THE PREVALENCE AND INCIDENCE OF *CRYTOSPORIDIUM* SPECIES IN NATURALLY INFECTED CALVES

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ABSTRACT

An investigation on the prevalence and incidence of *Cryptosporidium* spp. in faecal samples of calves was carried out. Of the 178 faecal samples examined from younger calves (2-3 months of age), 119 (66.85%) were found positive with *Cryptosporidium* spp. (Ziehl-Neelsen method) and 100 (56.18%) were found positive using the rapid staining technique. The study indicated that asymptomatic infection was more common in young calves above two months of age. The incidence of *Cryptosporidium* oocysts in faecal samples of calves was also investigated during twelve months period. 100% (Ziehl-Neelsen method) samples were found positive in June while a gradual decline in incidence occurred such that by May of the following year only 26.66% were positive. A similar trend was evident using the rapid staining method; in general this method identified fewer positives. Faecal samples from calves were collected and analyzed on the basis of their consistency. Generally a greater number of soft faeces were found positive with oocysts compared to diarrhoeic and diarrhoea faeces. However, a variation in the morphological characteristics of the oocysts of *Cryptosporidium* species was seen using the two techniques.

Keywords: *Cryptosporidium* spp., prevalence, incidence, infected calves.

INTRODUCTION

Cryptosporidium spp. are coccidian parasites which are similar to *Eimeria* species, since members of both genera cause diarrhoea in domestic, as well as wild, animals and birds. However, they differ in that *Eimeria* species do not occur in primates, which are infected with related *Isospora* species. Members of the genus *Cryptosporidium* are intracellular protozoan parasites that inhabit the microvilli of the epithelial surfaces of the human, domestic and wild animals, birds, fish and reptiles (Dubey *et al.*, 1990). In view of the wide host specificity, these parasites infect a wide range of hosts, including man, and are now recognized as an important zoonosis (Current et al. 1983).

Numerous epidemiological studies have shown that *Cryptosporidium* infection in the developed world can be attributed to contaminated water (Hayes *et al.*, 1989), and oocysts have been recovered from variety of fresh water sources (Galahar *et al.*, 1989). Oocysts have also been obtained from mammals, fish, birds and reptiles (Dubey *et al.*, 1990; Gajadhar, 1993; 1994). Animals are the main source of transmitting *Cryptosporidium* infection from animal to animal and from animal to man (Xiao *et al.*, 1994).

The occurrence of *Cryptosporidium* species in cattle varies from one geographical region to another. The prevalence of *Cryptosporidium* infection varies from 20-100% in young calves and 1.7-8.7% in dairy cows in the United States of America (Redman *et al.*, 1983; Leek and Fayer, 1984; Anderson, 1990). In UK (Reynolds *et al.*, 1986) and Russia (Manysheva, 1990) its prevalence in cattle was 23-100%. Lorenzo *et al.* (1993) also found 71.75% prevalence in asymptomatic adult cattle in Spain. However, little work has been carried out on the prevalence of *Cryptosporidium* infection in cattle in the United Kingdom except that of Reynolds *et al.* (1986), who recorded 23% prevalence in young calves from South England.

There is lack of information regarding the prevalence and zoonotic importance of *Cryptosporidium* species, particularly in cattle of North Wales, though the species had been investigated in sheep in North Wales (Ball, 1989; Barber, 1989). Barber (1989) examined only two faecal samples from calves in North Wales and found 100% prevalence of *Cryptosporidium* species. Therefore, the present study was designed with the following aims:

1. To assess the prevalence of *Cryptosporidium* species in naturally infected calves of The University College Farm, North Wales, Bangor, UK.
2. To investigate the presence of *Cryptosporidium* species in different months of the year and under two rearing systems

3. To identify the possible diagnostic characteristics, size and shape of the *Cryptosporidium* species present.

MATERIALS AND METHODS

For this study, 178 faecal samples were collected over a 12 months period from 15 calves, aged 2-3 months and kept at the Farm of University College, North Wales, Bangor, UK. Keeping in view the prevailing climatic conditions at the farm, the calves were housed indoor during the period from December to May, while from June to November they were kept outdoor on a free range. The consistency of faecal samples was recorded.

The faecal samples were processed to determine the *Cryptosporidium* oocysts through concentration technique, as suggested by Casmore (1987). For this purpose, approximately 2 g of faeces was suspended in 26 ml water. From this suspension 1 ml was taken, mixed with 5 ml of 10% formaline in a test tube and left for 24 hours to settle. The supernatant was added to a clean test tube, using a Pasteur's pipette and allowed to stand for 7 days. The supernatant was carefully removed with a Pasteur pipette and discarded. The deposit was used to make a faecal smear on a glass slide and stained using modified Ziehl-Neelsen cold carbol fuchsin or rapid staining technique.

Ziehl-Neelsen method

The modified Ziehl-Neeelsen cold carbol fuchsin method was adopted, as detailed below (Casmore, 1987).

Faecal smears were air dried, fixed in methanol for 3 minutes, stained in cold carbol fuchsin for 10 minutes, washed with tap water and decolourized in 3% hydrochloric acid for 1 minute. Then 1% methylene blue (counter stain) was applied for 30 seconds, rinsed in tap water and air-dried. The slides were then examined under oil immersion objective.

Rapid staining technique

A rapid staining method as recommended by Cross and Moorhead (1984) was used. For this purpose, thin faecal smears were allowed to air dry and were then fixed over a gas or spirit lamp flame. Any lumps on the slide were removed by scraping with the edge of another slide. A few drops of 1% methylene blue and 1% borax (disodium tetraborate) were added and allowed to stain for 30-60 seconds. After rinsing with tap water, smears were stained with a few drops of 0.1% eosin solution for 60 seconds. The slides were then dipped in acetone for 1-2 minutes for dehydration and examined under oil immersion (X1000). In both the procedures, the oocysts were counted in 10 microscopic fields and at least 100 oocysts were measured and their size recorded, using a micrometer slide (X 1000).

Since it is impossible to identify the different species of *Cryptosporidium* by simply examining their oocysts, all the oocysts counted were grouped together as *Cryptosporidium* spp. However, it is likely that they represent *Cryptosporidium parvum*. It is possible, however, that *C. muris* could be also present.

Statistical method

Data were analyzed by analysis of variance (one way ANOVA), using a minitab package, 1993/94.

RESULTS

The prevalence of oocysts of *Cryptosporidium* spp. in faeces of young calves of 2-3 months of age

Using the modified Ziehl-Neelsen method, 119 (66.85%) of the 178 faecal samples examined, were found positive for *Cryptosporidium* oocysts, while 59 (33.15%) samples were negative. The figures were slightly lower for the rapid staining method (56.18% positive and 43.82% negative, Table 1).

Month-wise incidence of oocysts of *Cryptosporidium* spp. in faeces of calves

Using both methods, the number of positive faecal samples decreased during the course of the twelve months. Thus starting in June when 100% of samples were positive (Ziehl-Neelsen method), a gradual decline in incidence occurred such that by May of the following year only 26.66% of the samples were positive. A similar trend was evident using the rapid staining method. Although in general this method identified fewer positive than the Ziehl-Neelsen method (Table 2).

The incidence of the mean number of *Cryptosporidium* oocysts recorded over the twelve months

The mean number of oocysts produced over the twelve months is shown in Table 3. The trend in reduction of positive samples seen above was also evident for the mean number of oocysts recorded each month. Thus, a significant reduction ($P<0.01$) in the mean number of oocysts (10 microscopic fields per sample) was observed and the mean number fell from 57.40 in June to 1.13 in the following May (Ziehl-Neelsen method). This was again seen using the rapid staining technique.

The percentage prevalence of *Cryptosporidium* oocysts in faecal samples of different consistency

Table 4 shows the numbers of positive samples in the three categories of faeces including soft, diarrhoeic and diarrhoea. As seen with the number in the younger

calves a greater proportion of soft faeces was positive compared with diarrhoeic faeces. This indicates that while diarrhoea is often a symptom of acute cryptosporidiosis, the presence of diarrhoea in animals shedding low grade of numbers of oocysts is not necessarily indicative of disease since those animals with diarrhoea are shedding fewer oocysts.

The prevalence of *Cryptosporidium* spp. in faeces of calves while housed and at grass

Table 5 shows that of the 178 samples, 79 (44.38%) were positive with *Cryptosporidium* oocysts from the faeces of calves at grass compared with 37 (20.78%) samples while housed indoors, using Ziehl-Neelsen method and whereas 68 (38.20%) samples were found positive with infection at grass and 28 (15.73%) positive when housed indoors, using rapid method. Thus, it seems that the greater risk of infection is available at grass compared with housing.

The incidence percentage of oocysts of *Cryptosporidium* of the total oocysts recorded from faeces of calves while housed and while at grass

Since the conditions for survival of oocysts and the chances of infection differ when animals are housed compared with when they are at free range, the results have been analyzed accordingly (Table 6). The samples (90 samples) which were collected from calves during six months indoor period (December-May) yielded 9.39% (Ziehl-Neelsen method) of the total oocysts recorded compared with 90.61% (88 samples) for the period from June to November when animals were at free range. This indicates, not withstanding a diluting effect of grazing, that the animals appear to be at greater risk of infection when grazing compared with the period of housing. The reasons for this greater risk of infection when calves are at grass could be the continuous contamination of pasture with faeces by infected animals or the possibility of oocysts leaching from fields where muck spreading has taken place.

The mean number of *Cryptosporidium* oocysts in faeces of calves during housing and at grass

The greater infection rate at grass was also seen when the total number of oocysts shed under the two husbandry conditions was examined (Table 7). The mean number of oocysts in 10 microscopic fields per animal in positive animals was significantly ($P<0.01$) greater for the animals at grass (25.0) compared with housed calves (2.5).

The size, shape and staining characteristics of *Cryptosporidium* oocysts

Using the modified Ziehl-Neelsen cold carbol fuchsin method, *Cryptosporidium* oocysts measured 4.9 ± 0.8 µm in diameter. The oocysts were spherical to ovoid in shape, stained red or pink with a granular appearance against a blue background and were surrounded by a halo. In the rapid staining method, oocysts measured 4.4 ± 0.8 µm in diameter, appeared as small bodies with a nucleus stained purple or blue in colour and was surrounded by a clear non-staining halo (Table 8 and Plate 1).

Table 1: The percentage prevalence of oocysts of *Cryptosporidium* spp. in faecal samples of calves

Technique used	Total samples examined	No. of positive samples (%)	No. of negative samples (%)
Ziehl-Neelsen	178	119 (66.85)	59 (33.15)
Rapid staining	178	100 (56.18)	78 (43.82)

Table 2: The incidence of oocysts of *Cryptosporidium* spp. in calves at monthly intervals as measured by percentage of positive samples

Technique used	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	April	May
Ziehl-Neelsen	100.0	100.0	73.33	85.71	86.66	93.33	60.0	66.66	57.14	26.66	26.66	26.66
Rapid staining	93.33	100.0	46.66	71.72	73.33	80.0	53.33	60.0	35.71	20.0	13.33	6.66

Table 3: The incidence of *Cryptosporidium* spp. in the faeces of calves (n=15) as measured by total number of oocysts

Technique used	Mean oocysts numbers in each month *													
	June	July	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	April	May	SED	P
Ziehl-Neelsen	57.40	35.73	13.0	10.43	19.07	10.87	4.53	3.80	3.93	1.47	0.80	1.13	4.57	0.000
Rapid staining	10.37	14.80	5.80	6.85	7.13	5.53	3.47	2.53	2.0	0.66	0.40	0.26	1.75	0.000

* = Mean calculated by examining 10 microscopic fields of each sample of each calf.

Table 4: The percentage prevalence of *Cryptosporidium* spp. in the faecal samples (178 samples) of various consistency

Technique used	Faecal consistency	No. of samples examined (%)	No. of positive samples (%)	No. of negative samples (%)
Ziehl-Neelsen	Soft	91 (51.12)	50 (28.09)	41(23.03)
	Diarrhoeic	55 (30.9)	45 (25.28)	10 (5.62)
	Diarrhoea	32 (17.98)	21 (11.80)	11 (6.18)
Rapid staining	Soft	91 (51.12)	40 (22.47)	51(28.65)
	Diarrhoeic	55 (30.90)	40 (22.47)	15 (8.43)
	Diarrhoea	32 (17.98)	19 (10.67)	13 (7.30)

Table 5: The percentage prevalence of *Cryptosporidium* spp. in the faeces (178 samples) of calves (n=15) housed and at grass

Technique used	Housed calves (December to May months)			Calves at grass (June to November months)		
	No. of samples examined (%)	No. of samples positive (%)	No. of samples negative (%)	No. of samples examined (%)	No. of samples positive (%)	No. of samples negative (%)
Ziehl-Neelsen	90 (50.56)	37 (20.78)	53 (29.78)	88 (49.43)	79 (44. 38)	9 (5.06)
Rapid staining	90 (50.56)	28 (15.73)	62 (34.83)	88 (49.43)	68 (38.20)	20 (11.24)

Table 6: The percentage of oocysts of the total number recorded from samples of calves while housed and at grass

Technique used	Calves housed (December-May, months % of total oocysts recorded while indoors (90 samples)	Calves at grass (June to Novemer, months) % of total oocysts recorded while at grass (88 samples)
Ziehl-Neelsen	9.39	90.61
Rapid staining	15.68	84.32

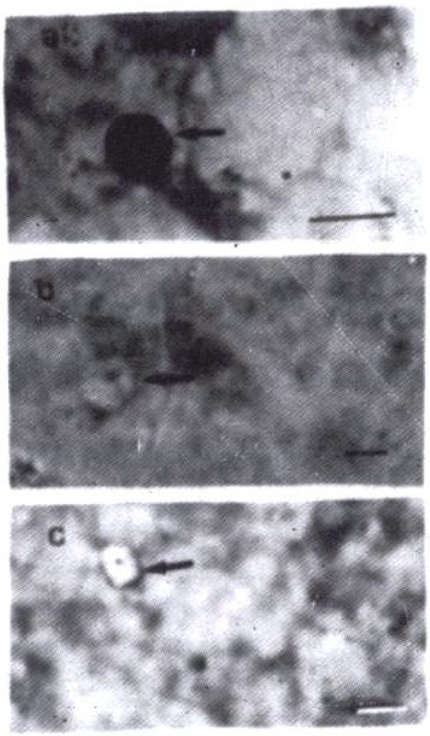

Plate 1: Staining characteristics of *Cryptosporidium* oocysts. (a). *Cryptosporidium* oocyst (←) in a faecal smear stained by the modified Ziehl-Neelsen method X 2520; (b). *Cryptosporidium* oocyst (←) in a faecal smear stained by the rapid staining technique X 1600; (c). *Cryptosporidium* oocyst (←) in a faecal smear of calf stained by the rapid staining technique X 1000. Scale bars represent 5 µm.

Table 7: The mean number of oocysts of *Cryptosporidium* spp. in the faecal samples of calves while housed and at grass

Technique used	Calves housed indoor	Calves at grass		
	Mean oocysts *	Mean oocysts *	SED	P
Ziehl-Neelsen	2.53	25.0	2.55	0.000
Rapid staining	1.57	8.34	0.77	0.000

* = Mean based on 10 microscopic fields of each sample of each animal.

Table 8: The size, shape and staining characteristics of *Cryptosporidium* spp. oocysts recorded from faeces of calves

Technique used	Size (µm)	Shape	Staining Characteristics
Ziehl-Neelsen	4.9 ± 0.8	Spherical to ovoidal	Red or pink against blue background, oocysts contain granular contents and surrounded by a clear halo
Rapid staining	4 4 ± 0.8	Spherical	Oocysts appear as small bodies with a blue or purple nucleus surrounded by a clear non-staining halo.

DISCUSSION

An investigation of *Cryptosporidium* spp. in 2-3 months old calves was carried out. Of the 178 faecal samples examined, 119 (66.58%) were found positive for *Cryptosporidium* spp. (Ziehl-Neelsen method) and 100 (56.18%) were positive with the rapid staining technique. These results are in agreement with those recorded by Manysheva (1990), who found 45.7% *Cryptosporidium* infection in calves under one month of age, while Rajkova and Jurasek (1989) found 76% prevalence in calves with diarrhoeal enteritis. However, no clinical cryptosporidiosis was observed in the present study.

Pohjola *et al.* (1986) reported 40% infection in asymptomatic calves under one month of age during their investigation which is also in line with the results obtained in this survey from asymptomatic older calves.

Our study indicates that asymptomatic *Cryptosporidium* infection is more common in young calves above one month of age. It is clear from the present study that the subclinical infection is always evident in young calves. However, according to Henriksen and Krogh (1985), subclinical cryptosporidiosis is often seen in calves less than 1 month of age.

During June, at the commencement of our survey, 100% (Ziehl-Neelsen method) incidence of *Cryptosporidium* was seen in calves, which decreased to 26.66% over a period of twelve months (May). It is difficult to compare these results with those of pervious workers since such studies could not be traced in the available literature. However, they do fit the general findings that initially animals with higher infection rates then decreased to lower, this is probably due to the development of immunity with age.

Faecal samples from a group of calves were collected and analyzed on the basis of their consistency. Higher number of soft faeces were found to be positive with oocysts compared to diarrhoeic and diarrhoea faeces. Manysheva (1990) recorded higher number of oocysts in diarrhoeic faeces. Sterba and Sulcova (1989) recorded 14% infection in non-diarrhoeic faeces (soft faeces) as compared to 41% in diarrhoea faeces.

Since a greater proportion of samples were positive when taken from the same animals when at grass compared with when housed, it appears that animals are at greater risk of infection when they are free grazing. However, Kaminjolo *et al.* (1993) found no significant difference in prevalence of *Cryptosporidium* infection in semi-intensive and extensive husbandry systems of management of calves in the West Indies. Similarly, Pavlasek *et al.* (1989) found no difference in the prevalence of *Cryptosporidium* infection between the calves under the two conditions i.e., inside and outside of the sheds. It is difficult to conclude from these findings whether animals are at greater risk of infection when housed indooors compared with those kept on free grazing since this is dependent on a number of factors. Thus under conditions of excellent hygiene, individually penned animals, which are well fed, are likely to have low infection rates. On the other hand, intensive indoor rearing under poor hygiene may contribute to heavy infection. It obviously depends on the previous history of infection and the husbandry management involved.

A variation in the morphological characteristics of oocysts of *Cryptosporidium* species was seen using two techniques. The size and shape of *Cryptosporidium* are similar to those recorded by Upton and Current (1985) for *Cryptosporidium parvum* and are also in consistent with the descriptions of Ball (1989) and Cross and Moorhead (1984).

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