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CERTAIN ENTEROPATHOGENS IN CALVES OF FINNISH DAIRY HERDS WITH RECURRENT OUTBREAKS OF DIARRHEA

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ABSTRACT

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The occurrence and distribution of certain enteropathogenic organisms and the manifestation of diarrhea, especially in relation to colostral management, were studied in neonatal calves of eight southern Finnish dairy herds with recurrent outbreaks of enteric disturbances. The fecal samples were obtained from the calves and were provided by the farmers. Potentially pathogenic agents were demonstrated in 52 of the 68 calves studied. *Cryptosporidium* was found in 36 of them while rotavirus was found in 29 and *Escherichia coli* in 15 of them, either singly or in combination. *Salmonella*, coronavirus or parvovirus were not detected in any of the calves. Twenty-six of the 36 calves contracted their *Cryptosporidium* infection and 18 of the 29 calves their rotavirus infection within the first week of life.

Eighteen of the 52 infected calves developed diarrhea of microbial origin within 20 days of age; the most frequent finding was mixed infection with *Cryptosporidium* and rotavirus. In addition there were six diarrheic calves where no infection could be demonstrated. Only 10 of the 68 calves had neither infections nor diarrhea. There was a highly significant difference between the mean times of the first colostral feeding of the uninfected non-diarrheic and diarrheic calves and a significant difference between the mean times of the first colostral feeding of the subclinically infected and diarrheic calves. The importance of sucking of colostrum at the first feeding was observed. The smallest amount protecting the calves from infections was 0.75 l given within 1 h of delivery.

INTRODUCTION

The environment of neonatal calves is rich in microorganisms and within a few hours after delivery microbial colonization begins in the gastrointestinal tract (Ruckebusch et al., 1983). The development of colonization is complex (Tzipori et al., 1983) and depends partly on the health of the dam, the type

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of delivery, the time of the first colostral feeding, the method of feeding, the volume of colostrum given and its immunoglobulin concentration. Inadequate colostrum-derived passive immunity during the monogastric phase may lead to enteric disturbances (Kruse, 1983; Gay, 1984a).

Calves born into a highly contaminated environment may become infected during delivery with enteropathogens carried by their dams or other cows. In spite of shedding the organisms some calves may remain clinically normal. Diarrheic calves constitute the main source of infection or contamination for the whole herd and for the environment (Radostits and Acres, 1980).

Infectious agents can cause alterations in intestinal structures and functions; they may promote the loss of normal defence mechanisms against secondary opportunistic pathogens leading to malabsorption and malnutrition (Archer, 1984). Death may occur in older calves, when the primary causal agents can no longer be detected.

The purpose of the present study was (1) to evaluate the occurrence and distribution of certain common enteropathogens of neonatal calves in Finland, with special reference to *Cryptosporidium*, whose etiological role in calf diarrhea has been established only recently (Tzipori, 1983; Pohlenz et al., 1984); (2) to determine whether the subclinical or clinical neonatal enteric infections detected depend on the management, and in particular on the colostral feeding, of the calves; (3) to analyze data on the farm-adopted management programs as related to enteric infections, and (4) to determine how clinical signs of diarrhea as evaluated by the farmers, are related to laboratory findings of enteropathogens in feces.

MATERIALS AND METHODS

Animals

Sixty-seven dairy cows, 51 Ayrshire, 14 Friesian and 2 Finncattle, originating from eight housed herds (A—H) from southern and southwestern Finland, were selected on the grounds of previous history of diarrhea. The mean size of the herds was 37 cows (range 12—230). A total of 68 calves (33 females and 35 bulls) were delivered naturally during the period dealt with from March to July.

Clinical observations

The calves were observed during the first 3 weeks of life. Questionnaires were distributed for each calf, dealing with the health condition and weight at birth, the time and the method of the first colostral feeding and the amount offered. The farmers were asked to maintain the general management of the herds during the period. Observations including the color and consistency of feces (diarrhea was defined as an abnormally loose consistency of feces), intake of food and any abnormalities were recorded daily and the length of the dry period and the previous number of calvings of the dams were noted.

Sampling procedure

Fecal samples were obtained at 1, 3, 5, 7, 10, 15, and 20 days of age from each calf by stimulation of defecation with a gloved finger and collected directly from the rectum into a plastic vial. The samples were stored deep-frozen until examined.

Fecal analyses

Parasitology

For the detection of oocysts of *Cryptosporidium*, each fecal specimen was smeared onto a defatted microscope slide. After air-drying overnight the thin smears were fixed with absolute methanol for 10 min and allowed to air-dry. A total of 1428 fixed smears were stained with an automatic staining device (Varistain 24-2, Shandon Southern Products Ltd., Cheshire, U.K.) according to the modified Ziehl-Neelsen technique (Henriksen and Pohlenz, 1981). The sequential steps of the procedure were; immersion in concentrated carbol fuchsin (Merck 1358, 206, 1.0 g of fuchsin, 10 ml of ethanol, 90 ml of 5% phenol) for 20 min, two 2-min rinses with tap water, immersion in 7% sulphuric acid for 1 min, a 2-min rinse with tap water, and counterstaining with 5% malachite green (Merck 1398, 5.0 g of malachite green, 100 ml ethanol) for 1.5 min and four 1-min rinses with tap water. After air-drying the slides were thoroughly examined at a magnification of 400× and focused when necessary with an oil-immersion lens at a magnification of 1000×. The grade of infection was scored semiquantitatively as follows: 0 = no detected oocysts, + = 1 oocyst, ++ = 2—5 oocysts, +++ = 6—20 oocysts and ++++ = > 21 oocysts per field at a magnification of 400×. The oocysts were identified by comparing their size and internal structure with those of reference slides of *Cryptosporidium*, kindly supplied by Dr. Sv.Aa. Henriksen (State Veterinary Serum Laboratory, Copenhagen, Denmark). Specific tests for other intestinal parasites were not conducted.

Virology

For the detection of rotaviruses, coronaviruses and parvoviruses each fecal specimen was diluted with phosphate buffered saline, pH 7.3 so as to form a 10% suspension. After centrifugation at 600× *g* for 30 min the supernatants were collected and stored at −20°C until use. Two methods were used for the detection of rotaviruses and coronaviruses. If either of the methods gave a positive result the sample was considered to be positive for the virus in question. Each sample was studied by electron microscopy (EM), using a negative staining technique (Ritchie and Fernelius, 1969). The supernatants were placed on carbon coated formvar grids and stained with 2% potassium phosphotungstate, pH 7.0. The grids were examined with a Jeol-Jem-100S electron microscope (Japanese Electron Optics, Beabody, Mass., U.S.A.) at a magnification of 40 000×. A latex agglutination (LA) method was used to

detect rotavirus antigen (Haikala et al., 1983; Morinet et al., 1984): 20 μl of the fecal suspension from each sample was mixed with an equal amount of LA reagent on a glass slide from each sample. Negative controls were also made. The test was read after 3—4 min and was considered to be positive if a distinct agglutination was observed, provided that control suspensions remained milky. Coronavirus was studied with the haemadsorption-haemagglutination assay (HEHA) (van Balken et al., 1978/1979). A titer >1:32 was considered positive.

Bacteriology

Each fecal specimen was initially plated on 5% bovine blood agar (BBL, Becton Dickinson Co, Cockeysville, U.S.A.) and eosin-methylene-blue-agar (Difco, Detroit, U.S.A.) plates and incubated aerobically at 37°C for 24 h. Massive overgrowth of *Escherichia coli* in a pure or almost pure culture was taken to indicate an enteric infection. Four colonies from each plate were examined serologically for antigens O 55, O 78 and O 86, which have been found to be the most common antigens in Finnish calves (H. Vasenius, personal communication, 1984, National Veterinary Institute, Helsinki, Finland). The assay for the detection of the antigen K 99 of *E. coli* was not available at that time. For the enrichment of *Salmonella*, the fecal material was inoculated into tubes of tetrathionate broth. After 24 h at 37°C the broths were plated onto brilliant green (E. Merck, Darmstadt, Federal Republic of Germany) and BROLAC-agar (E. Merck) and incubated aerobically at 37°C for 24 h. Conventional biochemical tests were used for the final identification of *Salmonella* and *E. coli* isolates (Kaufman, 1966).

Statistical methods

Statistical comparisons were made with the Kruskal-Wallis Test (Sokal and Rohlf, 1969).

RESULTS

Fecal samples

A total of 476 fecal samples from 68 calves were analyzed. According to the farmers' reports 24 of the calves were diarrheic and 44 non-diarrheic (Table I). *Cryptosporidium* was found in 46% (11/24) of the former and 57% (25/44) of the latter. A total of 53% of all calves harbored *Cryptosporidium*, making this the most frequent finding. Rotavirus was found in 54% (13/24) of the diarrheic and 36% (16/44) of the non-diarrheic calves, a total of 43%. *E. coli* was isolated from 17% (4/24) of the diarrheic and from 25% (11/44) of the non-diarrheic calves, totalling 22%. The *E. coli* isolates represented antigenic types other than O 55, O 78 and O 86.

Table II presents the fecal findings of 18 diarrheic and 34 non-diarrheic

TABLE I

Distribution of enteropathogens in 24 diarrheic (a)* and 44 non-diarrheic (b) calves among 8 Finnish dairy herds

Herd	Number of calves	Rotavirus a)	Rotavirus b)	*E. coli* a)	*E. coli* b)	*Cryptosporidium* a)	*Cryptosporidium* b)
A	5	0	1	0	0	0	4
B	8	3	2	1	2	3	4
C	5	1	4	1	0	0	3
D	13	1	1	0	2	2	6
E	4	0	1	0	2	0	2
F	19	7	2	2	1	5	1
G	7	1	1	0	1	1	1
H	7	0	4	0	3	0	4
Total	68	13	16	4	11	11	25
		29		15		36	
%		43		22		53	

*A diarrheic calf may have been infected with more than one species of enteropathogen.

TABLE II

Etiological findings of the 68 calves

	Number of calves	%
Cryptosporidium only	15	29
Cryptosporidum + rotavirus	14	27
Rotavirus only	8	15
Rotavirus + *E. coli*	5	10
Cryptosporidium + *E. coli*	5	10
E. coli only	3	6
Cryptosporidium + rotavirus + *E. coli*	2	4
Total	52	76

calves. In addition, six calves out of the 68 (9%) were reported to have signs of scouring but no enteropathogens were detected. Ten calves (15%) were without enteric symptoms or enteropathogens as detected by the present methods.

The rotavirus antigen detection and the grade of *Cryptosporidium* infection, manifestation of diarrhea and onset of shedding of the virus and oocysts are summarized in Table III. 62% of the calves with rotavirus, 72% of the calves with *Cryptosporidium* and 86% of the calves with *E. coli* were found to be already infected during the first week of life. In 94% of the cases the grade of cryptosporidiosis was low (+).

TABLE III

Rotavirus antigen detection[a] and grade of *Cryptosporidium* infection[b]; manifestation of diarrhea and onset of shedding in infected calves

Days after birth	Rotavirus Number of infected calves (of whom diarrheic)	*Cryptosporidium* Grade of infection and number of infected calves (of whom diarrheic)		
		+	++	+++
1	3 (1)	0	0	0
3	3 (0)	4 (1)	0	0
5	7 (5)	11 (6)	1 (0)	0
7	5 (3)	9 (2)	0	1 (0)
10	7 (2)	4 (0)	0	0
15	2 (1)	3 (2)	0	0
20	2 (0)	3 (0)	0	0
	29 (12)	34 (11)	1 (0)	1 (0)

[a]Latex agglutination. Rotalex® test, Orion Diagnostica, Espoo, Finland.
[b]Fecal smears stained by the modified Ziehl-Neelsen technique, semiquantitative scale: 0 = no detected oocysts, + = 1 oocyst, ++ = 2—5 oocysts, +++ = 6—20 oocysts per field at magnification of 400×.

Twenty-four of the calves had scours. *Cryptosporidium* alone was found in 21% of these cases, while rotavirus alone accounted for 17% of the cases. *E. coli* alone seemed not to be associated with clinical symptoms. Mixed infection with *Cryptosporidium* and rotavirus was found in 21% of the cases, in all of which rotavirus alone was detected at 1—7 days of age, prior to the shedding of *Cryptosporidium* oocysts and the onset of diarrhea. Concurrent infection with *Cryptosporidium* and *E. coli* was not found in diarrheic calves. In all of the infected but non-diarrheic calves *E. coli* was detected at the earliest at 5 days of age, followed by *Cryptosporidium* at 5—20 days of age. Both rotavirus and *E. coli* were found in 13% of the diarrheic cases; in all of these *E. coli* was isolated primarily at an age of 1—3 days followed by rotavirus at 5—10 days of age. A mixed infection with all the three agents was found in only one calf. In this case *Cryptosporidium* and *E. coli* were detected at 3 days of age, followed by rotavirus at 5 days of age.

The appearance of the feces in association with *Cryptosporidium* infection without diarrhea was greenish and mucoid, usually semisolid in consistency. Rotavirus infection resulted in yellowish-white feces of normal consistency.

All of the fecal samples from the 68 calves were negative for *Salmonella*, coronavirus and parvovirus.

The 68 calves can be divided into four groups based on enteropathogen status on postnatal day 15: Group 1 consists of 10 non-diarrheic calves in whom no enteropathogens were detected and Group 2 of 34 subclinically infected calves. Eighteen diarrheic calves with enteropathogens form Group

3, and six diarrheic calves, in whom enteropathogens were not detected, Group 4.

Postnatal care of calves

The mean time of the first colostral feeding of all 68 calves was 3.1 h (median (M) 2.2, standard deviation (S.D.) 1.9, range 0.5—8.0) after delivery. The means for the different groups were 1.0 h (M 0.7, S.D. 0.6, range 0.5—2.0 h) for Group 1, 2.9 h (M 1.8, S.D. 1.9, range 0.25—8.0 h) for Group 2, 4.0 h (M 3.8, S.D. 1.8, range 1.5—7.0 h) for Group 3 and 4.9 h (M 4.8, S.D. 0.3, range 4.5—5.0 h) for Group 4. The Kruskal-Wallis Test was applied to compare the groups. A significant difference was found between Groups 1 and 2 ($p < 0.05$), and a highly significant difference was found between Groups 1 and 3 ($p < 0.01$). The means of the Groups 2 and 3 do not differ ($p < 0.1$). The details of colostral management and occurrence of scouring in calves from different herds are given in Table IV. The sex, breed or birthweight of the calves seemed not to be related to the manifestation of scouring. All calves received the first milking colostrum. The medians of the lengths of the dam's dry period were 50 days (range 37—60 days) for Group 1, 53 days (range 30—75 days) for Group 2, 72 days (range 50—110 days)

TABLE IV

The colostral management and manifestation of scouring in 68 calves of 8 Finnish dairy herds

	Time from birth to first feeding (h)	Method of feeding	Volume of colostrum (l)	Number of diarrheic calves	Total number of calves
Herds					
E	0.6[a] 0.25—2.0[b]	Assisted sucking	Ad. lib.	0	4
A	0.7 0.5—1.0	Nursing bottle	0.7[a] 0[b]	0	5
G	0.7 0.5—2.0	Bucket	1.5 1.5—2.0	1	7
D	2.2 0.5—8.0	Bucket	1.7 1.0—2.0	3	13
H	2.5 2.0—5.0	Assisted sucking	Ad. lib.	0	7
B	3.5 1.5—7.0	Bucket	1.2 1.0—2.0	3	8
C	4.1 2.0—5.0	Bucket	1.8 1.0—1.5	1	5
F	5.2 2.0—6.0	Bucket	2.0 0	16	19

[a]Medians.
[b]Ranges.

TABLE V

Manifestation of scours of the 68 calves as related to lactation period of dams

	Number of the lactation period of the dams								Total
	I	II	III	IV	V	VI	VII	VIII	
Number of calves in Group 1[a]	1	4	1	3	1				10
2[b]	5	7	7	7	5	2		1	34
3[c]	4	5	5	0	2	1	1		18
4[d]	2	2	0	2	0	0	0	0	6
Total	12	18	13	12	8	3	1	1	68
Number of diarrheic calves (%)	6 (50)	7 (39)	5 (38)	2 (17)	2 (25)	1 (33)	1	0	24 (35)

[a]Calves without clinical symptoms and enteropathogens.
[b]Subclinically infected calves.
[c]Diarrheic calves, in whom enteropathogens were detected.
[d]Diarrheic calves, in whom no enteropathogens were detected.

for Group 3 and 60 days (range 30—84 days) for Group 4. The means of Groups 1 and 2 do not differ, but there is a significant difference between Groups 1 and 3 ($p < 0.05$) and a highly significant difference between Groups 2 and 3 ($p < 0.01$). The manifestation of scours in the calves in relation to the lactation period of the dams is presented in Table V. 50% of the calves of heifers had diarrhea, but the occurrence decreased on subsequent lactation periods.

DISCUSSION

The most common enteropathogens in neonatal calves are enteropathogenic *E. coli*, rotavirus, coronavirus, and more recently, *Cryptosporidium* (Morin et al., 1978; Moon et al., 1978; Tzipori, 1981; Bulgin et al., 1982). In the present study *Cryptosporidium* and rotavirus were detected in all eight herds and *E. coli* in seven of them; all of the agents were found also in clinically healthy calves (Group 2), reflecting the complex aetiology of neonatal calf diarrhea (Tzipori, 1981; Myers et al., 1984).

Clinical diarrhea in calves was observed in 63% (5/8) of the herds. Infectious agents associated with symptoms were found in 75% (18/24) of cases. The failure to detect infectious agents in the other six diarrheic calves may be due to one or more of the following factors: (1) the shedding of the agent did not coincide with the sampling schedule. (2) The methods of detection were inadequate for the agent in question (e.g. *Campylobacter* or *Yersinia*).

(3) The diarrhea was not of microbial origin. These six calves were from the two largest herds. It is conceivable that in small herds, where the animals are managed by the owners themselves, the calves receive more careful treatment (Martin et al., 1975). Nutritional, immunological and even genetic factors may also be involved in precipitating the disease (Tzipori, 1981).

Cryptosporidium was detected in calves of all herds, with a frequency of occurrence from 29 to 88%, and was nearly as frequent in diarrheic as in clinically normal calves up to 3 weeks of age. Since the first report (Panciera et al., 1971), extensive surveys have shown this protozoan to be involved in outbreaks of diarrhea in large numbers of calves (Morin et al., 1978; Bulgin et al., 1982; Stein et al., 1983). An incidence of infection as high as 95.8% has been recorded in calves of four herds observed for the first 5-week period (Stein et al., 1983).

Our results seem to indicate the importance of infection during the first week of life; the largest number of positive oocyst findings was that for the fifth postpartum day. Nearly 75% of the infected calves started to shed oocysts during this initial period, in >80% of the infected and diarrheic calves symptoms also began during this initial period. *Cryptosporidium* alone was found in two thirds of the diarrhea cases during the first week while both *Cryptosporidium* and rotavirus were found in the other third.

Involvement of *Cryptosporidium* along with another agent in 40% (21/52) of the infected calves, versus the occurrence of *Cryptosporidium* alone in 29% (15/52), confirms the suggestion that infection with more than one agent is common under field conditions (Tzipori, 1981; Morin et al., 1978; Tzipori et al., 1983). The most frequent combination of infectious agents in the present study was *Cryptosporidium* and rotavirus. As suggested by Angus (1983), *Cryptosporidium* appears to represent a complication of virus-induced enteritis. The shared anatomic location of both agents is the distal half of the small intestine (Pearson and Logan, 1983). The data obtained from mixed infection with *Cryptosporidium* and *E. coli* or rotavirus and *E. coli* confirm the suggestion that under field conditions the enteric colibacillosis in which *E. coli* occurs either alone or combined with rotavirus is a disease of calves <4 days of age (Morin et al., 1978; Tzipori, 1981). In experimental studies gnotobiotic and specific-pathogen-free-calves aged 7—12 days and orally infected with rotavirus alone showed an age-related increase in resistance against this agent; dual infection with *E. coli* and rotavirus tended to prolong the period of susceptibility. The interaction is considered to be synergistic (Tzipori et al., 1983).

Rotavirus accounted for 15.3% of single agent infections and for 40% of mixed infections. The importance of the first week of life was again observed. More than 60% of the calves were found to be already infected during the first week of life, and the symptoms began in >60% of infected calves during this time. Rotavirus may be an aetiological agent of calf diarrhea in all age groups, but the highest incidences have generally been observed in calves between 4 and 8 days of age (Morin et al., 1978; Moon et

al., 1978; McNulty, 1983). The age and immunity dependent incubation period ranges from 15 h to 3 or 4 days (Tzipori, 1981), suggesting the primary role of rotavirus in mixed infections with *Cryptosporidium*. The shortest prepatent period for *Cryptosporidium* is 3—4 days (Stein et al., 1983).

All of the negative LA tests for rotavirus were also negative in EM but of the 29 positive LA reactions only 14 were positive in EM. The LA test appears to be a sensitive screening method for the detection of rotavirus, but differences in the positive results clearly calls for reappraisal of the methods.

Coronavirus was not found in any of the calves. The EM method of the detection may be hampered by the fringed particles sometimes found in feces (Tzipori, 1981), and the extent to which these negative results account for the six unresolved cases of diarrhea from the two largest herds is a matter of conjecture. Of course the possible involvement of other enteropathogenic agents not covered in this study, such as *Campylobacter jejuni* or *Yersinia enterocolitica*, cannot be disregarded (Myers et al., 1984).

Parvovirus was not found in any of the calves, although a high incidence of infection among cattle in U.S.A. has been reported. The aetiological role of parvovirus in neonatal diarrhea has yet to be evaluated (Tzipori, 1981).

All the non-diarrheic calves in which no enteropathogens were found had received colostrum within roughly the first 2 h of life. The non-selective absorption of colostral immunoglobulins has been observed to decline linearly when feeding is delayed by more than 2 h (Busch and Staley, 1980), however, it is not humoral antibodies but the lactogenic immunoglobulins remaining in the intestine that play a local protective role soon after birth (Busch and Staley, 1980; Gay, 1984a). Calves that were assisted in sucking or fed with a nursing bottle for the first time remained non-diarrheic. Natural sucking with a functioning esophageal groove reflex leads the colostrum into abomasum. An esophageal feeder or bucket feeding deposits colostrum in the forestomachs, where it is delayed for some hours (Gay, 1984a). Early sucking ensures normal gastrointestinal motility (Ruckebusch et al., 1983) and the transfer of the lactoglobulins to form a prophylactic barrier against infection in the intestine (Busch and Staley, 1980; Gay, 1984a).

The amount of colostrum in the first feed did not seem to be a critical determinant; no diarrhea was observed in Herd A, where only 0.75 l for each calf was offered. Small amounts of colostrum offered frequently may be beneficial in retaining the pinocytic activity of the enterocytes for a longer period rather than a single large amount immediately after birth (Petrie, 1984). The immunoglobulin concentrations in colostrum varies between cows, tending to increase until 5 years of age (Berger, 1979). This may explain the decreased occurrence of diarrhea among first-, second- and third-born calves. The colostral immunoglobulin concentration decreases as much as 47% from the initial level during the first day of lactation (Berger, 1979), emphasizing the importance of using first milking colostrum. Poor mothering, the pendulous udders of some cows and difficulties in calving have also been incriminated in a weak sucking drive and a failure in the passive transfer

of immunoglobulins (Gay, 1984b). Lower IgG_1 and IgG_2 concentrations in the colostrum during the first postparturient day have been observed in cows with intramammary treatment at drying off (Geene, 1984). A prolonged dry period also appears to decrease the average concentrations of IgG_1 and IgA in the first milking colostrum (Geene, 1984). The dams of the non-diarrheic calves (Groups 1 and 2) in the present study were found to have a shorter dry period than dams of the diarrheic calves (Group 3).

All herds had a history of recurrent outbreaks of scouring. It seems reasonable to assume that the newborn calves had an elevated infectious pressure, which was reflected in the degree and multiplicity of enteric infections encountered. In addition to the epidemiological and microbiological insight into neonatal calf diarrhea in Finland, this study yields some suggestions as to prophylactic measures, including assisted sucking, preferably of 1—2 l of the first milking colostrum within 2 h after delivery. These recommendations agree entirely with those of Gay (1984b), who also suggests avoiding the use of heifer colostrum for the first day and continuing the colostral feeding for the following day.

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