Cryptosporidium and concurrent infections with other major enterophatogens in 1 to 30-day-old diarrheic dairy calves in central Spain

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Abstract

Faeces samples from 218, 1 to 30-day-old, diarrheic dairy calves in 65 dairy herds were screened for the presence of *Cryptosporidium* and concurrent infections with rotavirus, coronavirus, F5[+] *Escherichia coli* and *Salmonella* spp. Calves were grouped according to their age as follows: 1–7, 8–14, 15–21 and 22–30 days. *Cryptosporidium* infection was detected in 43.8%, 71.9%, 63.2% and 6.9% of the calves in the respective age groups. Significant differences in the detection rate of *Cryptosporidium* were found between the age group 22–30 days and all other age groups, and between the age group 1–7 days and the age groups 8–14 days and 15–21 days. *Cryptosporidium* was the only enteropathogen detected in 60 of the 114 (52.6%) diarrheic calves. Concurrent infections with other enteropathogen(s) were detected in 64.3%, 46.3%, 39.5% and 0% of the *Cryptosporidium*-infected calves in the age groups 1–7, 8–14, 15–21 and 22–30 days, respectively. A significant age-associated decrease in the detection rate of mixed infections ($p < 0.05$) was found. The detection rates of the other enteropathogens considered in calves with *Cryptosporidium* infection were 87% for rotavirus, 11.1% for coronavirus, 27.8% for F5[+] *E. coli* and 1.8% for *Salmonella*. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Cattle-protozoa; *Cryptosporidium* sp.; Neonatal calf diarrhea; Rotavirus; Coronavirus; *Escherichia coli*; *Salmonella*

1. Introduction

Diarrhea of neonatal calves causes major economic loss directly through mortality and therapy, and indirectly from poor growth. The diarrheal disease syndrome has a complex

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etiopathogenesis, because various infectious agents, either single or in combination, may be associated with field outbreaks. In addition, environmental, managemental, and nutritional factors may influence the severity and outcome of the disease.

Cryptosporidium parvum, an intestinal protozoan parasite that causes enteric infection and diarrhea in many species of mammals (O'Donoghue, 1995), has emerged as one of the major enteropathogens associated with neonatal calf diarrhea together with rotavirus, coronavirus, F5[+] *Escherichia coli* and *Salmonella*. *Cryptosporidium* infection of calves may be asymptomatic or causes clinical signs range from mild intermittent diarrhea to profuse watery diarrhea with concomitant dehydration (Fayer and Ungar, 1986).

Most studies carried out in different countries have found *Cryptosporidium* and rotavirus to be the most commonly detected agents in calves with diarrhea. In some reports *Cryptosporidium* was the most prevalent (Rosati et al., 1991; McDonough et al., 1994; Fagan et al., 1995; Otto et al., 1995), while in other studies rotavirus was the agent most frequently detected (Sherwood et al., 1983; Reynolds et al., 1986; Snodgrass et al., 1986; Zrelli et al., 1990; Moore and Zeman, 1991; Brenner et al., 1993). In microbiological surveys of diarrheic calves, the detection rate of mixed infections with two or more of the main enteropathogens ranged between 5% and 20%, and in most of them *Cryptosporidium* was involved (Bulgin et al., 1982; Sherwood et al., 1983; Reynolds et al., 1986; Snodgrass et al., 1986; Bellinzoni et al., 1990; Zrelli et al., 1990; Fagan et al., 1995).

Some reports have described the detection rate of *Cryptosporidium* infection in field cases of neonatal calf diarrhoea in Spain (Martín et al., 1995; Quílez et al., 1996). However, no studies involving other recognized enteropathogens simultaneously has been conducted. This study reports the detection rates of *Cryptosporidium* and concurrent infections with four other major enteropathogens in different age groups (1–7, 8–14, 15–21 and 22–30 days) of diarrheic dairy calves in central Spain.

2. Materials and methods

2.1. Sampling procedure

In the autumn of 1993, a letter was sent to 33 veterinarians working in the dairy industry in central Spain and members of the National Association of Specialists in Bovine Medicine (ANEMBE), asking for their collaboration for sampling natural cases of neonatal calf diarrhea. The diagnosis of diarrhea was made by veterinarians. Faecal samples were collected directly from the rectum in sterile plastic bottles and submitted on the day of sampling to the laboratory by express mail. Submitted samples were accompanied by a record sheet containing information on the size of herd, number of scouring calves and number of calves (< 30 days) in the herd at sampling time, and age of each sampled calf. Only fecal samples obtained within 48 h of onset of clinical signs from non-treated calves up to 30 days of age were included in this study. Samples were processed within 24 h of reception.

2.2. Detection of Cryptosporidium

Cryptosporidium infection was diagnosed in fresh faecal smears. For oocysts detection smears were made from non-concentrated faecal samples on glass slides and oocysts were detected microscopically using extemporaneous fuchsin staining method (Heine, 1982). A modified Ziehl-Neelsen's acid-fast method (Casemore et al., 1985) was also used to confirm Heine-stain negative results. Moreover, *Cryptosporidium* was also detected by a commercial ELISA kit (Tetravalent, Vétoquinol, Magny-Vernois, France).

2.3. Detection of other enteropathogens

All the faecal samples positive to *Cryptosporidium* were tested for the presence of rotavirus, coronavirus and $F5^+$ *E. coli* by a commercial ELISA kit (Tetravalent). The ELISA test was performed according to the manufacturer's instructions. Moreover, faecal samples were also tested for the presence of rotavirus by polyacrylamide gel electrophoresis (PAGE) as described by Herring et al. (1982), and for the presence of $F5^+$ *E. coli* by bacterial culture as follows. The faeces samples were plated on MacConkey agar. After overnight incubation, four colonies with the typical appearance of *E. coli* from each sample were randomly chosen. *E. coli* strains were identified by biochemical tests. The strains were tested for F5 fimbriae by the slide agglutination method with live bacteria grown on Minca-Isovitalex solid media. The production of absorbed antiserum used to detect this fimbrial antigen has been described (Morris et al., 1983; Contrepois et al., 1985). *Salmonella* species were isolated by enrichment of faeces in selective broths. Approximately 1 g of faeces was added both to 9 ml of tetrathionate broth and to 7.5 ml of selenite broth. After 24 h of incubation at 37°C, samples were plated out on brilliant green agar, and incubated for 24 h at 37°C. Lactose-negative colonies were tested on triple sugar iron, urea, and *o*-nitrophenyl-β-D-galactopyranoside media. Those that had *Salmonella*-type reactions were tested for agglutination, using commercial polyvalent O-antisera (*Salmonella* antiserum, Difco).

2.4. Statistical analysis

Data were computed using Epi Info Version 6.04 (Dean et al., 1994). Calves were grouped according to their age as follows: 1–7, 8–14, 15–21 and 22–30 days. Odds ratios (OR) and 95% confidence interval (CI) for *Cryptosporidium* infection in the different age groups were calculated using each age group as reference. The relative risk of infection was considered significant if 95% CI for OR did not include 1.0 (Fletcher et al., 1996). Mantel-Haenszel χ^2 was used for linear trends.

3. Results

From November 1993 to September 1995, 18 of the 33 veterinarians submitted samples from at least two herds (range 2–7). Altogether, 218 faecal samples from diarrheic dairy calves in 65 herds were submitted. Herd sizes ranged from 12 to 650 cows (median 49

Table 1
Detection rate of *Cryptosporidium* infection in the different age groups of diarrheic calves and odd ratios using age-group 1–7 days as reference

Age group (no. of animals)	Animals infected with *Cryptosporidium*				
	Number	%	OR	95% CI[a]	*p*-Value
1–7 days ($n = 64$)	28	43.8	1		
8–14 days ($n = 57$)	41	71.9	3.29	1.54–7.05	< 0.01
15–21 days ($n = 68$)	43	63.2	2.21	1.10–4.44	< 0.05
22–30 days ($n = 29$)	2	6.9	0.10	0.02–0.43	< 0.001

[a]Confidence interval.

cows). Of 218 calves, 64, 57, 68 and 29 calves were 1–7, 8–14, 15–21 and 22–30-days old, respectively. The mean age of the studied calves was 13.3 days (median 12 days).

Cryptosporidium infection was detected in 43.8%, 71.9%, 63.2% and 6.9% of the calves in the age groups 1–7, 8–14, 15–21 and 22–30 days, respectively (Table 1). The risk for *Cryptosporidium* infection in the age group 22–30 days was significantly lower in comparison with age group 1–7 days (OR = 0.10; 95% CI, 0.02–0.43; $p < 0.001$; Table 1), with age group 8–14 days (OR = 0.03; 95% CI, 0.01–0.14; $p < 0.0001$), and with age group 15–21 days (OR = 0.04; 95% CI, 0.01–0.20; $p < 0.0001$). The risk for *Cryptosporidium* infection in the age group 1–7 days was also significantly lower in comparison with age group 8–14 days (OR = 0.30; 95% CI, 0.14–0.65; $p < 0.01$) and with age group 15–21 days (OR = 0.45; 95% CI, 0.23–0.91; $p < 0.05$). The mean age of the *Cryptosporidium*-infected calves was 11.8 days (range 2–30 days).

Only *Cryptosporidium* was detected in 60 of the 114 (52.6%) diarrheic calves. Concomitant infections with other enteropathogen(s) were detected in 64.3%, 46.3%, 39.5% and 0% of the *Cryptosporidium* infected calves in the age groups 1–7, 8–14, 15–21 and 22–30 days, respectively (Table 2). A significant age-associated decrease in the detection rate of mixed infections ($p < 0.05$) was found. The detection rates of the other

Table 2
Detection of *Cryptosporidium* and its combination with other enteropathogens in the different age groups of diarrheic calves

Enteropathogen(s) detected	1–7 days ($n = 64$)		8–14 days ($n = 57$)		15–21 days ($n = 68$)		22–30 days ($n = 29$)	
	Number positive	%	Number positive	%	Number positive	%	Number positive	%
Cryptosporidium only	10	15.6	22	38.6	26	38.2	2	6.9
Cryptos. + Rota.	9	14.1	14	24.6	10	14.7	0	0
Cryptos. + *E. coli* F5	1	1.6	2	3.5	4	5.9	0	0
Cryptos. + Rota. + *E.coli* F5	2	3.1	3	5.3	2	2.9	0	0
Cryptos. + Rota. + *Salmonella* sp.	1	1.6	0	0	0	0	0	0
Cryptos. + Rota. + Corona	5	7.8	0	0	0	0	0	0
Cryptos. + Rota. + Corona + *E. coli* F5	0	0	0	0	1	1.5	0	0
Total	28	43.8	41	71.9	43	63.2	2	6.9

enteropathogens considered in calves with *Cryptosporidium* infection were 87% for rotavirus (47/54), 11.1% for coronavirus (6/54), 27.8% for F5$^+$ *E. coli* (15/54) and 1.8% for *Salmonella* (1/54) (Table 2). Thus, the combination *Cryptosporidium*-rotavirus was found in all but seven of the calves with mixed infection, and in 14 of the 47 calves with *Cryptosporidium*–rotavirus infection at least one of the other agents was also detected (Table 2).

4. Discussion

This study reports the detection rates of *Cryptosporidium* and concurrent infections with other enteropathogens in different age groups (1–7, 8–14, 15–21 and 22–30 days) of diarrheic dairy calves. Given the sampling procedure, we cannot exclude that the results are biased, since veterinarians may have tended to select severe or persistent cases of diarrhea for sampling, and, thus, mild or transient cases may be under-represented in this study. This same drawback frequently exists for other studies involving diarrheic calves. Therefore, it seems reasonable to compare our results with those reported in similar studies.

The overall detection rate of *Cryptosporidium* in this study (52.3%) is higher than those found in neonatal diarrheic calves in other countries (from 14.1% to 44.4%) (Reynolds et al., 1986; Snodgrass et al., 1986; Zrelli et al., 1990; Moore and Zeman, 1991; Rosati et al., 1991; Brenner et al., 1993; Fagan et al., 1995), and in Spain (35.7%) (Martín et al., 1995), but similar to that reported by Otto et al. (1995) (52.5%) in diarrheic calves up to 3 weeks of age in Germany. Rotavirus is traditionally considered the most prevalent agent in diarrheic calves. However, recent surveys tend to find higher *Cryptosporidium* than rotavirus prevalence. Thus, it is possible that the incidence of *Cryptosporidium* had increased in the last years.

The detection rates of *Cryptosporidium* infection in the different age groups are generally in accordance with the pattern of *Cryptosporidium* oocysts shedding reported for calves (Tzipori et al., 1983; Markovics and Pipano, 1987). Thus, the highest risk of being infected with *Cryptosporidium* in diarrheic calves occurred in the second week of life, although this risk was not significantly higher in comparison with that of the third week. The percentage of calves in the second week of life positive to *Cryptosporidium* in our study was slightly higher than the result reported by Fagan et al. (1995) in a similar study (71.9% vs. 62.5%), while that of the third week was much higher (63.2% vs. 33.3%) than their's.

The percentage of calves infected with *Cryptosporidium* in the first week of life was higher than expected, since the experimentally determined pre-patent period for the parasite in cattle is 2–7 days (Tzipori et al., 1983), and 4–8 days is the usual pre-patent period in naturally infected calves (Markovics and Pipano, 1987). Nevertheless, broadly similar high detection rate of *Cryptosporidium* infection (37.3%) in 0–7-day-old diarrheic calves was found by Fagan et al. (1995) in Ireland. On the other hand, very different results have been reported in prevalence surveys. Thus, in a survey carried out in Aragón (Spain), Quílez et al. (1996) found as much as 44.4% of calves aged 3–4 days infected with *Cryptosporidium*, while in a nation-wide survey performed in USA only 2.5% of < 4

days old calves were positive to *Cryptosporidium* (Garber et al., 1994). Two to 4 days are typically required for the first shedding of oocysts in feces. Therefore, the high detection rate found by us in age group 1–7 days indicates that most of these calves were infected immediately after birth, and that the environment was heavily contaminated with oocysts.

Cryptosporidium was the only enteropathogen detected in 52.6% of the diarrhoeic calves. Therefore, although concurrent infection with other agents as well as environmental, managemental, and nutritional factors may influence the outcome of disease for a calf with *Cryptosporidium* infection, the results of this study indicate the importance of this organism as primary pathogen causing acute diarrhea in neonatal calves.

Mixed infections were detected in nearly half (47.4%) of the *Cryptosporidium*-infected calves, and, in agreement with the results published in most surveys all over the world, the most common mixed infection found in this study was rotavirus–*Cryptosporidium*. A significant age-associated decrease in the detection rate of mixed infections ($p < 0.05$) was found. That is in accordance with the age-dependent susceptibility of calves to the other enteropathogens considered except *Salmonella*. The pathogenic significance of concurrent infections in diarrheic calves, however, is not yet clear. Whether additive or synergistic interactions, that give rise to clinical effect or increase the severity of disease, occur in calves infected with *Cryptosporidium* and any other agent, or whether other agents extend the risk period for clinical cryptosporidiosis remain to be proved experimentally. Nevertheless, in microbiological surveys of diarrheic and healthy neonatal calves, mixed infections were much more commonly detected in diarrheic than in healthy calves (Morin et al., 1980; Reynolds et al., 1986). Reynolds et al. (1986) suggested that the presence of more than one enteropathogen may be one of the factors determining whether an infection results in a clinical or subclinical effect. Moreover, mixed infections have been associated with more severe disease (Morin et al., 1980).

Acknowledgements

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