



Antigen based diagnosis of *Cryptosporidium parvum* infection in faeces of cattle and buffalo calves

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Cryptosporidium parvum is a microscopic protozoan parasite that causes gastrointestinal infection in a wide variety of animals, including humans. The disease is of zoonotic importance (Goh *et al.* 2004, Sulaiman *et al.* 2005) and is also reported to be prevalent in India (Singh *et al.* 2006, Saha *et al.* 2006, Nagamani *et al.* 2007, Paul *et al.* 2008). In cattle, the clinical form of the disease (Randhawa *et al.* 2012) and the shedding of oocysts in faeces is usually limited to calves of few months of age (Bhat *et al.* 2012), but there are some reports of sub-clinical oocysts shedding in adult cattle as well as immunocompromised cattle (Lorenzo *et al.* 1993). In routine, for detection of oocysts in faecal samples, floatation and staining techniques are employed but these are time consuming and require trained personnel.

In the present study, a commercially available rapid and simple strip-kit based chromatographic lateral flow immunoassay was utilized for detection of *C. parvum* antigen in faecal samples collected from buffalo and crossbred cattle calves. The diagnostic method constitutes a new rapid enzyme immunoassay (EIA) that identifies the *C. parvum* specific antigen (CSA), qualitatively as well as semi-quantitatively. The results obtained were further compared with a conventional microscopy method using the modified Ziehl-Neelsen (mZN) staining.

Fresh faecal samples (34 from buffalo calves and 16 from crossbred cattle calves) were collected from 5 organized dairy farms in and around Ludhiana district of Punjab (Table 1). Immediately after collection, the samples were transported to laboratory and were examined within 24 h. The consistency of the faecal samples was scored as diarrhoic and non-diarrhoic. The samples were analyzed by using strip assay according to the manufacturer's instructions. Results obtained

from chromatographic lateral flow immunoassay were graded as negative, weak (+), moderate (++) or strong (+++), depending on absence or presence of color development along with its intensity in T-line of the test strip. Each test strip additionally had a positive control line (C-line) that will always appear regardless of the presence of *C. parvum* oocysts in faeces and hence served as an internal qualitative control, whilst the test line (T line) will only develop if *C. parvum* oocysts are present in the faecal sample. So, only C line will be observed in negative samples whereas in positive samples both C and T lines will develop. When the results of positive tests were graded semi-quantitatively depending on the intensity of color development in T line (with the help of the chart provided by the manufacturer along with test kit) different degrees of intensity were found within the positive samples. Faecal smears, prepared on a clean microscopic glass slide were subjected to mZN staining technique (Henriksen and Pohlenz 1981) for detection of *Cryptosporidium* spp. oocysts.

Out of the 50 faecal samples subjected to chromatographic lateral flow immunoassay, 10 samples found to be positive for CSA giving an overall percentage of positivity of 20%. From the 10 positive samples, 8 corresponded to buffalo calves (23.52%) and 2 were crossbred cattle calves samples (12.50%) (Table 1). All the positive cases corresponded to animals less than 1 month of age. Positive samples from buffalo calves were either from diarrhoic or dysenteric faeces, whereas in crossbred cattle calves non-diarrhoic faeces also gave positive results. Oocysts of *Cryptosporidium* were detected in faecal samples of both diarrhoic and non-diarrhoic cattle and buffalo calves at Izatnagar, Uttar Pradesh (Jeyabal and Ray 2005). The virulence of bovine cryptosporidiosis caused by *C. parvum* is enhanced in young unweaned dairy calves having premature immune system resulting in scour, dehydration and death (Moore *et al.* 2003, Trotz *et al.* 2007). Several studies have recorded the prevalence of *C. parvum* in cattle and buffaloes from different regions of the world (Dubey *et al.* 1990, Becher *et al.* 2004, Geurden *et al.* 2005, Rinaldi *et al.* 2007). In India, the prevalence of *C. parvum* in

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Table 1. Antigenic prevalence of *Cryptosporidium parvum* in buffalo and crossbred cow calves

Dairy farm	Age of calf (months)	Positive/screened		Percent positivity	
		Buffalo calves	Crossbred calves cattle	Buffalo calves	Crossbred cattle calves
GADVASU, Ludhiana	<1 (buffalo calves)	3/9	1/6	33.34	16.67
	1–2 (crossbred cattle calves)	0/4	0/4	-	-
Apra village, Ludhiana	<1 (buffalo calves)	0/1	0/0	-	-
	1–2 (crossbred cattle calves)	0/3	0/0	-	-
Harnek, Threekay village, Ludhiana	<1 (buffalo calves)	2/2	1/3	100.00	33.34
	1–2 (crossbred cattle calves)	0/0	0/0	-	-
Parkash, Ashok and Bittu, Haibowal, Ludhiana city	<1 (buffalo calves)	1/7	0/1	14.28	-
	1–2 (crossbred cattle calves)	0/1	0/0	-	-
Major and Jagjit, Threekay village, Ludhiana	<1 (buffalo calves)	2/6	0/2	33.34	-
	1–2 (crossbred cattle calves)	0/1	0/0	-	-
Total		8/34	2/16	23.52	12.50
		10/50	20		

cattle and buffaloes was recorded from Kolkata, Maharashtra Punjab and Uttar Pradesh states (Khubnani *et al.* 1997, Das *et al.* 2003, Jeyabal and Ray 2005, Singh *et al.* 2006, Bhat *et al.* 2012). Reported infection in bovines ranged from 11.32 to 69.32% with maximum positive cases as well as oocyst shedding in diarrhoeic calves up to 30 days of age.

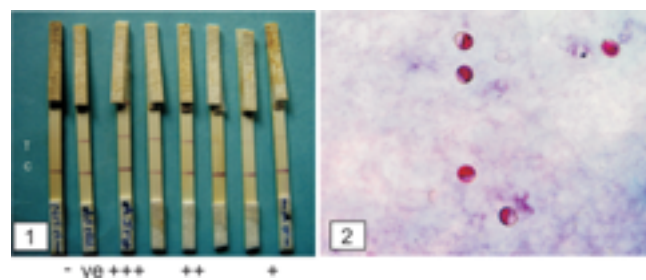
Amongst 8 positive faecal samples from buffalo calves, 2 came to scale of +++ (strongly positive for CSA, color on T line matches with that on C line), 4 ++ (moderately positive for CSA, color on T line is less intense than that on C line) and 2 + (weakly positive for CSA, color on T line is fainter than that on C line); whereas out of 2 positive samples of crossbred cow calves, 1 was scored as moderate positive (++) and the other as weakly positive (+). In all the negative samples there was development of color only on T line (Fig. 1).

Using the modified Ziehl-Neelsen staining, oocysts from the positive samples appeared as pink red colored granules with varying diameter of 4–5 µm (Fig. 2) and the percentage of positivity was recorded as 14% (17.65% corresponded to

samples from buffalo calves and 6.25% from cattle). When the results obtained with both techniques were compared, weakly positive samples in chromatographic lateral immunoassay were found not to be detected by mZN staining method. The sensitivity of mZN staining was 70% compared to lateral flow immuno assay.

During the past decade, *Cryptosporidium* spp. have become an important pathogen of animals and humans. Numerous stool concentration and staining techniques that allow direct visualization of oocysts in fecal specimens were developed. All microscopic diagnoses rely on direct visualization and morphological recognition of small-sized oocysts, which may be scanty in number, intermittently shed, or inconsistently stained. Although microscopy remains the gold standard test for the detection of many diseases, the lack of sensitivity and the different interpretation of the results depending on the observer make this method less reliable when compared to immunological and molecular based tests. As chromatographic lateral immunoassay for detection of *C. parvum* utilizes monoclonal antibody specific for *C. parvum* oocyst antigen, the method is highly specific as there is no cross reactivity with other gastrointestinal parasites (Lise *et al.* 2005) and sensitive too when compared with mZN staining test. This test can be used to screen even small number of samples when it proves to be problematic to utilize enzyme linked immunosorbent assay, hence, this classical technique can be used as replacement of ELISA technology (Lise *et al.* 2005).

To summarize, this immunological based diagnostic test is simple, rapid to perform, easy to analyze, requires limited laboratory equipments and can also be used even for screening of a single sample. So for detection of cryptosporidiosis in bovines, it can be used as single test in place of conventionally used microscopic technique. The assay will also eliminate some of the skill needed in



Figs 1–2. 1. Chromatographic lateral flow immunoassay graded as negative, strong (+++), moderate (++) and weak (+) positive based on intensity of color development along with T-line of the test strip. 2. Modified Ziehl-Neelsen stained oocysts from the positive samples with varying diameter of 4.3–5.2 µm.

performing complicated staining procedures and recognizing the morphology of the small oocysts of *C. parvum*.

SUMMARY

Cryptosporidium parvum, an obligatory intracellular apicomplexan enteric protozoan parasite causes cryptosporidiosis, a disease of substantial veterinary and medical importance. In the present study, 50 faecal samples from 5 organized dairy farms were examined for cryptosporidiosis by employing simple and rapid chromatographic lateral flow immunoassay for antigenic detection of cryptosporidial infections in buffalo (34) and crossbred cattle (16) calves in Punjab state. The percentage of positivity from buffalo and crossbred cattle calves observed by lateral flow immunoassay was 23.52 and 12.50, respectively, while by modified Ziehl-Neelsen (mZN) staining percentages of 17.65 and 6.25, respectively, were recorded. The sensitivity of mZN staining was 70% as compared to chromatographic lateral flow immunoassay.

REFERENCES

- Becher K A, Robertson I D, Fraser D M, Palme D G and Thompson R C. 2004. Molecular epidemiology of *Giardia* and *Cryptosporidium* infections in dairy calves originating from three sources in Western Australia. *Veterinary Parasitology* **123**: 1–9.
- Bhat S A, Juyal P D and Singla L D. 2012. Prevalence of cryptosporidiosis in neonatal buffalo calves in Ludhiana district of Punjab, India. *Asian Journal of Animal and Veterinary Advances* **7**: 512–20.
- Das G, Sarkar S, Das P and Panja P. 2003. *Cryptosporidium* infection of cattle in and around Kolkata, West Bengal. *Indian Journal of Animal Health* **42**: 142–44.
- Dubey J P, Fayer, R and Speer C R. 1990. *Cryptosporidium in Man and Animals*. CRC Press, Boca Raton, Florida.
- Geurden T, Claerebout E and Vercruysse J. 2005. Protozoan infection causes diarrhea in calves. *Tijdschr Diergeneeskde* **130**: 734–37.
- Goh S, Reacher M, Cassemore D P, Verlande N Q, Chalmers R, Knowles M, Pennington A, Williams J, Osborn K and Richard S. 2004. *Sporadic Cryptosporidiosis*, North Cumbria, England, 1996–2000. *Emerging and Infectious Diseases* **10**: 1007–15.
- Henriksen A and Pohlenz J F L. 1981. Staining of cryptosporidia by a modified Ziehl-Neelsen Technique. *Acta Veterinaria Scandinavica* **22**: 594–96.
- Jeyabal L and Ray D D. 2005. Cryptosporidial infection in cattle and buffaloes. *Journal of Veterinary Parasitology* **19**: 165–66.
- Khubnani H, Sivarajan K and Khubnani A H. 1997. Study of cryptosporidiosis in a rural area of Maharashtra. *Indian Journal of Pathology and Microbiology* **40**: 33–36.
- Lise A, Trotz-Williams S, Wayne M, Donald M, Todd Duffield, Kenneth E. Leslie, Daryl V. Nydam, Frames J and Peregrine A S. 2005. Multiattribute evaluation of two simple tests for the detection of *Cryptosporidium parvum* in calf faeces. *Veterinary Parasitology* **134**: 15–23.
- Lorenzo LMJ, Ares-Mazás E and Villacorta M de M I. 1993. Detection of oocysts and IgG antibodies to *Cryptosporidium parvum* in asymptomatic adult cattle. *Veterinary Parasitology* **47**: 9–15.
- Moore D A, Atwill R E, Kirk J H, Brahmabhatt D, Herrera L, Alonso L H, Singer M D and Miller T D. 2003. Prophylactic use of decoquinate for infections with *Cryptosporidium parvum* in experimentally challenged neonatal calves. *Journal of American Veterinary Medical Association* **223**: 839–45.
- Nagamani K, Pavuluri P R R, Gyaneshwari M, Prasanthi K, Rao M I S and Saxena N K. 2007. Molecular characterization of *Cryptosporidium*: An emerging parasite. *Indian Journal of Medical Microbiology* **25**: 133–36.
- Paul S, Chandra D, Ray D D, Tewari A K, Rao J R, Banerjee P S, Baidya S and Raina O K. 2008. Prevalence and molecular characterization of bovine *Cryptosporidium* isolates in India. *Veterinary Parasitology* **153**: 143–46.
- Randhawa S S, Zahid U N, Randhawa Swarn S, Juyal P D, Singla L D and Uppal S K. 2012. Therapeutic management of cryptosporidiosis in cross bred dairy calves. *Indian Veterinary Journal* **89**: 17–19.
- Rinaldi L, Musella V, Condoleo R, Saralli G, Veneziano V, Bruni G, Condoleo RU and Cringoli G. 2007. *Giardia* and *Cryptosporidium* in water buffaloes (*Bubalus bubalis*). *Parasitology Research* **100**: 1113–18.
- Saha R S, Sarkar S, Batabyal S, Pramanik A K and Das P. 2006. Observations on the epidemiology of bovine cryptosporidiosis in India. *Veterinary Parasitology* **141**: 330–33.
- Singh B B, Sharma R, Kumar H, Banga H S, Aulakh R S, Gill J P and Sharma J K. 2006. Prevalence of *Cryptosporidium parvum* infection in Punjab (India) and its association with diarrhea in neonatal dairy calves. *Veterinary Parasitology* **140**: 162–65.
- Sulaiman I M, Hira P R, Zhou I, Al-Ali F M, Shelahi F A, Shweiki H M, Iqbal J, Khalid N and Xiao L. 2005. Unique endemicity of cryptosporidiosis in children in Kuwait. *Journal of Clinical Microbiology* **43**: 2805–09.
- Trotz W L A, Martin S W, Leslie K E, Duffield T, Nydam D V and Peregrine A S. 2007. Calf-level risk factors for neonatal diarrhoea and shedding of *Cryptosporidium parvum* in Ontario dairy calves. *Preventive Veterinary Medicine* **82**: 12–28.