Factors influencing on prevalence of *Cryptosporidium* infection in south Indian dairy calves

R. Venu · B. R. Latha · S. Abdul Basith ·
C. Sreekumar · G. Dhinakar Raj · M. Raman

Received: 26 June 2012 / Accepted: 16 July 2012 / Published online: 2 August 2012
© Indian Society for Parasitology 2012

Abstract The objectives of the present study were to report the influence of factors like age, sex, breed, dung consistency and rearing system on prevalence of *Cryptosporidium* spp. in south Indian cattle. Two-step nested PCR was employed for detection of *Cryptosporidium* infection in dairy calves of south Indian states viz., Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and union territory i.e., Puducherry. A total of 459 dung samples from the calves were subjected to nested PCR, 182 were found positive with prevalence percent of 39.65. Age wise comparison showed a high prevalence of *Cryptosporidium* in the age group of one month old calves. This concludes that the cryptosporidiosis is highly age dependent with young calves showed the highest prevalence. Depending on the group had consistency of dung, the highest prevalence of *Cryptosporidium* was observed in semi-solid dung, followed by formed and the diarrhoeic group animals. Female calves showed slightly higher prevalence rate than male animals. Cow calves had an overall prevalence percent of 40.75 and the infection rate in buffalo calves was 36.28 %.

In relation to rearing system, individual animals had 42.18 % and farm animals showed 38.46 % of *Cryptosporidium* infection. In conclusion, the prevalence of *Cryptosporidium* in dairy calves should be correlated with the factors like age, sex, breed, dung consistency and rearing system of the animal to arrive at a reliable epidemiological data on bovine cryptosporidiosis.

Keywords *Cryptosporidium* · Cattle · Nested PCR · Prevalence · India

Introduction

Cryptosporidium is an obligate protozoan parasite that commonly infects calves and other mammalian hosts. Cryptosporidiosis poses a significant problem in dairy calves where the prevalence of infection is high, with losses due to increased treatment costs and occasionally causes mortality. In India, since the first report of bovine cryptosporidiosis by Nooruddin and Sarma (1987), most of the studies have been based on the morphological identification of oocysts in faeces (Dubey et al. 1992; Chattopadhyay et al. 2000; Das et al. 2004; Kumar et al. 2004; Jeyabal and Ray 2005; Singh et al. 2006). Microscopic method of detection can provide generalized prevalence data for the presence of *Cryptosporidium* spp. but this method of detection is entirely not suitable for the detection of *Cryptosporidium* oocysts. The oocysts are much smaller than those of other coccidian parasites and they differ in many of their staining and buoyancy characteristics (O'Donoghue 1995). Molecular techniques are being increasingly employed for the detection of *Cryptosporidium* during last two decades mainly because of its accuracy. The use of molecular tools has made significant

R. Venu · B. R. Latha · S. A. Basith · M. Raman
Department of Veterinary Parasitology, Madras Veterinary
College, Chennai 600 007, Tamil Nadu, India

C. Sreekumar
Sheep Breeding Research Station, Sandynallah, Ooty,
Tamil Nadu, India

G. D. Raj
Department of Animal Biotechnology, Madras Veterinary
College, Chennai 600 007, Tamil Nadu, India

R. Venu (✉)
Department of Veterinary Parasitology, College of Veterinary
Science, Proddatur 516 360, Kadapa District, A.P., India
e-mail: venuparas2001@yahoo.com

contributions to our understanding of the epidemiology of *Cryptosporidium* infection (Xiao and Ryan 2008) and became the new corner stone for taxonomic and epidemiologic studies (Fayer and Xiao 2008). Prevalence of *Cryptosporidium* infection may vary with factors like age, breed, sex, consistency of dung and management practices such as the rearing system. Some studies have showed its susceptibility depends on the age of the host: *C. parvum* in pre-weaned calves; *C. bovis*, and *C. ryanae* in post-weaned calves, and *C. andersoni* in older calves and adult cattle (Anderson 1998; Santin et al. 2004; Fayer et al. 2006), although *C. bovis* and *C. ryanae* have been found in all the age groups (Feng et al. 2007). The present study was undertaken: (1) to study the prevalence of *Cryptosporidium* spp. with special emphasis on different influencing factors from the dairy calves of Southern states of India, where this kind of information is lacking, (2) to study the influence of the related factors on prevalence of *Cryptosporidium* infection in south Indian dairy calves based on the molecular techniques to provide accurate data for the estimation of the prevalence.

Materials and methods

A total of 459 dung samples were collected from dairy calves aged less than 6 months from various dairy farms/small holdings in Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Puducherry of south India for screening *Cryptosporidium* infection during the period from February 2009 to June 2010. Dung samples were labelled with details of breed, age, sex, place and date of collection. Depending on the dung consistency at the time of collection, all the samples were categorized and placed into four groups viz., formed, semi-solid, diarrhoeic, and watery. Each animal was sampled only once and the collected dung samples were stored on ice and brought to the laboratory. When the samples could not be transported within a day of collection to the laboratory, 2.5 % potassium dichromate was added as preservative.

Genomic DNA was extracted individually from 459 dung samples using QIAamp® DNA stool mini kit (Qiagen, Germany) following the manufacturer's instructions, except in the final step wherein DNA was eluted with 60 µl of elution buffer (supplied with kit) instead of 200 µl. Eluted DNA was tested for its concentration and purity with Biophotometer plus® (Eppendorf). The purity of the DNA sample was estimated by observing the ratio between optical density value at 260 and 280 nm. DNA samples were considered to be of sufficient purity if the ratio was 1.7 and above. The eluted DNA samples were stored at -20 °C for further use.

A two-step nested PCR protocol was followed in the present study to diagnose *Cryptosporidium* DNA from the

collected dung samples to amplify ~830 bp fragment of the 18S rRNA gene as described by Xiao et al. (1999, 2001). Nested PCR products were analyzed by 1.2 % agarose gel in a submarine horizontal gel electrophoresis apparatus as described by Sambrook and Russel (2001) and the amplified DNA products were visualized using UV transilluminator (GeNei, Bangalore). The data obtained by nested PCR was categorized based on the age, sex, breed, dung consistency and the rearing system. The results of the present study were analyzed by Vetstat, χ^2 analysis.

Results

Genomic DNA obtained from 459 dung samples were screened by nested PCR for the detection of *Cryptosporidium* using 18S rRNA gene specific primers. *Cryptosporidium* DNA was found in 182 (39.65 %) samples and nested PCR amplicons were observed at ~830 bp (Fig. 1). Age wise prevalence of *Cryptosporidium* in the present study by nested PCR showed the highest prevalence among the calves having less than one month age group. The screening results of the nested PCR from 343 dung samples from dairy calves aged less than a month old showed 143 (41.69 %) positives followed by 13 (40.63 %) positives from 32 samples tested from the age group of 2–3 months old calves whereas from 1 to 2 months old calves aged 26 (31.33 %) positives, out of 83 samples screened.

Based on the consistency of dung samples, *Cryptosporidium* infection was observed in the current study by nested PCR and the infection was observed in diarrhoeic, non-diarrhoeic and also animals passing dung of semi-solid in consistency. The highest rate of infection (44.05 %) was observed in semi-solid dung samples group followed by formed (38.69 %) and diarrhoeic (35.23 %) animals

Fig. 1 Agarose gel electrophoresis of nested PCR amplified products of 18S rRNA gene of *Cryptosporidium*. M 100 bp DNA ladder, L1–L8 nested PCR amplicons of ~830 bp size, and L9 non-template control

Table 1 Prevalence of *Cryptosporidium* infection in relation to age group and dung consistency

Number positive/number examined					
Dung consistency*					
Age (months)**	Formed	Semi-solid	Diarrhoeic	Watery	Total
<1	45/112 (40.18)	68/152 (44.74)	30/75 (40.0)	0/4 (0.0)	143/343 (41.69)
1–2	22/61 (36.07)	4/12 (33.33)	0/10 (0.0)	–	26/83 (31.33)
2–3	10/26 (38.46)	2/4 (50.0)	1/2 (50.0)	–	13/32 (40.63)
3–6	–	–	0/1 (0.0)	–	0/1 (0.0)
Total	77/199 (38.69)	74/168 (44.05)	31/88 (35.23)	0/4 (0.0)	182/459 (39.65)

Figure in parentheses indicates percentage

* Between dung consistency groups χ^2 value = 1.72^{NS}

** Between age groups χ^2 value = 3.01^{NS}

Table 2 Sex-wise prevalence of *Cryptosporidium* infection

Dung samples	Male	Female	Total
Number tested	202	257	459
Number infected	77	105	182
Percent positive	38.19	40.86	39.65

χ^2 value = 0.35^{NS}

Table 3 Prevalence of *Cryptosporidium* infection in cow calves and buffalo calves

Dung samples	Cow calves	Buffalo calves	Total
Number screened	346	113	459
Number positive	141	41	182
Prevalence (%)	40.75	36.28	39.65

χ^2 value = 0.71^{NS}

(Table 1). The prevalence of *Cryptosporidium* was assessed based on sex and showed the prevalence rate of 38.19 % (77 male dairy calves) out of 202 tested. Among female calves, 105 (40.86 %) were infected out of 257 dung samples screened. Statistically there was no significant difference observed between the prevalence rate among male and female animals (Table 2). *Cryptosporidium* infection in dairy cattle was studied based on breed particularly for cattle and buffalo calves. The highest prevalence rate of 40.75 % was reported in cow calves where as in buffalo calves, it was 36.28 %. No significant difference was observed between these two groups (Table 3). Out of 312 dung samples screened from farm animal, 120 (38.46 %) were found positive for *Cryptosporidium* infection whereas in case of 147 individual animals, 62 (42.18 %) were positive. These results showed no significant difference between farm and non-farm animals in relation to *Cryptosporidium* infection detected by nested PCR (Table 4).

Table 4 Prevalence of *Cryptosporidium* infection in relation to rearing system

Dung samples	Farm	Individual	Total
Number tested	312	147	459
Number positive	120	62	182
Prevalence (%)	38.46	42.18	39.65

χ^2 value = 0.58^{NS}

Discussion

The age related pattern of *Cryptosporidium* species in young calves reported in other studies (Santin et al. 2004; Langkjaer et al. 2007; Bhat et al. 2012) was seen in this study as well, although changes in distribution with age were not as dramatic. Prevalence of *Cryptosporidium* related to different ages have been reported worldwide (Garber et al. 1994; de la Fuente et al. 1999; Castro-Hermida et al. 2002; Trotz-Williams et al. 2005; Geurden et al. 2006; Kvac et al. 2006; Fayer et al. 2007). Data from the available literature concluded that the prevalence of *Cryptosporidium* infection in cattle reduces as the progress of the age of the animal (Fayer et al. 2007, 2010; Santin et al. 2008). In the present study an overall percent of 39.74 (182/458) of *Cryptosporidium* infection was recorded in calves less than 3 months of age. Lower prevalence data have been reported in other studies of cattle (Wade et al. 2000) possibly reflected on the detection method used for oocyst but showed a decreased prevalence. Higher prevalence rate reported by Starkey et al. (2005) might be due to their study conducted over a prolonged period for 2 years and all positive specimens were from less than 2 month old calves and the number of samples screened was very high (9,914).

In the current study, the highest prevalence of *Cryptosporidium* infection was observed in semi-solid dung consistency group. Further, out of 24 positive dung samples

for *Cryptosporidium* DNA in the first round PCR, 19 (79.17 %) samples belonged to the semi-solid dung consistency group. Sensitivity of first round PCR in the present study was 10,000 oocysts per sample whereas for nested PCR, it was 100 oocysts per sample. These facts explain that the species of *Cryptosporidium* and the number of oocysts shedding have influence on the consistency of dung. Thus it may be recommended to correlate PCR results with dung consistency to arrive at a relevant clinical diagnosis and these findings corroborate with those of Roy et al. (2006). Robinson et al. (2006) observed that all of the *C. andersoni* positive faecal samples from the cattle from United Kingdom were with normal dung consistency and no obvious clinical symptoms. Different species of *Cryptosporidium* and number of oocyst shedding in the faeces may have influence on the consistency of dung. Sex of the animal is not having any influence on the prevalence rate of *Cryptosporidium* infection in south Indian dairy cattle. Both male and female calves are equally susceptible and there is no significant change in the prevalence. The results of the present study were comparable to Karanis et al. (2010). The present study involved both cattle and buffalo calves to record the influence of breed on the prevalence rate of *Cryptosporidium* infection. No significant difference was observed. No such comparative study was traced out in the available literature for discussion. Both farm and individually reared animals are equally susceptible in acquiring the *Cryptosporidium* infection and statistically there is no significant difference between these two groups in relation to prevalence rate. This might be due to the contamination of *Cryptosporidium* oocyst in both farm and non-farm areas. Several authors have reported *Cryptosporidium* infection in dairy calves reared in farms (Geurden et al. 2006; Robinson et al. 2006; Fayer et al. 2007).

In conclusion, this is the first report on the influence of age, sex, breed, dung consistency and rearing system on prevalence of *Cryptosporidium* in south Indian dairy cattle. The results obtained in the present study documents a sequential association of *Cryptosporidium* infection in cattle as they increase in age thereby provides a guide for preventing, managing and tracking sources of *Cryptosporidium*. Clinically, relevant diagnosis of *Cryptosporidium* can be achieved by coupling the PCR results with the different influencing factors of the affected animals. In the epidemiological studies of *Cryptosporidium*, factors like age, sex, breed, dung consistency and rearing system needs to be included.

Acknowledgments The authors are grateful to Tamil Nadu Veterinary and Animal Sciences University, Chennai for providing facilities.

Conflict of interest None.

References

- Anderson BC (1998) Cryptosporidiosis in bovine and human health. *J Dairy Res* 81:3036–3041
- Bhat SA, Juyal PD, Singla LD (2012) Prevalence of cryptosporidiosis in neonatal buffalo calves in Ludhiana district of Punjab, India. *Asian J Anim Vet Adv* 7(6):512–520
- Castro-Hermida JA, Yolanda A, Gonzalez-Losada A, Ares-Mazas E (2002) Prevalence of and risk factors involved in the spread of neonatal bovine cryptosporidiosis in Galicia (NW Spain). *Vet Parasitol* 106:1–10
- Chattopadhyay UKD, Chowdhury CK, Dasgupta, Pramanik AK (2000) Prevalence of cryptosporidiosis in man and animals in and around Calcutta. *J Vet Parasitol* 14:167–168
- Das G, Sarkar S, Das P, Sheikh GN, Sarkar S (2004) Prevalence of *Cryptosporidium* infection in cattle. *J Vet Pub Health* 2:15–17
- de la Fuente R, Luzon M, Ruiz-Santa-Quiteria JA, Garcia A, Cid D, Orden JA, Garcia S, Sanz R, Gomez- Bautista M (1999) *Cryptosporidium* and concurrent infections with other major enteropathogens in 1–30-day-old diarrhoeic dairy calves in central Spain. *Vet Parasitol* 80:179–185
- Dubey JP, Fayer R, Rao JR (1992) Cryptosporidial oocysts in faeces of water buffalo and zebu calves in India. *J Vet Parasitol* 6:55–56
- Fayer R, Xiao L (2008) *Cryptosporidium* and cryptosporidiosis. CRC Press, Boca Raton
- Fayer R, Santin M, Trout JM, Greiner E (2006) Prevalence of species and genotypes of *Cryptosporidium* found in 1–2-year-old dairy cattle in the eastern United States. *Vet Parasitol* 135:105–112
- Fayer R, Santin M, Trout JM (2007) Prevalence of *Cryptosporidium* species and genotypes in mature dairy cattle on farms in eastern United States compared with younger cattle from the same locations. *Vet Parasitol* 145:260–266
- Fayer R, Santin M, Dargatz D (2010) Species of *Cryptosporidium* detected in weaned cattle on cow-calf operations in the United States. *Vet Parasitol* 170:187–192
- Feng Y, Ortega Y, He G, Das P, Xu M, Zhang X, Fayer R, Gatei W, Cama V, Xiao L (2007) Wide geographic distribution of *Cryptosporidium parvum* and deer like genotype in bovines. *Vet Parasitol* 144:1–9
- Garber LP, Salman MD, Hurd HS, Keefe T, Schlatter JL (1994) Potential risk factors for *Cryptosporidium* infection in dairy calves. *J Am Vet Med Assoc* 205:86–91
- Geurden T, Goma FY, Siwila J, Phiri IKG, Mwanza AM, Gabriel S, Claerebout E, Vercruysse J (2006) Prevalence and genotyping of *Cryptosporidium* in three cattle husbandry systems in Zambia. *Vet Parasitol* 138:217–222
- Jeyabal L, Ray DD (2005) Cryptosporidial infection in cattle and buffaloes. *J Vet Parasitol* 19:165–166
- Karanis P, Eiji T, Palomino L, Boonrod K, Plutzer J, Ongerth J, Igarashi I (2010) First description of *Cryptosporidium bovis* in Japan and diagnosis and genotyping of *Cryptosporidium* spp. in diarrheic pre-weaned calves in Hokkaido. *Vet Parasitol* 169:387–390
- Kumar D, Sreekrishnan R, Das SS (2004) Cryptosporidiosis in man and animals in Pondicherry. *Indian J Anim Sci* 74:261–263
- Kvac M, Kouba M, Vitovec J (2006) Age-related and housing-dependence of *Cryptosporidium* infection of calves from dairy and beef herds in South Bohemia, Czech Republic. *Vet Parasitol* 137:202–209
- Langkjaer RB, Vigre H, Enemark HL, Maddox-Hyttel C (2007) Molecular and phylogenetic characterization of *Cryptosporidium* and *Giardia* from pigs and cattle in Denmark. *Parasitol* 134: 339–350
- Nooruddin M, Sarma DK (1987) Role of *Cryptosporidium* in calf diarrhoea. *Livest Advis* 12:49

- O'Donoghue PJ (1995) *Cryptosporidium* and cryptosporidiosis in man and animals. *Intl J Parasitol* 25(2):139–195
- Robinson G, Thomas AL, Daniel RG, Hadfield SJ, Elwin K, Chalmer RM (2006) Sample prevalence and molecular characterization of *Cryptosporidium andersoni* within a dairy herd in the United Kingdom. *Vet Parasitol* 142:163–167
- Roy SS, Sarkar S, Batabyal S, Pramanik AK, Das P (2006) Observations on the epidemiology of bovine Cryptosporidiosis in India. *Vet Parasitol* 141:330–333
- Sambrook J, Russel DW (2001) Molecular cloning—a laboratory manual. Cold Spring Harbor Laboratory Press, New York
- Santin M, Trout JM, Xiao L, Zhou L, Greiner E, Fayer R (2004) Prevalence and age-related variation of *Cryptosporidium* species and genotypes in dairy calves. *Vet Parasitol* 122:103–117
- Santin M, Trout JM, Fayer R (2008) A longitudinal study of cryptosporidiosis in dairy cattle from birth to 2 years of age. *Vet Parasitol* 155:15–23
- Singh BB, Sharma R, Kumar H, Banga HS, Aulakh RS, Gill JPS, Sharma JK (2006) Prevalence of *Cryptosporidium parvum* infection in Punjab (India) and its association with diarrhoea in neonatal dairy calves. *Vet Parasitol* 140:162–165
- Starkey SR, Wade SE, Schaaf S, Mohammed HO (2005) Incidence of *Cryptosporidium parvum* in the dairy cattle population in a New York City watershed. *Vet Parasitol* 131:197–205
- Trotz-Williams LA, Martin SW, Martin D, Duffield T, Leslie KE, Nydam DV, Jamieson F, Peregrine AS (2005) Multi-attribute evaluation of two simple tests for the detection of *Cryptosporidium parvum* in calf faeces. *Vet Parasitol* 134:15–23
- Wade SE, Mohammed HO, Schaaf SL (2000) Prevalence of *Giardia* sp. *Cryptosporidium parvum* and *Cryptosporidium muris* (*C. andersoni*) in 109 dairy herds in five counties of southeastern New York. *Vet Parasitol* 93:1–11
- Xiao L, Ryan UM (2008) Molecular epidemiology. In: Fayer R, Xiao L (eds) *Cryptosporidium* and Cryptosporidiosis. CRC Press/IWA Publishing, Boca Raton
- Xiao L, Escalante L, Yang C, Sulaiman I, Escalante AA, Monsali RJ, Fayer R, Lal AA (1999) Phylogenetic analysis of *Cryptosporidium* parasites based on the SSU rRNA gene locus. *Appl Environ Microbiol* 65:1578–1583
- Xiao L, Singh A, Limor J, Graczyk TK, Gradus S, Lal AA (2001) Molecular characterization of *Cryptosporidium* oocysts in samples of raw surface water and waste water. *Appl Environ Microbiol* 67:1097–1101