Coprological investigation on neonatal bovine cryptosporidiosis in Ludhiana, Punjab

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Abstract An overall prevalence of 38.90 % was recorded for bovine cryptosporidiosis on coprological examination of 144 faecal samples collected from neonatal cattle calves from organised dairy farms in and around Ludhiana, Punjab by modified Ziehl–Neelsen staining. Further, a gradual decline in the percent prevalence was seen with increase in the age of the host from <1 month (64.1 %) to 4–5 months (12.5 %). The highest prevalence was recorded during the monsoon season (47.06 %) followed by summer (37.73 %) and winter (30.0 %) season and the seasonal variation was statistically significant ($p \leq 0.05$). Female calves showed higher prevalence (44.32 %) than their male counterparts (27.66 %). Further, prevalence of cryptosporidiosis was significantly higher ($p \leq 0.05$) in the diarrhoeic calves (52.70 %) as compared to the non-diarrhoeic (24.28 %) thus indicating a relatively higher risk (1.75 times) of the disease in diarrhoeic than normal calves.

Keywords *Cryptosporidium* spp. · mZN staining · Prevalence

Introduction

Bovine cryptosporidiosis, a predominantly gastrointestinal disease of neonatal calves, is caused by a monoxenous, opportunistic intracellular but extra-cytoplasmic apicomplexan protozoan parasite belonging to genus *Cryptosporidium*. It is an important pathogen of domesticated livestock

and human beings (Santin et al. 2004) causing self-limiting watery diarrhoea in immunocompetent hosts but has far more devastating effects on immunocompromised individuals. In some cases the infection can be life threatening due to dehydration caused by chronic diarrhoea (Caccio 2005). *Cryptosporidium parvum* and *C. andersoni* have been identified as cosmopolitan species infecting cattle (Peng et al. 2003) and calves particularly of 1–3 weeks of age are highly susceptible (Leek and Fayer 1984) but it has also been recorded in older animals above 2 years of age (Henriksen and Krogh 1985; Singh et al. 2006).

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). Since the first report of bovine cryptosporidiosis in India by Nooruddin and Sarma (1987), several other sporadic reports using stained faecal smears of both diarrhoeic and normal cattle and buffaloes have been documented from different parts of the country (Dubey et al. 1992; Das et al. 2004; Jeyabal and Ray 2005; Roy et al. 2006; Singh et al. 2006; Paul et al. 2008; Mallinath et al. 2009). As there is only a single published report from the state of Punjab (Singh et al. 2006) the present study was undertaken to observe the current status of cryptosporidiosis in bovines in organised dairy farms in and around Ludhiana, Punjab.

Materials and methods

Study area

Ludhiana is located in the central plain zone of Punjab state with latitudes and longitudes of 30°55' N–75°54' E and has an estimated cattle population of ~755,536. Five

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organised dairy farms in and around Ludhiana were selected for collection of faecal samples from bovine calves using simple random sampling method.

Sample collection

A total of 144 faecal samples were collected from cross-bred cattle calves, below 5 months of age, directly from the rectum with or without diarrhoea from July 2009 to June 2010. The data related to risk factors like age, sex, breed, presence of diarrhoea and history of illness was collected from each animal through a questionnaire. The samples were quickly transported after collection to the Post-graduate Laboratory, Department of Veterinary Parasitology and were stored at 4 °C in 2.5 % potassium dichromate solution till further use.

Examination of faecal smears

For direct faecal smear examination, a thin and transparent faecal smear was prepared with the help of a sterilized ear bud on a clean microscopic glass slide and air dried, fixed in methanol for 3 min, air dried, stained by modified Ziehl–Neelsen (mZN) staining as per the method described by OIE (2008). In brief, after fixation, smears were stained with 1 % cold Carbol-Fuchsin solution for 15 min and rinsed thoroughly in tap water. Then decolorization was done in 1 % acid methanol for 15 s and again the smears were rinsed with tap water and then, the smears were counter-stained with 0.4 % Malachite Green for 30 s. The smears were finally washed in tap water, air-dried and were examined. Faecal samples negative by direct smear examination were further subjected to concentration methods as per Mallinath et al. (2009) for confirmation. For this purpose, the faecal samples (1–2 g) were subjected to floatation using Sheather's sugar solution for 20 min. After this, the meniscus was gently removed with a disposable pipette and dispensed gently on to a microscope slide to prepare faecal smears. Subsequently these were air dried, fixed in methanol and stained by mZN staining as described above.

Statistical analysis

Statistical analysis was performed on data by SPSS 13.0 software by applying Chi-Square test and statistical differences ($p \leq 0.01$ and $p \leq 0.05$) between various groups were calculated.

Results and discussion

Out of 144 faecal samples examined by direct smear examination, 28.47 % (41/144) samples were found to be

positive for *Cryptosporidium* spp oocysts. Further, the negative samples (103) when subjected to concentration method revealed 15 more samples positive for *Cryptosporidium* spp. oocysts thus increasing the overall percent positivity to 38.89 (56/144). The higher sensitivity recorded in the concentration method has also been previously reported by Paul et al. (2009). The oocysts stained bright pink to apple red on a pale green background in mZN staining (Fig. 1, 2). In similar studies from different parts of the country a variable positive percentage for cryptosporidiosis in cattle calves has been reported viz. 38.02 % from Bareilly, Uttar Pradesh (Paul et al. 2008), 9.05 % from Chennai (Prakash et al. 2009), 25 % from Puducherry (Kumar et al. 2004), 32.8 % from Tirupati (Shobhamani 2005), 50 % from Izatnagar, Uttar Pradesh (Jeyabal and Ray 2005). Further, from the state of Punjab almost similar prevalence rate of 38.31 % has been reported from cattle population in an earlier study (Singh et al. 2006).

Fig. 1 *Cryptosporidium* oocysts in direct faecal smear staining (mZN) at $\times 1,000$ magnification

Fig. 2 *Cryptosporidium* oocysts in mZN after floatation at $\times 1,000$ magnification

Fig. 3 Age related prevalence (%) of cryptosporidiosis in dairy cattle calves

Study of age wise prevalence revealed the highest infection rate in calves 0–30 days (64.10 %) which declined with an increase in age to a minimum of 12.50 % in 4–5 months age group. A negative correlation ($r = -0.95$) was recorded between the percentage positivity and age groups of the calves and the difference was statistically significant ($p \leq 0.05$) (Fig. 3).

Variation in susceptibility of different age groups to the *Cryptosporidium* spp infection has been reported by several workers from various parts of world (Olson et al. 1997; Sturdee et al. 2003; Santin et al. 2004) and from India (Roy et al. 2006; Singh et al. 2006; Paul et al. 2008). Maximum incidence rates among calves less than 1 months of age has been reported (Fayer et al. 2006; Roy et al. 2006; Paul et al. 2008). Similarly Singh et al. (2006) reported high (79.41 %) prevalence of *Cryptosporidium parvum* infection among 0–30-day-old dairy calves from Punjab state.

In terms of seasonal variation, cattle calves showed the prevalence of the cryptosporidiosis as maximum in monsoon (47.06 %), followed by summer (37.73 %) and winter (30 %) and the variation was statistically significant ($p \leq 0.05$) (Table 1). The results are in congruent with Paul et al. (2008) who recorded the prevalence of cryptosporidiosis in the monsoon months as 37.3 % which was higher than the dry pre-monsoon (25.6 %) and cooler post-monsoon months (19.6 %). The findings also corroborate the results obtained in the earlier studies done in India by Prasad et al. (1989) and Roy et al. (2006) who observed highest infection rates in warm and humid months. This may be due to the fact that high temperature and humidity along with frequent rains in the monsoon season enabled the faster transmission of the oocysts.

As regards sex wise distribution, female calves showed relatively higher prevalence (44.32 %) than male calves (27.66 %) (Table 1). This may be due to the practice of culling of male calves soon after birth by most of dairy farmers and thus the number of faecal samples collected from the male calves was comparatively lesser than the female calves. Results of Mallinath et al. (2009) are in congruent with the findings of present study whereas, higher incidence of the infection was recorded in male calves by Paul et al. (2008) but the difference was not significantly apart.

Prevalence of cryptosporidiosis was significantly higher ($p \leq 0.05$) in the diarrhoeic calves (52.70 %) as compared to the non-diarrhoeic (24.28 %) thus indicating a relatively higher risk (1.75 times) of the disease in diarrhoeic than normal calves. This may be probably due to the fact that the diarrhoeic calves provide a better chance for oocyst shedding and propagation as compared to the non-diarrhoeic calves. The results of the present study corroborate the earlier findings of various workers reporting higher prevalence in diarrhoeic calves from India (Singh et al. 2006; Paul et al. 2008; Roy et al. 2006) and abroad (Gracia

Table 1 Prevalence of cryptosporidiosis in crossbred calves of Ludhiana, Punjab

Risk factor	Diarrhoeic			Non-diarrhoeic			Total (%) positive
	No. of animals examined	No. of positive cases	% Positive	No. of animals examined	No. of positive cases	% Positive	
Season							
Summer (March–June)	25	12	48.0	28	8	28.57	37.73
Monsoon (July–September)	28	19	67.85	23	5	21.74	47.06
Winter (October–February)	21	8	38.09	19	4	21.05	30.0
χ^2 ($p \leq 0.05$)			4.599			0.469	0.110
Sex							
Male	19	8	42.10	28	5	17.86	27.66
Female	55	31	56.36	42	12	28.57	44.32
χ^2 ($p \leq 0.05$)			1.152			1.049	0.053

and Lima 1993; Kaminjolo et al. 1993). The shedding of *Cryptosporidium* oocysts by clinically asymptomatic calves (non-diarrhoeic) as recorded in the present study indicate a carrier status of cattle calves, which may act as reservoir and nidus for transmission to susceptible neonatal calves.

Results of current study further substantiate the ubiquitous distribution of *Cryptosporidium* particularly in developing country like India where the poor animal husbandry practices viz. improper spacing leading to very close association, poor hygiene, suckling of unsanitised udder lead to high prevalence values.

References

- Caccio SM (2005) Molecular epidemiology of human cryptosporidiosis. *Parasitologia* 47:185–192
- Das G, Sarkar S, Das P, Sheikh GN, Sarkar S (2004) Prevalence of *Cryptosporidium* infection in cattle. *J Vet Publ Health* 2:15–17
- 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, Santin M, Trout JM, Greiner E (2006) Prevalence of species and genotypes of *Cryptosporidium* found in 1–2 year old dairy cattle in eastern United States. *Vet Parasitol* 135:105–112
- Gracia AM, Lima JD (1993) Frequency of *Cryptosporidium* in suckling dairy calves. *Arq Bras Med Vet Zootec* 45:193–198
- Henriksen SA, Krogh HV (1985) Bovine cryptosporidiosis in Denmark: prevalence, age distribution, and seasonal variation. *Nord Vet Med* 37:34–41
- Jeyabal L, Ray DD (2005) Cryptosporidial infection in cattle and buffaloes. *J Vet Parasitol* 19:165–166
- Kaminjolo JS, Adesiyun AA, Loregnard R, Kitson-Piggott W (1993) Prevalence of *Cryptosporidium* oocysts in livestock in Trinidad and Tobago. *Vet Parasitol* 45:209–213
- Kumar D, Sreekrishnan R, Das SS (2004) Cryptosporidiosis in man and animals in Pondicherry. *Indian J Anim Sci* 74:261–263
- Kumar D, Sreekrishnan R, Das SS (2005) Cryptosporidiosis: an emerging disease of zoonotic importance. *Proc Nat Acad Sci India* 75:160–172
- Leek RG, Fayer R (1984) Prevalence of *Cryptosporidium* infections and their relation to diarrhea in calves on 12 dairy farms in Maryland. *P Helm Soc Wash* 51:360–361
- Mallinath RHK, Chikkachowdappa PG, Gowda AJK, D'souza PE (2009) Studies on the prevalence of cryptosporidiosis in bovines in organized dairy farms in and around Bangalore, South India. *Vet Archiv* 79:461–470
- Nooruddin M, Sarma DK (1987) Role of *Cryptosporidium* in calf diarrhoea: in annual report. *Livestock Advisor* 12:49
- OIE (2008) Cryptosporidiosis. In: *Terrestrial manual chapter 2.9.4*, pp 1192–1215
- Olson ME, Thorlakson CL, Deselliers L, Morck DW, McAllister TA (1997) *Giardia* and *Cryptosporidium* in Canadian farm animals. *Vet Parasitol* 68:375–381
- Paul S, Chandra D, Ray DD, Tewari AK, Rao JR, Banerjee PS, Baidya S, Raina OK (2008) Prevalence and molecular characterization of bovine *Cryptosporidium* isolates in India. *Vet Parasitol* 153:143–146
- Paul S, Chandra D, Tewari AK, Banerjee PS, Ray DD, Boral R, Rao JR (2009) Comparative evaluation and economic assessment of coprological diagnostic methods and PCR for detection of *Cryptosporidium* spp. in bovines. *Vet Parasitol* 164:291–295
- Peng MM, Wilson ML, Holland RE, Meshnick SR, Lal AA, Xiao L (2003) Genetic diversity of *Cryptosporidium* spp. in cattle in Michigan; implications for understanding the transmission dynamics. *Parasitol Res* 90:175–180
- Prakash S, Prabu K, Palanivel KM (2009) Prevalence of cryptosporidiosis in dairy calves in Chennai. *TN J Vet Anim Sci* 5:41–46
- Prasad KN, Chattopadhyay UK, Sanyal SC (1989) Cryptosporidiosis in calves. *Indian J Microbiol* 29:139–142
- 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
- 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
- Shobhamani B (2005) Epidemiological studies on diarrhoea in calves with particular reference to diagnosis and treatment of cryptosporidiosis. *J Vet Parasitol* 19:77
- 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
- Sturdee AP, Bodley-Tickell AT, Archer A, Chalmers RM (2003) Long-term study of *Cryptosporidium* prevalence on a lowland farm in the United Kingdom. *Vet Parasitol* 116:97–113