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DIARRHEA IN ONE TO THREE WEEK-OLD PIGLETS ASSOCIATED WITH CLOSTRIDIUM PERFRINGENS TYPE A.

M.J.A. NABUURS, J. HAAGSMA, E.J. v.d. MOLEN and Ph. J. v.d. HEIJDEN

Centraal Diergeneeskundig Instituut, Edelhertweg 15, 8219 PH Lelystad, The Netherlands

Abstract

Investigation on the aetiology of diarrhoea in piglets of one to three weeks of age revealed high numbers of Clostridium perfringens type A in intestinal contents of severely affected animals. Experimental infections with hysterectomy derived, colostrum deprived piglets performed in an isolator resulted in a clinical picture indistinguishable from the clinical signs observed under field conditions i.e. creamy diarrhoea, emaciation, abundant gas in the gut but usually low mortality rate. The predominant post-mortem findings were the presence of gas in the intestinal lumen and in the mucosa, superficial necrosis and villus atrophy.

Steatorrhoea, one to three weeks old scours, white scours, is a disease frequently affecting piglets in The Netherlands. The syndrome is clinically characterized by a diarrhea with white or yellow faeces of a consistency which varies from pasty to sometimes milky. The faeces has a high fat content (Mouwen et al., 1972). The disease is microscopically characterized by an atrophy of the villi of the small intestinal mucosa (Mouwen and Schotman, 1972).

Steatorrhoea causes a high morbidity on affected farms with a low mortality rate; the losses being mainly the result of wasting. Some authors suggest that there is an association between steatorrhoea and rotavirus (De Leeuw et al., 1979; Bohl, 1979), or between steatorrhoea and coccidiosis (Coussemant et al., 1981). This paper reports the results of:

- an investigation about the association of steatorrhoea and rotavirus under field conditions,
- an investigation about the association of steatorrhoea and Clostridium perfringens type A (CPA) in the absence of rotavirus,
- an experimental infection with Clostridium perfringens type A.

Materials and Methods

Farm

The relation of steatorrhoea and rotavirus was investigated on a commercial farm with a long history of white scours. On that farm were 150 sows and no fattening pigs.

Bacteriological and virological examinations

Rectal swabs and faecal samples were collected daily from all piglets of one litter during eight weeks.

Rectal swabs were, after collection, stored at 4 °C. They were transported three times a week to our institute for routine bacteriological examination; faecal samples were frozen at -20 °C until the day of examination.

Bacteriological examination was directed against E. coli and other gram negative pathogens.

Virological examination for rotavirus was carried out on ELISA®.

CPA in faeces was detected using a tryptose-sulfite-dextrose cycloserin agar (TSDC).

Bacterial counts were made by testing ten fold dilutions on TSDC. Three to five black colonies from the highest dilution were examined for purity. Toxicity and type of toxins were examined by a neutralisation test in mice and a blocking test on an egg yolk medium.

Diarrhea was scored using the following scale based on colour and consistency of the faeces (table 1).

Post mortem examination

Living animals were euthanised. Gut tissue samples were fixed in a 10% buffered formalin...
solution and embedded in paraplast. Slides (5 μm) were stained with haematoxylin-eosin.

**Experimental infection**

Four piglets were obtained by hysterectomy and kept under sterile conditions. They were immediately transported to the institute. The piglets were fed a commercial coffee milk (containing 7% of proteins; 8.2% fat and 10% of carbohydrate).

An infection with CPA was carried out four days after the hysterectomy. Diarrhea was scored in the same way as in the field investigation. Post mortem examination was performed seven days after the experimental infection.

**Results**

The results of the investigation on the commercial farm are given in figure 1. The lack of correlation between diarrhea score and excretion of rotavirus from day 8 until day 20 is striking, even more so the lack of correlation between the high rotavirus titres and low diarrhea score between day 24 and day 42. In figure 2 we have especially focused our attention on the period between day 8 and day 20 and presented the number of piglets with diarrhea without rotavirus excretion and the piglets with diarrhea and a positive rotavirus excretion in the faeces.

Pathogenic *E. coli* O141: K85 a, c were present in all the piglets with diarrhea between day 29 and day 34 and after weaning between day 44 and day 55. There also remains a period of 12 days with diarrhea in which no known aetiologic agent was isolated.

In the course of the investigation we examined some piglets from other litters on the same farm suffering from white scours and found abundant numbers of gram positive rods in the ileal and rectal contents. Therefore we extended our investigation on the commercial farm during one year with special attention on the diarrhea peak between day 8 and day 20. All piglets that were severely affected by diarrhea, or which died after a period of diarrhea were transported to the institute for examination.

<table>
<thead>
<tr>
<th>Colour</th>
<th>Consistency</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brown</td>
<td>normal</td>
<td>0</td>
</tr>
<tr>
<td>Yellow</td>
<td>normal</td>
<td>1</td>
</tr>
<tr>
<td>Yellow</td>
<td>pasty</td>
<td>2</td>
</tr>
<tr>
<td>Yellow-white</td>
<td>medium-liquid</td>
<td>3</td>
</tr>
<tr>
<td>Yellow-white</td>
<td>milky</td>
<td>4</td>
</tr>
<tr>
<td>Yellow-white</td>
<td>watery</td>
<td>5</td>
</tr>
</tbody>
</table>

**Table 1.**—Diarrhea score according to colour and consistency of the faeces.

In the course of the investigation we examined some piglets from other litters on the same farm suffering from white scours and found abundant numbers of gram positive rods in the ileal and rectal contents. Therefore we extended our investigation on the commercial farm during one year with special attention on the diarrhea peak between day 8 and day 20. All piglets that were severely affected by diarrhea, or which died after a period of diarrhea were transported to the institute for examination.

**Fig. 1.**—Diarrhea score and rotavirus excretion in a litter of piglets.
Twenty eight piglets were tested for the presence of pathogenic E. coli and *Clostridium perfringens*. Isolates were typed according to standard methods. Samples from different parts of the gut especially the ileum and the colon were examined. Pathogenic *E. coli* was not found, but *Clostridium perfringens* type A in 18 piglets; *Clostridium perfringens* type C in 4 piglets; *Clostridium perfringens* type D in 1 piglet. The following counts were scored in table 2.

<table>
<thead>
<tr>
<th>Number of piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td>10¹</td>
</tr>
<tr>
<td>Clostridium perfringens type A</td>
</tr>
<tr>
<td>Clostridium perfringens type C</td>
</tr>
<tr>
<td>Clostridium perfringens type D</td>
</tr>
</tbody>
</table>

Table 3. — Changes in faeces occurring after the experimental infection. Yellow pasty/milky faeces was present.

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Number of piglets</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 4. — Post mortem findings after the experimental infection.

- villus oedema in the duodenum
- inflammation of the serosal part of the colon with gas flegmonous character
- hydriotopic degeneration in the epithelium of the villus tip
- abundant gas in the lumen of the gut of the small as well as the large intestine
- intramural gas in the colon
- focal necrosis of the colon mucosa
- no coccidiosis
- no villus atrophy

**Fig. 2.** — Numbers of piglets with diarrhea in one litter related to rotavirus excretion between 8 and 22 days after birth. Diarrhea score in number of piglets with (---) and without (—) rotavirus in faecal samples.
In general post-mortem examinations revealed emaciated carcasses; the small intestine was atonic; a necrotizing enteritis was found in jejunum, ileum or colon; there was abundant gas in the gut lumen; and sometimes a peritonitis had developed from a gaseous phlegmon. Histologically the villus atrophy was obvious. To substantiate the significance of CPA for young piglets we carried out an experimental infection using four hysterectomy derived, colostrum deprived piglets. The infection was performed on day four after hysterectomy with 2 ml of a whole culture containing \( 6 \times 10^8 \) Clostridium perfringens type A /ml. Diarrhea scores are given in table 3. The histological findings were in agreement with the clinical symptoms as shown in table 4. Table 5 presents isolations of Clostridium perfringens type A.

In a period of three months we have examined five other farms for the presence of CPA in white scours. All the piglets were positive, that means CPA was present in a quantity of more than \( 10^3 / \)gram in the ileal content.

**Discussion**

It seems that white scours are not always associated with rotavirus infection nor with a pathogenic E. coli or coccidiosis.

However CPA has been isolated frequently from piglets suffering from white scours. It seems plausible that CPA can induce in HDCD piglets a clinical picture indistinguishable from the white scours syndrome under field conditions.

The significance of CPA for the aetiology of white scours is not elucidated, nor if there is interference between rotavirus and CPA infections. From these results it seems apparent that CPA is preceded by a rotavirus infection.

It may be possible that villus atrophy induced by rotavirus will predispose CPA adhesion.

In order to know more about the possible pathogenicity of CPA in white scours it will be necessary to determine free CPA toxins in the gut content.

**References**


**Question**

From Dr. Ducluzeau to Dr. Nabuurs

Did you check the healthy piglets on your farm for the presence of *C. perfringens* in faeces?

**Answer**

On the farm where our investigation was performed, we find CPA in healthy piglets between one and three weeks. On other farms, CPA was not detectable in healthy piglets.

**Question**

From Dr. Larvor to Dr. Nabuurs

In your second slide you presented parallel data on diarrhoea score in piglets and rotavirus score. There seems to be not only a lack of correlation, but may be a negative correlation. Could you comment on this?

**Answer**

Clostridium perfringens type A can produce an enzyme «neuraminidase». Neuraminidase destroys ganglioside which is a receptor for Rota adherence. Therefore it may be possible that CPA neuraminidase actively prevents the pathogenic effects of rotavirus.