concentrations (mean ± SEM) of the acute-phase proteins SAA and haptoglobin in 22 pigs that developed haemorrhagic dysentery after an experimental inoculation with \textit{Brachyspira hyodysenteriae}. Samples were collected prior to the start of the study (sample 0), at the first sign of diarrhoea (1), 12 h later (2), when haemorrhagic diarrhoea occurred (3) and once daily thereafter (4) until the day of euthanasia (5).](image-url)
After experimental inoculation with *Brachyspira hyodysenteriae*, with proteins SAA and haptoglobin in 12 pigs during the first 14 days after inoculation with *Brachyspira hyodysenteriae*, with the first day of inoculation designated day 0. Pigs were inoculated on 3 consecutive days. Three pigs (filled bars) were given dexamethasone i.m. prior to inoculation and nine pigs (open bars) were not. All pigs were clinically healthy. The cortisol values recorded at the initial sampling ranged from 99 to 347 nmol l\(^{-1}\) in weaners (mean 249 ± 106 nmol l\(^{-1}\)) and from 3 to 199 nmol l\(^{-1}\) (88 ± 51 nmol l\(^{-1}\)) in growers. In the pigs with non-haemorrhagic dysentery, the values varied from 11 to 199 nmol l\(^{-1}\) (81 ± 56 nmol l\(^{-1}\)) and, in those with haemorrhagic dysentery, from 7 to 460 nmol l\(^{-1}\) (151 ± 150 nmol l\(^{-1}\)); these differences were not statistically significant. The total and differential leucocyte counts are shown in Table 2. In one pig, an increase was seen 1 day prior to the development of haemorrhagic dysentery in the neutrophil (11.3 \(\times\) 10\(^9\) cells l\(^{-1}\)) and monocyte (4.4 \(\times\) 10\(^9\) cells l\(^{-1}\)) counts, compared with the pre-inoculation values.

Necropsy

Twenty of 22 pigs with clinical dysentery had gross and microscopic lesions in the large intestine, centred on the spiral colon. Eighteen pigs showed lesions compatible with swine dysentery. In addition, two pigs had lesions consistent with porcine proliferative enteropathy, which made it difficult to interpret lesions related to *Brachyspira hyodysenteriae*. The predominant gross change in most pigs was moderately to markedly increased mucous secretion. However, superficial necrotic foci with pseudomembranes were also prominent in many animals. Histological changes in the large bowel mucosa were usually characterized by mucous hypersecretion and crypt hyperplasia, and superficial mucosal necrosis and erosions with fibrinocellular exudate were observed in many pigs. In non-inoculated controls and inoculated pigs that had not developed clinical dysentery (n = 12), the morphological findings were normal. The remaining pigs (n = 13) were macroscopically normal.

**Table 2. Total and differential leucocyte counts in 19 pigs experimentally inoculated with *Brachyspira hyodysenteriae***

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Cell count (\times 10^9) cells l(^{-1})</th>
<th>Before</th>
<th>At euthanasia</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dysentery (n = 11)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>17.6 ± 4.6</td>
<td>26.0 ± 5.4<em>a</em></td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>7.2 ± 2.6</td>
<td>13.5 ± 3.5<em>b</em></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>8.1 ± 3.9</td>
<td>8.2 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>2.1 ± 1.0</td>
<td>4.0 ± 1.8<em>a</em></td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.2</td>
<td></td>
</tr>
<tr>
<td><strong>Healthy (n = 8)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>17.9 ± 5.1</td>
<td>18.8 ± 5.5</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>6.6 ± 1.8</td>
<td>8.9 ± 4.5</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>9.6 ± 5.0</td>
<td>8.0 ± 3.0</td>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
<td>1.7 ± 0.3</td>
<td>1.8 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.1 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td></td>
</tr>
</tbody>
</table>

*aStatistically significantly different from day 0: a, P < 0.01; b, P < 0.001.*

**DISCUSSION**

The present results underscore the multifactorial causality of infection with *Brachyspira hyodysenteriae*. Large quantities of soybean meal fed to group-housed pigs repeatedly predisposed to dysentery, whereas pigs fed soybean meal but kept in single pens all remained healthy. Furthermore, half of the pigs fed soya but with limited contact between them developed disease. Blaha *et al.* (1984) reported a success rate of 20% in animals kept in single pens and inoculated once. In the present study, among pigs concomitantly exposed to other bacteria, a measure that has previously been shown to predispose to dysentery or induce post-weaning diarrhoea (Harris *et al.*, 1978; Melin *et al.*, 2003), disease occurred in only about 30%. In addition, some pigs seemed to harbour *Brachyspira hyodysenteriae* in the intestine for several weeks without clinical signs or shedding of bacteria, as also observed previously (Kinyon *et al.*, 1980; Albassam *et al.*, 1985). In the present study, the use of isolates from animals with haemorrhagic dysentery did not reproduce the disease. In the study by Blaha *et al.* (1984), three in vivo passages of dysenteric colonic material were required for induction. In other studies, however, 60–100% of the pigs developed...
dysentery after one inoculation with low-passage strains (Whipp et al., 1979; Moreng et al., 1980). Neither the route of administration nor administration of antacids seemed to influence the onset of dysentery in the present study.

All pigs inoculated at weaning developed mucous dysentery. The stress caused by the environmental, social and dietary changes is thought to predispose to post-weaning diarrhoea (Nabuurs et al., 1993; Melin et al., 2003). However, weaning is not considered to predispose to dysentery, and repeated injections of the stress hormone dexamethasone in growers did not provoke clinical disease. The present result might possibly be explained by the abrupt dietary changes, which might cause a disturbance in the gut flora (Meyer et al., 1998). Furthermore, one pig started to shed the bacteria after antibiotic treatment.

Composition of feed has previously been shown to influence the course of dysentery (Durmic et al., 1998). In many countries, pig feed is based on corn or barley, with the addition of 20–30 % soybean meal (Nabuurs, 1986; Dewey, 1993; Neef et al., 1994b; Jensen et al., 1998), whereas the diet used in the present study was based on cereals, containing less than 2 % soybean meal. Studies on soya-associated diarrhoea have mainly focused on the small intestine (Li et al., 1991; Dréau et al., 1995) and few studies have addressed the large intestine (Neef et al., 1994b). The soybean meal used in the present study contained hulls composed of non-starch polysaccharides. It is proposed that non-starch polysaccharides may increase microbial fermentation and production of volatile fatty acids (VFA) in the large intestine, thereby predisposing to dysentery (Siba et al., 1996). However, reports regarding the fermentation rates of hemicellulose and cellulose and subsequent VFA production in the large intestine differ (Pluske et al., 1996; Durmic et al., 1998; Canh et al., 1998). In the present study, no changes in VFA production were seen during the experiments. Certain soybean proteins (glycinin and β-conglycinin) might also induce immunological reactions in the small intestine (Li et al., 1991; Dréau et al., 1994), and antinutritional factors such as trypsin inhibitors, phytates and lectins are considered to induce diarrhoea (Huisman & Jansman, 1991). However, these substances should be destroyed by the ethanol extraction and heat treatment during processing (Dréau et al., 1994; Reddy & Pierson, 1994), with the possible exception of heat-treated lectins (Huisman & Jansman, 1991; Reddy & Pierson, 1994). Also, osmotic mechanisms might cause diarrhoea (Makinde et al., 1996). In addition, it has been suggested that a predisposing diet containing 40 % soya may induce proliferation of certain attaching and effacing E. coli strains, probably by altering the balance of the commensal microflora in the large intestine. It is proposed that these strains may play a role in the pathogenesis of colitis and, together with other pathogenic agents, cause clinical diarrhoea (Neef et al., 1994b).

Some differences were noted in the other microbial activity characteristics measured. In response to feeding with soya, the microbial conversion of cholesterol to coprosterol increased. However, this was measured in only four pigs. The bacteria responsible for this conversion probably belong to the genus *Eubacterium*. Furthermore, the microbial conversion of bilirubin to urobilinogen and the microbial inactivation of trypsin decreased when dysentery had developed. The conversion of bilirubin is probably mainly achieved by *Clostridium ramosum*. Faecal trypsic activity is the net sum of the secretion of trypsinogen from the pancreas, activation of trypsinogen to trypsin and inactivation of trypsin by compounds derived from the diet and certain microbes such as *Bacteroides distasonis* (Midtvedt, 1999). The number of samples analysed was too small to allow any extensive conclusions to be drawn, but the results suggest that a change in the microbial activities occurs, which might indicate an alteration in the microbial flora of the large intestine.

Among the pigs of the present study, haptoglobin and SAA generally increased several-fold in response to haemorrhagic dysentery. In contrast, non-haemorrhagic dysentery was not associated with any acute-phase response (Fig. 1). Overall, the APP response to other enteric diseases in pigs is not known. In two cases, only haptoglobin increased (to 2.66 and 4.05 g l⁻¹), whereas SAA remained at baseline levels. Induction of the APP response is complex and is modulated by several cytokines (Mackiewicz et al., 1991; Jiang et al., 1995). For example, in cell lines SAA is induced by IL1, with a synergistic action of IL6, whereas IL6 alone is a poor inducer of SAA (Uhlar & Whitehead, 1999). On the other hand, haptoglobin is primarily induced by IL6 (Mackiewicz et al., 1991; Jiang et al., 1995). However, the cytokine pattern was not investigated in the present study. Two of nine pigs with non-haemorrhagic dysentery showed a slight increase in one or both APP (haptoglobin to 2.83 and 4.3 g l⁻¹ and SAA to 42.6 and 0 mg l⁻¹, respectively), one of which had necrotic lesions in the large intestine. Among eight pigs with necrotic lesions at necropsy, seven showed increased APPs and six had increased cortisol values, although there were large individual variations. Glucocorticoids play a complex role in the inflammatory response. They are upregulated by several cytokines and enhance the induction of APP synthesis, but also exert a negative feedback on cytokine production. Interestingly, a non-significant increase in glucocorticoids in response to haemorrhagic dysentery was seen in the present study. Dexamethasone increases haptoglobin and SAA levels in several species (Higuchi et al., 1994; Uhlar & Whitehead, 1999). The response in swine is unknown, but, in our three pigs given dexamethasone, an increase in both APPs was noted 1–2 days after injection (Fig. 2). This increase was smaller than that caused by haemorrhagic dysentery.

In the present study, the total white blood cell count increased and there was a relative rise in monocytes and neutrophils. Large lymphocytes and monocytes could sometimes be difficult to differentiate, but an increased recruitment of phagocytic cells could be expected in cases of tissue damage. In some previous studies, no increase in total leucocytes counts has been found (Olson 1974; Neef et al.,

CONCLUSION

IN THE PRESENT STUDY, FEED CONTAINING LARGE QUANTITIES OF SOYBEAN MEAL AND GROUP-HOUSING OF THE PIGS WERE MAJOR CONTRIBUTORY FACTORS IN EXPERIMENTAL INDUCTION OF SWINE DYSENTERY. THIS CONFIRMS THE MULTIFACTORIAL NATURE OF THE DISEASE. SIGNIFICANT, SEVERAL-FOLD INCREASES IN THE ACUTE-PHASE PROTEINS SAA AND HAPTOGLOBIN WERE NOTED IN RESPONSE TO HAEMORRHAGIC, BUT NOT TO NON-HAEMORRHAGIC DYSENTERY. IN ADDITION, RELATIVE INCREASES IN MONOCYTES AND NEUTROPHILS AND SIGNIFICANT ALTERATIONS IN THREE MICROFLORA-ASSOCIATED CHARACTERISTICS IN THE FEACES WERE SEEN.

ACKNOWLEDGEMENTS

THIS STUDY WAS FINANCED BY GRANTS FROM THE SWEDISH RESEARCH COUNCIL FOR ENVIRONMENT, AGRICULTURAL SCIENCES AND SPATIAL PLANNING. WE WISH TO EXPRESS OUR GRATITUDE TO MR. FELIKE LINDBERG, WHO WILLINGLY AND PROMPTLY SUPPORTED US WITH ANIMALS, AND TO DR. EJE COLLINDER, WHO ENTHUSIASTICALLY INITIATED THE STUDIES OF MACS.

REFERENCES


