

Some associated factors with *Cryptosporidium* infection in lambs and dogs

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Abstract

The use of MZN technique revealed the detection of *Cryptosporidium* spp. Oocysts in 26 out of 254 (10.24%) fecal samples collected from diarrheic and apparently healthy calves in Behera and Menoufia governorates. There were significant associations between infection of calves with *Cryptosporidium* spp. and their age and health status. On the other hand, there were no significant association between infection of calves and their sex and season of the year.

By using MZN technique, *Cryptosporidium* spp. Oocysts have been detected in 31 (12.4%) out of 250 lamb samples in both governorates. In studying the relation between infection with *Cryptosporidium* and the age of lambs, the relation was significant; denoting that the age of lamb is an important factor in susceptibility to infection. By the use of MZI technique, *Cryptosporidium* oocysts have been detected in 2 (3.84%) out of 52 clinically healthy dogs in Behera governorate. The positive MZN samples were retested by using ELISA. The sensitivity of the test was clear as 25 (96%) out of 26 calf samples; 29 (94%) out of 31 of lamb samples and 2 (100%) out of 2 dog samples were positives with ELISA. The overall sensitivity of the commercially available ELISA was (96.6%) for all samples.

Introduction:

Cryptosporidium is an enteric coccidian parasite that causes diarrhea in a wide range of vertebrates worldwide. *Cryptosporidium* infection of livestock may have an important economic impact to farmers because of high morbidity and sometimes mortality rates among farm animals (Casmore et al., 1997). Recently, the clinical picture of cryptosporidiosis was changed from that of rare and largely asymptomatic infection to an important cause of diarrhea in calves, lambs and also it is an important cause of diarrhea and enterocolitis in human beings especially in immunocompromised and children and in groups involved in cryptosporidiosis is widely considered a disease of neonates, however sub clinical infection have been reported in older and less frequently in adult animals (Nouri and Toroghi, 1991).

Cryptosporidium can occur either alone or together with other enteric pathogens: bacterial or viral (Bjorkman et al., 2003). The ileum always harbors the early developmental stages of the parasite, some of these stages could be found in the enterocytes of the duodenum, jejunum, caecum and colon (Current and Ree

A significant relationship was found between *C. parvum* infection and the age of calves (De La Fuente et al., 1999). Atwill et al. (1999) and O'Handley et al., (1999) that *Cryptosporidium parvum* shedding in dairy calves occurring at 8-14 days of age. Calves that infected with *C. parvum* had a significantly higher rate of diarrhea than non-infected calves (Uga et al., 2000). Calf to calf contact appeared to be the main source of transmission. *Cryptosporidium* oocysts excreted with feces of infected animals, particularly calves can be a source of human infection having a significant influence on public health (Mirion et al., 1991 and O' Handley et al., 1999).

In contrast, there is a less information on the occurrence of cryptosporidiosis in sheep. The infection often causes death of diarrheic lambs and the intensity of infection is higher in lambs than in sheep (Olseon et al., 1997 and Viera et al., 1997). The presence of *C. parvum* oocysts in feces of apparently healthy dogs help in maintaining the environmental contamination and provide infection for the other susceptible animals and human beings (Ederli et al., 2005).

Materials and methods

This study was carried out in some rural areas of Behera and West Bengal governorates throughout a period of one year. A total of 254 fecal samples were collected from diarrheic (150) and apparently healthy (104) calves ranging from 1 day- 3 months in age. Moreover, 250 fecal samples were collected from diarrheic and apparently healthy lambs (1 day – 3 months old) in the same areas. In addition, 250 fecal samples were collected from clinically healthy dogs ranging in age from 1 day – 2 years old) in Behera governorate only. All the collected samples were identified by recording the locality, sex, age, health state and character of fecal matter of the animal. In the laboratory, 1 g of each fecal sample was emulsified in 10% formalin solution and preserved until performing MZN technique, then approximately 5 g of emulsion sample was mixed with 2.5% potassium dichromate solution and kept at 4°C for the detection of *Cryptosporidium parvum* by ELISA.

Modified Ziel-Neelson staining:

Cryptosporidium spp. Oocysts were stained with modified Ziel-Neelson stain and examined microscopically according to (Henrikson and Pohlenz, 1981).

ELISA:

ELISA was performed for detection and confirmation of the MZN positive samples using (RIDIASCREEN test C-1201 GmbH, Darmstadt, Germany) according to (Uga et al., 1990).

Statistical analysis was done using Chi-square test "X²" according to (Hill, 1971).

Results and Discussion:

Cryptosporidium parvum is a ubiquitous coccidian parasite that cause di many mammalian species. It is the second most common pathogen from your with diarrhea (Hall et al., 1992).

Table (1) showed that the incidence of *Cryptosporidium parvum* in faecal from calves as examined by MZN staining was 26 (10.24%). Among the samples 25 (9.83%) were confirmed by using ELISA. Bogaaerts et al. (1987) al. (2000) and Isaacs et al. (1985) stated that MZN staining technique has been used as a reliable method for detection of *Cryptosporidium* spp. oocysts samples since it allows the observation of *C. parvum* oocysts at lower mag power and solves the problem of differential diagnosis related to the pre yeasts. Higher detection rates were recorded by Abo-El-Magd and Haiba (19 demonstrated the parasite in the faeces of 185 out of 246 clinical diarrheic calves (1-5 weeks old) at two different localities (Quena and Naga-Hamadi).

Furthermore, the 26 MZN (+ve) samples were retested and confirmed by Ridascreen *Cryptosporidium* (an enzyme linked immunoassay for detection of *Cryptosporidium*). Out of 26 MZN (+ve) samples, 25 samples were (+ve) by ELISA. Different workers compared the sensitivity and specificity of ELISA to the microscopic detection of *Cryptosporidium* oocysts in faecal samples. McClusky et al. (1997) indicated a moderate agreement between the two diagnostic methods, with ELISA being the more sensitive of the two. Majewska et al. (2000) showed that both methods showed the same sensitivity. Moreover, Sreter and Varga (2000) and Marquardt et al. (2003) stated that the sensitivity and specificity of some ELISA tests render ELISA a good tool in serology based epidemiological investigations or screening of environmental samples. Table (2) showed that a nearly similar number of samples were collected from both Governorates (Behera and Menoufia) and the incidence rate was nearly the same. Out of 128 and 126 faecal samples collected from calves at Bahariya and Menoufia Governorates, 14 (10.94%) and 12 (9.52%), respectively tested positive for *C. parvum* oocysts.

The association between *C. parvum* infection and age of the examined calves was demonstrated in table (3) and Fig. (1). A total of 254 calves were grouped according to their age during the course of the study into three age groups: 132 calves (<1 month), 59 (1-2 month) and 63 (2-3 month). *C. parvum* oocysts were detected in 20 (15.15%) and 3 (4.76%) of the examined calf groups respectively with incidence rate being in the 1st age group (<1 month of age). The Chi square value was 7.2339*. In cattle, clinical infections seem largely confined to new born calves 7-21 days of age (Garber et al., 1994). The prepatent period of 5-12 days follows oocyst shedding which is usually coincident with the onset of diarrhea. The results agreed with those of Ares-Mazas et al. (1999) who examined faecal

from 101 bovine calves in a farm in northern Spain. They detected that 26% were infected and the incidence of infection was 81% in age group I (2-36 days) and 0 in age group III (20-24 months). This primary infection occurred early in the neonatal period and the environment was contaminated with oocysts. Initial exposure to infective oocysts appears to occur in a maternity pen or shortly after placement in outdoor cages. However, the role of apparently healthy carriers in the epidemiology of the disease has more recently been described. Excretion of oocysts has been found in apparently healthy adult calves (Villacorta et al., 1991 and Scott et al., 1994).

Out of 150 diarrheic calves, 21 (14%) excreted *C. parvum* oocysts in their faeces, while out of 104 apparently healthy calves, only 5 (4.8%) excreted *C. parvum* oocysts in their feces (table 4 and figure 2). Statistical analysis of these results revealed that there was a significant association between infection with *C. parvum* and the presence of diarrhea in calves ($P < 0.05$) denoting that *C. parvum* is an important cause of calf diarrhea. Hall et al. (1992) stated that *C. Parvum* is the second most common pathogen from young calves with diarrhea. The obtained results in the present study were in agreement with those of Naciri et al. (1999) who recorded the presence of *C. parvum* oocysts in the feces of diarrheic suckling and dairy calves in France at percentages of 34.7 and 2.4, respectively, while *C. parvum* were detected in non diarrheic suckling and dairy calves at percentages of 13.8 and 3.9, respectively. Furthermore, Uga et al. (2000) reported that calves infected with *Cryptosporidium parvum* had a significant higher rate of diarrhea (33%) than non infected calves ($P < 0.05$) suggesting that *C. parvum* infection was likely the cause.

The incidence of *C. parvum* infection in calves were, 5 (10%); 8 (10.34%) and 7 (7.29%) during winter, spring, summer and autumn, respectively (table 5). Statistical analysis of these results revealed no significant association ($P > 0.05$) between infection with *C. parvum* in calves and season in the two investigated Governorates. These results agreed with those reported by Wade et al. (2000) and Becher et al. (2004) who reported that there was no significant association between the parasite occurrence and season. On the other hand, Mann et al. (1986) and Sahal et al. (2005) in Turkey recorded that the occurrence of the disease was more common in winter (56.4%) than during other seasons (autumn 0%, summer 15.4% and spring 28.2%).

Among 250 faecal samples collected from lambs, the protozoan oocysts were detected in 31 (12.4%) of the examined samples as examined by MZN technique. Of the 31 (+ve) faecal samples were further tested using ELISA which revealed the presence of Cryptosporidial antigens at a percentage of 29 (11.6%). Nearly similar results were obtained by Majewska et al. (2000) who detected 16 (10.1%) out of 159 different ages by using MZN staining technique and ELISA.

Out of 127 and 123 faecal samples from lambs in Behera and Governorates, 15 (11.81%) and 16 (13%), respectively were positive Cryptosporidial oocysts. There was no significant difference in the detected between both Governorates (table 7). The examined lambs were categorized into three age groups 107 (< 1 month of age); 80 (<2 month of age) and 63 (< 3 month of age). Cryptosporidial oocyst were detected in the faeces of the examined age groups with incidence rates of 21 (19.63%); 7 (8.75%) and 3 (4.76%), respectively (table 8). The results agreed with those of Olson et al. (1997) in Canada, Majewaska et al. (1997) in the West-Central region of Poland and Noordeen et al. (2000) in Sri Lanka. The present work indicated that there was a highly significant association between infection with *Cryptosporidium parvum* and the age of lambs. Concerning the association between infection with *Cryptosporidium parvum* and sex of the examined lambs, table (13) showed that there was no significant association between sex of lambs and infection with the protozoan.

The incidence of *Cryptosporidium Parvum* Infection in faecal samples from lambs examined by MZN staining technique and confirmed by ELISA was shown in table (11). By the use of MZN staining technique, *Cryptosporidium* spp. oocysts were first detected in 2 (3.84%) out of 52 clinically healthy dogs from Behera Governorate. The results reported in the present study are in agreement with Huber et al. (2002) who reported a prevalence of 2.41% among clinically healthy dogs living in two types of environments (animal shelters and household pets) in Brazil. The presence of *C. parvum* in faecal samples from apparently healthy dogs from Behera Governorate indicates the maintaining of the environmental contamination and provide infection for susceptible animals and human beings.

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Table [1]: Incidence of *Cryptosporidium Parvum* Infection in fecal samples from dairy calves as examined by MZN staining technique and confirmed by ELISA

Test	+ve cases		-ve cases		Total	
	No	%	No.	%	No.	%
MZN	26	10.24%	228	89.76%	254	100%
ELISA	25	9.83%	1	3.8%	26	100%

Table [2]: The association between the infection with *C. parvum* and locality of the examined calves.

locality	n =	+ ve cases		- ve cases	
		No.	%	No.	%
Behera governorate	128	14	10.94%	114	89.06%
Menoufia governorate	126	12	9.52%	114	91.48%
Chi sq. value $X^2 = 0.58$				P > 0.05	

Table [3]: The association between infection with *C. parvum* and age of the examined calves.

Age group	n =254	+ve cases		-ve cases	
		No.	%	No.	%
1 st age group (<1 month)	132	20	15.15 %	112	84.85
2 nd age group (<2month)	59	3	5.08%	56	94.92
3 rd age group (<3month)	63	3	4.76%	60	95.24
Chi sq. value $X^2 = 7.2339^*$				P < 0.05	

Table [4]: The association between infection with *C. parvum* and health status of the examined calves.

Health status	n =254	+ ve cases		- ve cases	
		No.	%	No.	%
Diarrheic calves	150	21	14%	129	86%
Non-diarrheic calves	104	5	4.8%	99	95.2%
Chi sq. value $X^2 = 5.647^*$				P < 0.05	

Table [5] the association between infection with *C. Parvum* and sex of the examined calves.

Sex	n =	+ ve cases		- ve cases	
		No.	%	No.	%
Samples with ♂ sex.	138	14	10.14%	124	89.86%
Samples with ♀ sex.	116	12	10.34%	104	89.66%
Chi sq. value $X^2 = 0.001$				P > 0.05	

Table [6] The association between infection with *C. Parvum* in calves and season of the year.

season	n =	+ve cases		-ve cases	
		No.	%	No.	%
winter	50	5	10%	45	90%
spring	50	8	16%	42	84%
summer	58	6	10.34%	52	89.46%
autumn	96	7	7.29%	89	92.71%
Chi sq. value $X^2 = 2.81$				P > 0.05	

Table (7) Incidence of *Cryptosporidium Parvum* Infection in fecal samples of lambs as examined by MZN staining technique and confirmed by ELISA

Test	+ve cases		-ve cases		Total	
	No.	%	No.	%	No.	%
MZN	31	12.4%	219	87.6%	250	100
ELISA	29	11.6%	2	6.45%	31	100

Table [8] The association between the infection with *C. parvum* and localities of the examined lambs.

locality	n =	+ ve cases		- ve cases	
		No.	%	No.	%
Behera governorate	127	15	11.81%	112	88.19%
Menoufia governorate	123	16	13%	107	87%
Chi sq. value $X^2 = 0.023$				P > 0.05	

Table [9] The association between the infection with *C. parvum* and the age groups of the examined lambs.

Age group	n =	+ve cases		-ve cases	
		No.	%	No.	%
1 st age group (<1 month)	107	21	19.63 %	86	80.37
2 nd age group (<2month)	80	7	8.75%	73	91.25
3 rd age group (<3month)	63	3	4.76%	60	95.24
Chi sq. value $X^2 = 9.5^{**}$				P < 0.05	

Table [10] The association between the infection with *C. parvum* and of the examined lambs.

Sex	n =	+ ve cases		- ve cases	
		No.	%	No.	%
♂ Samples.	121	14	11.57%	107	88.43%
♀ Samples.	129	17	13.17%	112	86.83%
Chi sq. value $X^2 = 0.148$				P > 0.05	

Table (11) Incidence of *Cryptosporidium Parvum* Infection in fecal samples from dogs as examined by MZN staining technique and confirmed by

Test	+ve cases		-ve cases		Total	
	No.	%	No.	%	No.	%
MZN	2	3.84%	50	96.16%	52	100%
ELISA	2	3.84%	0	0%	2	100%

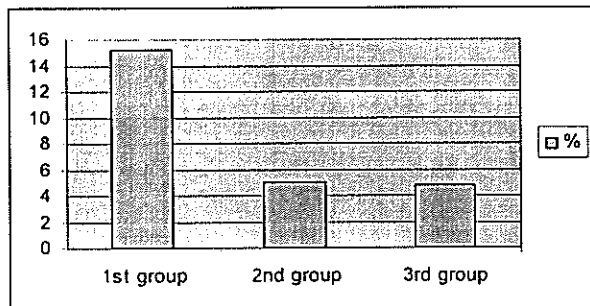
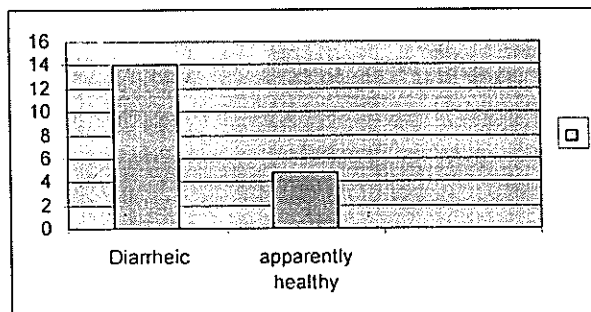
Fig. [1]: The association between infection with *C. parvum* and age of the examined calves.Fig. [2]: The association between infection with *C. parvum* and health status of the examined calves.

Fig. [3]: The association between the infection with *C. parvum* and the age examined lambs.

