PREVALENCE OF CRYPTOSPORIDIOSIS IN BUFFALO CALVES OF JABALPUR, INDIA

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ABSTRACT

Faecal samples from buffalo calves of Jabalpur district were studied from September 2011 to August 2012 using modified Ziehl-Neelsen technique (mZN). An overall positivity of 21.4% was recorded. The prevalence was higher (25.5%) in diarrhoeic as compared to non-diarrhoeic (17.0%) calves. The risk of infection was two times higher in diarrhoeic than non-diarrhoeic calves. As per age, highest prevalence (29.7%) was observed in calves of <1 month and it varied significantly (P<0.05) from >3 months age group. The chances of occurance of *Cryptosporidium* spp. in calves of <1 month age group was three times higher than >3 month age group. The prevalence in male calves was significantly (P<0.05) higher (32.2%) than female calves (15.9%). The risk was ~3 times higher in male than female. Season wise highest prevalence was observed in winter (30.1%) and lowest in summer (7.94%). The risk of infection was five times higher in winter and four times higher in rainy season as compared to summer. Diarrhoeic faecal samples having mucus (32.8%) showed significantly (P<0.05) higher prevalence than those having blood (7.40%).

Keywords: *Bubalus bubalis*, buffaloes, *Cryptosporidium*, buffalo calves, modified Ziehl-Neelsen technique

INTRODUCTION

Cryptosporidiosis is a widespread parasitic disease caused by obligate and opportunistic parasite of the genus *Cryptosporidium* (Tyzzer, 1907), which develop and multiply in the epithelial cells of the intestines and respiratory tracts of vertebrates, and for which 152 mammalian hosts have been described (Fayer *et al*., 2000). *Cryptosporidium* spp. is considered an important co-factor in neonatal diarrhoea in cattle, sheep, goats, and buffaloes (*Bubalus bubalis*). This protozoon species has been detected in buffaloes in Italy (Condoleo *et al*., 2007), Spain (Gomez-Couso

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et al., 2005), Egypt (El-Khodery and Osman, 2008), Cuba (Rodriguez-Diego *et al*., 1991), India (Dubey *et al*., 1992) and Brazil (Araujo *et al*., 1996). However, data on the distribution of this protozoan in buffaloes are quite fragmentary and studied with a systematic epidemiological approach in Central India are lacking. For these reasons, a cross-sectional survey aimed to study the presence and distribution of *Cryptosporidium* spp. in buffaloes of the Jabalpur, Madhya Pradesh was done.

MATERIALS AND METHODS

Study area

The study was undertaken in the most densely populated buffalo tract of Asia, located in Jabalpur, Madhya Pradesh. It is situated at 23.17^{o} latitude and 79.57^{o} longitude at 410.87 MSL (metres above sea level) in Southern part of agro-climatic zone *viz*., Kymore plateau and Satpura hills and has an average rainfall of 1241 mm.

Collection of samples

A total of 182 faecal samples (diarrhoeic and non-diarrhoeic) from buffalo calves of three age groups (<1 month, 1 to 3 month, >3 months) were collected from government and private dairy farms located in and around Jabalpur during the period from September 2011 to August 2012. Faecal sample was collected from rectum of each animal in clean, sterile stool container using separate gloves. The samples were transported on ice and stored at 4^{o}C until analysis. Whenever immediate processing was not possible, the samples were put in 2.5% potassium dichromate solution, kept at 4^{o}C and then brought to laboratory. At the time of faecal sample collection, data related to age, sex along with the season and consistency of faeces were recorded. Permission to carry out the work was taken from institutional Animal Ethical Committee of Nanaji Deshmukh Veterinary Science University, Jabalpur (M.P.).

Sample examination

Fecal smears were prepared and stained using modified Ziehl-Neelsen stain (Henricksen and Pohlenz, 1981). Intensity of infection was scored by counting the cryptosporidial oocysts semi-quantitatively according to Castro-Hermida *et al*. (2002) by finding the average number of oocysts in 20 randomly selected fields at 100x; Score 0 (0 oocysts), +1 (1-5 oocysts), +2 (6-10 oocysts) and +3 (more than 10 oocysts).

Statistical analysis

A chi-square test of independence was used to investigate possible associations between categorical variables and a positive result. Results were considered to be significant if $P<0.05$. Odds ratios (OR) and their 95% confidence intervals were calculated as per the methods described by Thrusfield (2007).

RESULTS AND DISCUSSION

In positive faecal samples at microscopy the oocysts appeared dark pinkish, 4 to 7 µm in diameter having crescentric forms of sporozoite against a blue background of methylene blue (Figure 1). The overall prevalence of *Cryptosporidium* spp. in buffalo calves of Jabalpur was 21.4% (Table 1). The prevalence of *Cryptosporidium* infection in buffalo calves recorded in the present study was higher than those reported from India (11.94%, Yadav *et al*., 2012), Egypt (14.19%, El- Khodery and Osman, 2008) and the Italy (14.7%, Condoleo *et al*. 2007) but lower than that reported by Bhat

Table 1. Prevalence, risk and intensity of *Cryptosporidium* spp. in buffalo calves of Jabalpur district.

Parameters	Category	Total number of animals			Odd ratio (95% CI)	Intensity of *Cryptosporidium* oocyst		
		Examined	Positive	% Positive		+1 (Low score)	+2 (Medium score)	+3 (High score)
Age	<1 month	64	19[p]	29.7	3.02 (1.07-8.79)	6 (31.6)	9 (47.4)	4 (21.0)
	1-3 months	61	13[pq]	21.3	1.93 (0.65-5.92)	5 (38.5)	6 (46.1)	2 (15.4)
	>3 months	57	7[q]	12.3	0.33 (0.11-0.94)	3 (42.8)	3 (42.8)	1 (14.3)
Sex	Male	56	19[p]	32.2	2.72 (1.23-6.02)	6 (31.6)	10 (52.6)	3 (15.8)
	Female	126	20[q]	15.9	0.37 (0.17-0.81)	8 (40.0)	8 (40.0)	4 (20.0)
Season	Summer	63	5[p]	7.94	0.20 (0.06-0.61)	5 (100.0)	0 (0.0)	0 (0.0)
	Rainy	46	12[q]	26.1	4.09 (1.19-14.75)	5 (41.7)	7 (58.3)	0 (0.0)
	Winter	73	22[q]	30.1	5.00 (1.63-16.37)	4 (18.2)	11 (50.0)	7 (31.8)
Faecal consistency	Diarrhoeic	94	24[p]	25.5	1.67 (0.76-3.66)	9 (37.5)	11 (45.8)	4 (16.7)
	Non-diarrhoeic	88	15[p]	17.0	0.59 (0.27-1.31)	5 (33.3)	7 (46.7)	3 (20.0)
Diarrhoea	with mucus	67	22[p]	32.8	6.11 (1.23-41.03)	-	-	-
	with blood	27	2[q]	7.40	0.16 (0.02-0.81)	-	-	-

[p,q] Different superscripts indicate significant ($P<0.05$) difference between the categories within a parameter.

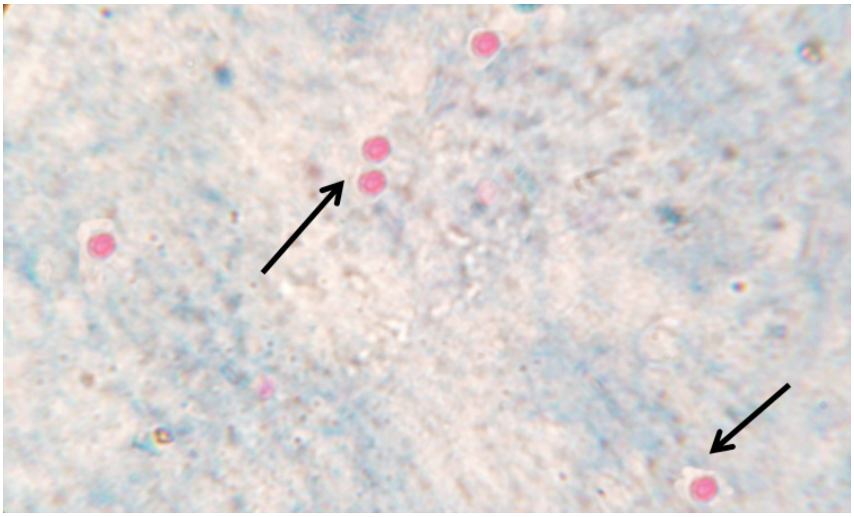

Figure 1. *Cryptosporidium* oocyst in faeces, modified Ziehl Neelsen staining (100x).

et al. (2012) from Punjab (38.3%). The variation in prevalence rates may be due to differences in management, climate, study design and screening methods used. The actual prevalence of cryptosporidiosis among the buffalo calves of the target area could also be influenced by the fact that only one sample per calf was examined which could be negative during a period when the animal was experiencing intermittent oocyst excretion (Fayer *et al*., 2007).

The prevalence of *Cryptosporidium* spp. in diarrhoeic calves was higher (25.5%) than non-diarrhoeic calves (17.0%). The risk/odd ratio indicated that the diarrhoeic calves were at higher risk (OR=1.67) than non-diarrhoeic calves (Table 1). The constant association of diarrhoea and presence of oocyst of *Cryptosporidium* in the faeces has been recorded by many other workers (Quilez *et al*., 1996; Wade *et al*., 2000; Lise *et al*., 2005; Roy *et al*., 2006; Singh *et al*., 2006; Maddox-Hyttel *et al*., 2006; Geurden *et al*., 2006; Roy *et al*., 2010; Safavi *et al*., 2011; Yadav *et al*., 2012). The intensity score of oocyst in both diarrhoeic and non-diarrhoeic faecal samples showed similar trend, the medium intensity (+2) score being higher than low and high score. Castro-Hermida *et al*. (2002) on the other hand found a significant association between the intensity of infection and the consistency of the faeces ($P<0.001$). None of the calves with solid faeces had severe infection. Wu *et al*. (2010) observed positive relationship between the change in faecal consistency and oocyst per gram. They found diarrhoea was significantly higher during the oocyst-positive period than during either oocyst-pre-excretion or oocyst disappearance periods. Such relation could not be recorded in our study because it requires a detailed follow up of the dynamics of cryptosporidial oocysts excretion on daily basis.

The prevalence in <1 month age group was significantly ($P<0.05$) higher from >3 months age group. The higher prevalence observed in 1 to 3 months age group than >3 months age group was found non-significant. The odd ratio indicated that the chance of occurrence of cryptosporidiosis in <1 month age group was 3.02 times higher as compared to >3 months age group whereas in 1 to 3 months age group calves it was 1.93 times higher than >3 months age group (Table 1). Many studies have reported an inverse association between age and cryptosporidiosis with the highest prevalence reported in pre-weaned calves (Maldonado-Camargo *et al*., 1998; Santin *et al*., 2004; Geurden *et al*., 2006; Trotz *et al*., 2007; Brook *et al*., 2008; Paul *et al*., 2008; Santin *et al*., 2008). This observation is consistent with the hypothesis that dairy calves become infected with *C. parvum* either from the dam while in the calving pen or from pens contaminated with oocysts. In addition, large groups, increase the potential for transmission through close physical contact and the tendency of calves to lick surfaces contaminated with faeces resulting in the ingestion of pathogens, thus completing the faecal-oral transmission pathway (Pell, 1997; Becher *et al*., 2004). Other sources of infection may be through farm staff moving from calf to calf or via mice and house-flies (Graczyk *et al*., 1999). Uninfected young calves typically have low percentages of CD4+ and CD8+ lymphocytes in the intraepithelial and lamina propria regions, although following infection, total intraepithelial lymphocytes and the percentages of CD4+ and CD8+ cells increase (Ruest *et al*., 1997; Fayer *et al*., 1998). These T-lymphocyte subsets have been reported to control cryptosporidial infections in mice (Ungar *et al*., 1991; Chen *et al*., 1993; McDonald *et al*., 1994); thus, the relative lack of these cells in young calves could explain their high susceptibility to *C. parvum* infection. The rate of prevalence decreased proportionally with the increase in age of the target animals with lowest prevalence recorded in calves of 3 to 6 months of age. The present observation is supported by the earlier findings of Kumar *et al.* (2004); Shobhamani *et al.* (2006) from India. In present study, the calves of less than 1 month of age group showed higher medium intensity score (47.4%) than low (31.6%) and high score (21.0%). In calves of 1 to 3 and >3 months of age group, the medium intensity score of oocyst was higher followed by low and high score (Table 1). About 68% calves of <1 month of age group had oocyst intensity score of ≥+2 while lesser, 61% and 57% calves of 1 to 3 and >3 months of age, respectively showed ≥+2 oocyst intensity score which indicated that oocyst excretion intensity decreases with the age. Likewise, Saha *et al*. (2006) reported a decline in the rate of infection with increase in the age of calves after 1 month of age. Del Coco *et al*. (2008); Singh *et al*. (2006) also recorded similar relationship between intensity of infection and age group. Uga *et al*. (2000) suggested that the oocyst detection rate can vary greatly depending on the age of the calves. The younger the animal when it acquired infection, the longer the period of oocyst shedding. Hamnes *et al*. (2006); Wu *et al*. (2010) also reported that the intensity of *Cryptosporidium* infection decline with age.

The prevalence of *Cryptosporidium* spp. infection in buffalo male calves was significantly ($P<0.05$) higher (32.2%) than female calves (15.9%). The risk of occurrence was ≈3 times higher (OR=2.72) in male than female (Table 1). The results are in accordance with the findings of Paul *et al.* (2008); Nouri and Toroghi (1994) but are in contrary to Prakash *et al.* (2009); Maurya *et al*. (2013); Bhat *et al*. (2012) who observed higher prevalence in female calves. Rehman *et al*. (1985); Shobhamani (2005) on the other hand observed that *Cryptosporidium* infection among the dairy calves was unrelated to sex. It was found that in both male and female calves the medium intensity score (+2) was more common than low (+1) and high (+3) score. Approximately 68% male calves and 60% female calves had an intensity score of ≥+2 indicating greater intensity of infection in male calves as compared to females. The reason for higher prevalence and intensity of infection in male calves may be that they are generally neglected as present day agriculture has become more mechanized, so males are not used for draught purpose. Further, due to religious sentiments their slaughter is prohibited in many states. All these factors predispose male bovine calves to poor feeding and management, which in turn lowers their immunity and predisposes them to various pathogens of biological origin (Yadav, 2010). Prevalence of *Cryptosporidium* spp. was highest during winter season (30.1%) followed by rainy (26.1%) and lowest in summer season (7.94%). The risk of occurrence was five times higher (OR=5.00) in winter and 4.09 times higher in rainy season as compared to summer (Table 1). One of the reasons for higher prevalence in winter may be that the temperature is suitable for viability and survival of *Cryptosporidium* oocysts. This can be supported by observation of Fayer *et al.* (1998) and Jenkins *et al.* (2003) who observed that *Cryptosporidium* oocysts can remain viable and infective for 4 to 5 months at 5 to 20^{o}C. Garber *et al.* (1994) attributed the high prevalence of cryptosporidiosis in winter (December to February) to presence of large number of calves at risk as a result of concentration of calving in winter months. It was observed that animals in organised farms were overcrowded in winter months, resulting in easy availability of infective *Cryptosporidium* oocysts to susceptible calves. Overcrowding has been suggested for increased prevalence of *Cryptosporidium* by many other workers (Garber *et al*., 1994; Quigley *et al*., 1994; Mohammed *et al*., 1999). They reported that higher the density of animals, greater the chances of infection and consequently the higher prevalence.

Infective faecal material run off to the drinking water sources during rains and thus increases the chances of contact between infective oocysts and host. This may be the reason for higher infection in rainy than summer season as observed in the present study and has been reported by other workers from India (Roy *et al*., 2006; Paul *et al*., 2008) and abroad (Chai *et al*., 2001). Lowest prevalence in summer found in the present study may be supported by the observation of Anderson (1986) who recorded that warm temperature of 18 to 29^{o}C is partially responsible for loss of infectivity of oocyst. The results of higher prevalence in winter season observed in the present study are similar to those reported by Das *et al.* (2004); Roy *et al*. (2010) from West Bengal. Similar findings were recorded by many workers (Tzipori *et al*., 1983; Lefay *et al*., 2000; El-Khodrey and Osman, 2008).

Season wise analysis showed that in summer season, low (+1) intensity infection was most common and none of the samples showed medium (+2) and high (+3) intensity score. In rainy season medium intensity (+2) infection seemed to be more common than low (+1) score. No sample showed a high (+3) score in rainy season. In winter season, the most common intensity score was medium (+2) followed by high (+3); the total of ≥+2 score being recorded in 82% faecal samples (Table 1). Thus, seasonal analysis revealed that calves excreted higher oocyst in winter season than rainy and summer season. Hamnes *et al*. (2006) recorded similar *Cryptosporidium* oocyst shedding intensities; higher in winter season than in summer.

Calves having mucus in the diarrhoeic faeces showed significantly ($P<0.05$) higher prevalence of cryptosporidiosis (32.8%) than those having blood in the faeces (7.40%). Diarrhoeic calves with mucus in faeces had six times more chances (OR=6.11) of occurrence of *Cryptosporidium* spp. infection than diarrhoeic animals having blood in faeces (Table 1). Association of mucus with *Cryptosporidium* infection as noticed in present study has also been reported by Del Coco *et al.* (2008); Yadav *et al*. (2012). Castro-Hermida *et al*. (2002); El-Khodery and Osman (2008) found a significant association (P<0.01) between *Cryptosporidium* infection and the presence of mucus in the faeces. *Cryptosporidium* infection usually does not cause bloody diarrhoea due to the very superficial location of the parasite (Heine *et al*., 1984; Aurich *et al*., 1990; Blewett and Angus, 1994). The co-existence of other enteropathogens (rotavirus, coronavirus and *Salomonella*) with *C. parvum* in calves is already on records (de la Fuente, 1999) and they may be responsible for association of blood and *Cryptosporidium* infection as observed in some calves in present study.

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