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Studies on the prevalence of cryptosporidiosis in bovines in organized dairy farms in and around Bangalore, South India

Rekha Hoskere K. Mallinath, Puttalakshmamma G. Chikkachowdappa, Ananda K. Javare Gowda*, and Placid E. D'Souza

Centre of Advanced Studies, Department of Veterinary Parasitology, Veterinary College, Karnataka Veterinary, Animal and Fisheries Sciences University, Bangalore, India

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ABSTRACT

The authors studied the prevalence of cryptosporidiosis by screening 455 bovine fecal samples collected from five different organized dairy farms and veterinary hospitals located in and around Bangalore, South India. The fecal samples were examined by Sheather's sugar flotation method for detection of oocysts, 5.71 percent were found positive for cryptosporidiosis. The species were identified as *Cryptosporidium parvum* and *Cryptosporidium andersoni* based on the morphology and micrometry of the oocysts by Modified Ziehl-Neelsen staining**,** Kinyoun's staining method and Safranin methylene blue staining methods. The prevalence and intensity of cryptosporidiosis was found more in calves of less than one month of age compared to adults and more frequently seen in diarrheic than in non-diarrheic samples. The highest prevalence of cryptosporidiosis was found in the HF crossbreeds followed by Jersey cross. No cryptosporidium oocysts were found in Deoni, Surti buffalo calves and non-descript breeds. The sex wise prevalence of cryptosporidiosis was observed more in females compared to males. A statistically significant difference was found between sexes, age and breed prevalence of cryptosporidiosis. Finally it was concluded that the prevalence of cryptosporidiosis in bovines in this region is under diagnosed and the sub clinical status of infection is potentially high.

Key words: cryptosporidiosis, prevalence, bovines, oocysts, *C. parvum, C. andersoni*

Introduction

Cryptosporidiosis is an emerging zoonotic disease of global importance caused by the apicomplexan protozoan parasite. *Cryptosporidium* inhabits the microvilli of the epithelial surface of the gastrointestinal and respiratory tracts of a wide variety of vertebrates, including humans, causing significant morbidity and mortality, which now represents the third major cause of diarrhoeal disease world wide (SPANO and CRISANTI, 2000).

*Corresponding author:

Dr. K. J. Ananda, M.V.Sc, PhD, Assistant Professor and Head, Department of Parasitology, Veterinary College, Vidyanagar, Shimoga-577203, Karnataka, India, Phone: +09845313244, +08182320920, Fax: +08182240330, E-mail: dranandakj@yahoo.co.in

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This monoxenous parasite completes its entire life cycle within a single host. The common feature in this species is the presence of four naked sporozoites, which are contained within a thick walled oocyst without sporocysts. Transmission is mainly by ingestion of sporulated oocysts through contaminated feed and water. Cross infection occurs between domestic and laboratory animals and man.

Cryptosporidium infection is well known as a major cause of morbidity and mortality particularly in immunocompromised hosts and young animals (GRAFF et al., 1999). It causes self-limited watery diarrhoea in immunocompetent subjects but has far more devastating effects on immunocompromised patients and in some cases can be life-threatening due to dehydration caused by chronic diarrhoea (CACCIO, 2005; CHEN et al., 2005). The economic losses associated with this disease are not only due to the resulting mortality, but also to the retarded growth of the animals, the cost of drugs, veterinary assistance and the increased labour involved.

Cryptosporidiosis in cattle has been reported from different parts of the world with approximately 45.5% incidence in USA, 24.5% in UK, 26% in USSR, 40% in Germany and 27% in Hungary (KUMAR et al., 2005). In India the *Cryptosporidium* oocysts were first demonstrated by (DUBEY et al., 1992) in the faeces of buffaloes and zebu cattle. The present study was undertaken to observe the actual status of cryptosporidiosis in bovines in organized dairy farms in and around Bangalore, South India.

Materials and methods

To study the prevalence of cryptosporidiosis, faecal samples of bovines were collected from five different organized dairy farms and veterinary hospitals in 2007 in and around Bangalore.

The faecal samples were collected directly from the rectum in a plastic container with a detailed history about age group, breed and sex and then labelled with particulars of individual animals on the container. Each sample was studied macroscopically to establish its consistency as liquid, soft or solid, and the presence of mucus or blood was noted. The samples were then stored at 4 °C in a refrigerator until examination. Later the samples were examined by Sheather's sugar flotation method for detection of cryptosporidium oocysts. The Potassium hydroxide treatment of faecal samples was done as per the procedure described by (GARCIA et al., 1983) to clear the mucus content of faeces.

The positive samples were then subjected to Modified Ziehl-Neelsen staining; Kinyoun's staining method and Safranin methylene blue staining methods for morphological studies. The positive samples were stored in 2.5% potassium dichromate at 4 °C or 10% formalin.

Modified Ziehl-Neelsen staining. The hot and cold method of Modified Ziehl-Neelsen staining of faecal smears was used as per the procedure described by (HENRIKSEN and POHLENZ, 1981) with slight modifications.

Thin smears of faecal sediment were made on a clean, grease free glass slide and air-dried. Then the smears were fixed transiently over a flame. The smears were then stained with a strong carbol fuchsin solution for 5 minutes. In the hot method, after pouring the stain the slide was heated until steam appeared but boiling was avoided. Then an additional stain was poured if the slide was dried. In the cold method the slides were not heated. After staining the smears were washed in running tap water for 1-2 minutes. Then the slides were subsequently decolorized in 5% sulphuric acid for 30 seconds. Again the smears were washed in tap water for 1-2 min and then, the smears were counterstained with 3% methylene blue for 1 minute. The smears were finally washed in tap water and air-dried. Then the smears were examined microscopically under oil immersion (100×) for *Cryptosporidium* oocysts.

Kinyoun's staining method. Kinyoun's staining was done as per the procedure described by GARCIA and BRUCKNER (1993).

Safranin methylene blue staining. The staining technique was followed as per the method described by the BAXBY et al. (1984).

Statistical analysis. The data were analysed using chi-square test and *t*-test and ANOVA as per the procedure of DANIEL (1987).

Results

The prevalence of cryptosporidiosis was studied on the basis of the detection of oocysts in the faecal materials collected from 5 different organized dairy farms and veterinary hospitals located in and around Bangalore. Out of 455 faecal samples screened, 26 (5.71%) animals were found positive for cryptosporidiosis by Sheather's sugar flotation method. The other three methods were employed in the present study to confirm the cryptosporidia oocysts from fungal spores and also to study the morphological characters and micrometry of oocysts for species identification. The prevalence of cryptosporidiosis was higher in diarrheic (24.20%) samples compared to non-diarrheic (16.60%) (Table 1).

The age wise prevalence was studied under four different age groups. Among 108 calves aged between 0-1 month, 10 (9.25%) were positive for cryptosporidiosis. Of the 112 calves screened in the 1-6 month age group, 8 (3.57%) were found positive for cryptosporidiosis. Out of 85 heifers from the 6 months to 1-year age group, 4 (4.71%) were found to be positive for cryptosporidiosis and among 150 adult animals of more than one year of age, 4 (2.66%) were positive for cryptosporidiosis (Table 1).

Table 1. Age wise prevalence of cryptosporidiosis in diarrhoeic and non-diarrhoeic calves

Age group	Nº of animals screened	Nº positive	Diarrhoeic			Non-diarrhoeic		
			Nº tested	Nº positive	% positive	Nº tested	Nº positive	% positive
0<1 month	108	10	95	10	10.53	13	0	0.00
1-6 months	112	8	90	6	6.67	22	2	9.09
7-12 months	85	4	42	2	4.8	43	2	4.65
Adults	150	4	45	1	2.2	105	3	2.86
Total	455	26	272	19	24.20	183	7	16.6

The values with age wise prevalence and diarrhoeic and non- diarrhoeic calves of cryptosporidiosis differ significantly at X^2= 9.5 and X^2=9.29 ($P<0.05$) respectively

Four different breeds of calves and non-descript animals were screened during the present study. Out of 310 HF cross calves screened, 21 (6.77%) were found positive for cryptosporidiosis and 5 (5.88%) were found positive among 85 Jersey cross calves screened. Among 30 Deoni calves, 10 Surti buffalo calves and 20 non-descript calves screened, none of them were found positive for the cryptosporidiosis. The result indicated that highest prevalence of cryptosporidiosis was found in the HF cross followed by the Jersey cross (Table 2).

Table 2. Breed wise prevalence of cryptosporidiosis

Breed	Total screened	Nº positive	% positive
HF cross	310	21	6.77
Jersey cross	85	5	5.88
Deoni	30	0	0.00
Surti	10	0	0.00
Non-descript	20	0	0.00
Total	455		

X^2 = 5.02 ($P<0.05$)

A sex wise prevalence was found in the present study with the prevalence rate of 3.64% and 6.0% in male and female respectively (Table 3). The prevalence of cryptosporidiosis according to age, sex and breeds of bovines was found to be statistically significant at $P\leq0.05$.

A sex wise prevalence was found in the present study with the prevalence rate of 3.64% and 6.0% in male and female respectively (Table 3). The prevalence of cryptosporidiosis according to age, sex and breeds of bovines was found to be statistically significant at $P \leq 0.05$.

Table 3. Sex wise prevalence of cryptosporidiosis in bovines

Sex	Total screened	N° positive	% positive
Male	55	2	3.64
Female	400	24	6.0
Total	455	26	

$X^2=0.495$ ($P<0.05$)

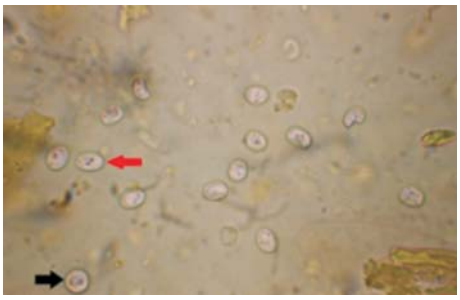

Fig. 1. *C. parvum* (round) *and C. andersoni* (oval) oocysts under Sheather's sugar flotation

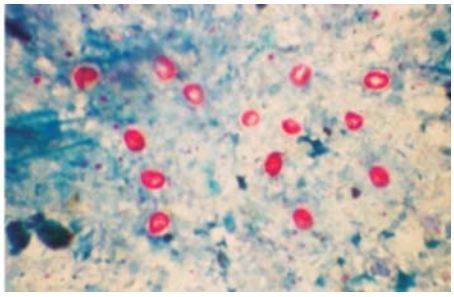

Fig. 2. *Cryptosporidium* oocysts in modified Ziehl-Neelsen staining

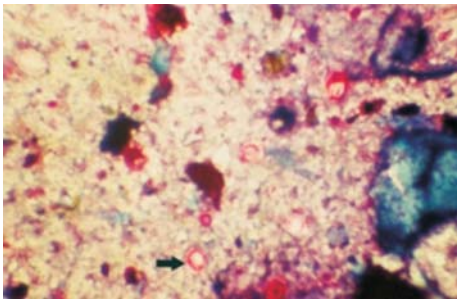

Fig. 3. *Cryptosporidium* oocysts in Kinyoun's acid-fast method

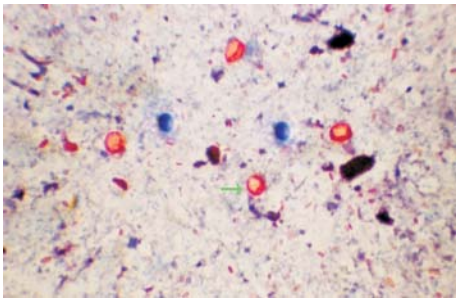

Fig. 4. *Cryptosporidium* oocysts in Kinyoun's acid-fast method oocysts in safranin methylene blue staining

During the present study two types of oocysts were recognized and were identified as *Cryptosporidium parvum* and *Cryptosporidium andersoni* based on morphology and micrometry. One was oval in shape, most commonly seen in the faecal samples of adult milking animals and heifers. The other type was spherical in shape found in the faecal samples of young calves. The species wise prevalence revealed 10.8% for *C.parvum*, 5.9% for *C.andersoni* and 5.98% for mixed infection.

Micrometrically larger oocysts had lengths of 7.2±0.835 µm and widths of 5.7±0.835 µm and conformed to *Cryptopsoridium andersoni*, whereas smaller oocysts with average length of 5.2 ±0.422 µm and width of 4.05±0.052 µm were considered as *Cryptopsoridium parvum*.

Morphologically in Sheather's sugar flotation, the oocysts appeared as round or oval, refractile bodies with a thin cytoplasmic membrane, finely granular cytoplasm and a prominent black dot (Fig. 1). The oocysts in modified Ziehl-Neelsens staining appeared as densely stained red bodies against a dark blue background, with a clear halo around the oocyst (Fig. 2). The oocysts appeared as pink to red to deep purple bodies against the dark blue background and in some four sporozoites were visible in Kinyoun's staining method (Fig. 3). In safranin methylene blue-staining oocysts appeared as orange-pink bodies and the sporozoites with in the oocysts stained slightly darker and sometimes were found arranged around the periphery (Fig. 4).

Discussion

Although cryptosporidiosis in animals and birds has been reported from Egypt, Brazil, USA, Czech Republic, Malaysia, Tanzania, Spain, Morocco, France, UK, Canada, Japan, Oman, Poland, Iran and China, only a few published reports of cryptosporidiosis in animals are available from India (KUMAR et al., 2005).

In India the disease was reported for the first time in Uttar Pradesh (DUBEY et al., 1992) later in Calcutta (CHATTOPADHYAY et al., 2000; DAS et al., 2003) Pondicherry (KUMAR et al., 2004), Andra Pradesh (SHOBHAMANI, 2005), UP (JAYABAL and RAY, 2005) West Bengal (ROY et al., 2006), Punjab (SINGH et al., 2006). The prevalence varied depending upon the age of the animal and other geographical and management practices.

It was observed that the majority of the animals between 1-6 months of age were found to have cryptosporidiosis caused by *Cryptosporidium parvum,* compared to those above six months and one year of age. Similar observations were also made by ONGERTH and STIBBS (1989), SHOBHAMANI (2005), JAYABAL and RAY (2005), ROY et al. (2006), and MEHDIAZAMI (2007) who reported higher rates of infection among calves less than 6 months of age. The study indicated that the younger animals were highly susceptible to infection with cryptosporidiosis compared to adult animals.

In the present study, out of 150 adult cattle screened, only four were found to be infected with cryptosporidiosis, with the low prevalence of 2.66%. KOYAMA et al. (2005) also recorded a low prevalence of 1.5% in adult cattle. GOW and WALDNER (2006) recorded a lower prevalence of 1.1% in beef cows and 3.1% in other cows. Much of the published reports indicate that bovine cryptosporidiosis is a disease of neonates and the low prevalence in adults indicates that they act as asymptomatic carriers of infection and will be a source of infection for younger animals.

In the present study more HF cross bred animals (6.77%) were found to be infected with cryptosporidium as compared to 5.88% in Jersey cross breeds and zero prevalence in Deoni, Surti and non-descript animals. Similar observations were made by SANFORD and JOSEPHSON (1982) and SHOBHAMANI (2005). The higher prevalence of cryptosporidiosis in HF cross breeds may be attributed to the greater number of HF cross animals screened in the present study compared to other breeds of animals.

No sex preponderance was observed in C*ryptosporidium* infection amongst the calves, as per REHAMAN et al. (1985) and SHOBHAMANI (2005). However in the present study it was found that females were more infected when compared to male animals. In contrast NOURI and TOROGHI (1991) recorded a higher rate of infection in male diarrhoeic calves than in female calves. The low prevalence of cryptosporidiosis in male animals in the present study may be attributed to the smaller number of male calves screened as most were culled after birth.

KAMINJOLO et al. (1993), DAS et al. (2003), ROY et al. (2006), REHAMAN et al. (1985), SINGH et al. (2006) and MEHDIAZAMI (2007) reported a higher prevalence of cryptosporidiosis in diarrhoeic calves compared to non-diarrhoeic animals. Similarly in the present study, although there was no significant difference between diarrhoeic and non-diarrhoeic animals, the percentage prevalence of cryptosporidiosis was higher in diarrhoeic animals at 24.2% compared to non-diarrhoeic animals 16.6%. Occurrence of cryptosporidiosis in clinically asymptomatic animals indicated that that particular age group of animals might be a reservoir for the parasite (MTAMBO et al., 1997). KAMINJOLO et al. (1993) found a statistically significant relationship between infection and diarrhoea, although cryptosporidia were detected more frequently in diarrhoeic than in healthy calves.

In the present study two species of cryptosporidium were identified namely *C. parvum* in young calves and *C. andersoni* in adults based on morphology and micrometry. These findings were in accordance with the UPTON and CURRENT (1985), LINDSAY et al. (2000), and FAYER et al. (2001).

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MALLINATH, R. H. K., P. G. CHIKKACHOWDAPPA, A. K. J. GOWDA, P. E.D'SOUZA: Istraživanje prevalencije kriptosporidioze u goveda na farmama mliječnih krava na području Bangalore u Južnoj Indiji. Vet. arhiv 79, 461-470, 2009.

SAŽETAK

Probirnim testom 455 uzoraka goveđih izmetina prikupljenih s pet različitih organiziranih farmi mliječnih krava i veterinarskih bolnica istražena je prevalencija kriptosporidioze u Bangaloru i okolici u Južnoj Indiji. Uzorci izmetina bili su pretraženi Sheatherovom metodom flotacije za dokaz oocista. Ustanovljeno je 5,71% pozitivnih na kriptosporidiozu. Na osnovi morfologije i mikrometrije oocista preinačenom metodom bojenja po Ziehl-Neelsenu, bojenjem po Kinyounu i safranin-metilenskim modrilom bile su identificirane vrste *Cryptosporidium parvum* i *Cryptosporidium andersoni*. Veća prevalencija i intenzitet kriptosporidioze ustanovljeni su u teladi mlađe od mjesec dana u odnosu na odrasle, a učestalost je također bila veća u životinja s proljevom. Najveća prevalencija ustanovljena je u križane holštajnsko-frizijske pasmine, a potom u pasmine Jersey. Oociste kriptosporidija nisu bile ustanovljene u teladi pasmine Deoni, Surtii i nekih neodređenih pasmina. Kriptosporidioza je bila češća u ženki. Statistički značajna razlika u osjetljivosti na kriptosporidiozu bila je ustanovljena s obzirom na spol, dob i pasminu. Zaključuje se da se kriptosporidioza u goveda u pretraživanom području ne dijagnosticira uvijek te da su supkliničke invazije vjerojatno česte.

Ključne riječi: kriptosporidioza, prevalencija, govedo, oociste, *C. parvum, C. andersoni*