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

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

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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 diarrhœa, 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.

Steatorrhœa, 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).

Steatorrhœa 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 steatorrhœa and rotavirus (De Leeuw *et al.*, 1979; Bohl, 1979), or between steatorrhœa and coccidiosis (Coussemant *et al.*, 1981). This paper reports the results of:

— an investigation about the association of steatorrhœa and rotavirus under field conditions,

— an investigation about the association of steatorrhœa 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 steatorrhœa 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[6].

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 rota 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 rota excretion and the piglets with diarrhea and a positive rota excretion in the faeces.

Pathogenic *E. coli*'s 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 1. — Diarrhea score according to colour and consistency of the faeces.

Colour	Consistency	Points
Brown	normal	0
Yellow	normal	1
Yellow	pasty	2
Yellow-white	medium-liquid	3
Yellow-white	milky	4
Yellow-white	watery	5

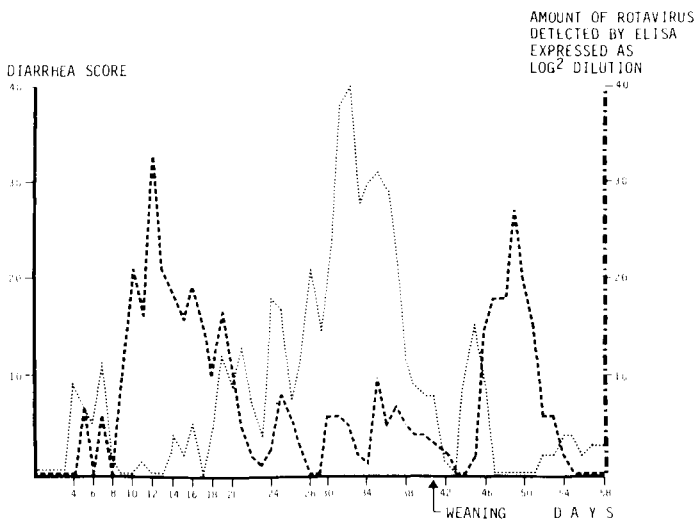

Fig. 1.— Diarrhea score and rotavirus excretion in a litter of piglets.

Table 2. — Concentration of *Clostridium perfringens* per piglet.

	Dilution								
	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
Clostridium perfringens type A Number of piglets	1	0	3	3	3	5	12	0	1
Clostridium perfringens type C Number of piglets	...	...	...	...	1	1	2	...	...
Clostridium perfringens type D Number of piglets	...	...	...	...	1	...	...	...	...

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

Days after infection	Number of piglets
2	1
3	1
4	2

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 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
hydropic 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

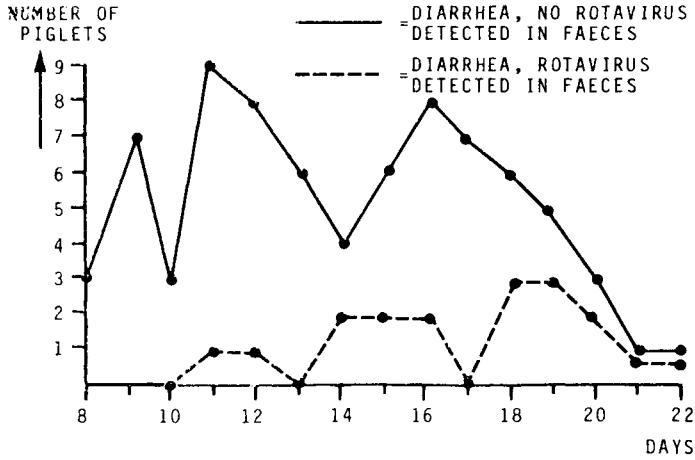

Fig. 2.— Numbers of piglets with diarrhea in one litter related to rotavirus excretion between 8 and 22 days after birth. Diarrhea score in number of piglets with (---) and without (—) rotavirus in faecal samples.

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 6×10^9 *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.

Table 5. — Number of *Clostridium perfringens* type A per gramme of feces seven days after the experimental infection.

Animals	Number of *Clostridium perfringens* type A
Pig no. 1	8×10^7
Pig no. 2	6.4×10^7
Pig no. 3	4.8×10^7
Pig no. 4	0.4×10^7

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 preceeded 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 f CPA in white scours it will be necessary to determine free CPA toxins in the gut content.

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References

BOHL E.H., 1979. Rotaviral diarrhea in pigs: brief review. *J. Am. Vet. Med. Assoc.*, **174**, 613-615.

COUSSEMANT W., HOORENS A.M., DUCATELLE R., BERGHEN P., GEERAERTS J., 1981. Diarrhee bij zuigende biggen geassocieerd met *Eimeria neodebliecki. Vlaam. Diergeneeskd. Tijdschr.*, **50**, 384-395.

ELLENS D.J., DE LEEUW P.W., 1977. Enzyme-linked immunosorbent assay for diagnosis of rotavirus infections in calves. *J. Clin. Microbiol.*, **6**, 530-532.

MOUWEN J.M.V.M., SCHOTMAN A.J.H., 1970. Steatorrhœa in piglets. *Vet. Rec.*, **87**, 172-173.

MOUWEN J.M.V.M., SCHOTMAN A.J.H., WENSING TH., KIJKUIT C.J., 1972. Some biochemical aspects of white scours in piglets. *Tijdschr. Diergeneesk.*, **97**, 65-90.

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 diarrhœa 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 rotarivus.