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# Occurrence of Zoonotic Genotype of *Cryptosporidium parvum* in Cattle and Buffaloes Managed Extensively in Mixed Livestock Farms in the Dry Zone

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*ABSTRACT. The occurrence of Cryptosporidium oocysts in faeces of cattle, buffaloes and goats managed extensively in a dry zone mixed farm was examined during early 2002. The farm had a history of fatal diarrhoea of buffalo calves associated with C. parvum. The oocysts were demonstrated using the salt flotation method followed by the modified Ziehl Neelsen staining technique. Of the cattle (n=20), buffaloe (n=20) and goat (n=10) faecal samples examined, 65, 25 and 0% were excreting Cryptosporidium oocysts, respectively. The mean Cryptosporidium oocyst counts in cattle and buffaloes were 1900±770 and 190±123 oocysts per gram of faeces, respectively. Cryptosporidium oocysts positive samples from cattle and buffaloes were pooled separately and were subjected to genotypic identification. The DNA extracted from pooled Cryptosporidium oocyst were amplified by PCR using a set of primers (5' CCTGATCCTGTACCACCTCC 3' and 5' GTCATTTCTGATGAGCACGG 3') designed from the β-tubulin gene of C. parvum to identify the species. The expected band size of 460 bp in the PCR product appeared on agarose gel confirming the presence of C. parvum in cattle and buffaloes in the farm. All the infected cattle and buffaloes were asymptomatic. These animals are likely to play an important role in the epidemiology of cryptosporidiosis in animals and human being since these animals are known to harbour exclusively Genotype 2 of C. parvum, which is zoonotic.*

## INTRODUCTION

Cryptosporidiosis caused by *Cryptosporidium parvum* has been recognized as a significant enteropathogen in human being and animals (Graff *et al.*, 1999). In human *C. parvum* associated cryptosporidiosis results from either zoonotic or anthroponotic transmission of infectious oocysts (Awad-El-Kariem, 1999). The zoonotic Genotype 2 is cross transmissible between animals and human being while human adapted Genotype 1 is known to cycle restrictively within the human population (Awad-El-Kariem, 1999). In Sri Lanka, *Cryptosporidium* oocysts were identified in the faeces of diarrhoeic children (Perera, 1988; Perera and Lucas, 1990) and calves (Bahirathan *et al.*, 1987). The widespread occurrence of *C. parvum* in goats has been reported (Noordeen *et al.*, 2000) and its association in fatal diarrhoea in buffalo calves has been recorded (Senasinghe *et al.*, 2002).

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Information on occurrence and transmission of *C. parvum* infection in livestock that may serve as reservoir hosts of the parasite, is a necessity to prevent infections.

Thus, the objective of the study was to identify the occurrence of *Cryptosporidium* species in cattle, buffaloes and goats raised in a mixed livestock farm and to determine existence of *C. parvum* oocysts in infected animals to assess their zoonotic potential.

## MATERIALS AND METHODS

### Study area and livestock management

The study was carried out in a mixed livestock farm situated in the dry zone of Sri Lanka where the average annual rainfall ranges from 1000–1750 mm and temperature varies from 21–38°C. The farm consist of indigenous and crosses of exotic breeds of cattle (n=100), buffaloes (n=99) and goats (n=80) and had a history of fatal diarrhoea of buffalo calves associated with *C. parvum* (Senasinghe *et al.*, 2002). The animals were turned out as a single herd in the morning to the shrub jungles, dry tank beds and communal grazing lands for 6–8 h and confined during the night in crowded night paddocks. The hygienic condition of the night holding areas for cattle and buffaloes which were separated only by a fence, was poor with excreta accumulating and soiling calves. The goats were housed in stilted sheds with wooden slatted floors during the night and that precluded cross transmission of pathogens from nearby cattle and buffalo night paddocks through faecal contamination. Since the goats were browsing in the field, the chance of infection in the field also was unlikely. However, the potential infection of goats through the water source could be observed.

### Design of the experiment

The total number of faecal samples collected from this mixed livestock farm were 50; cattle (n=20), buffalo (n=20) and goat (n=10) and the samples were randomly collected from young and adult animals. The oocysts excreted from each animal were demonstrated using salt flotation method followed by the modified Ziehl-Neelsen (MZN) acid fast staining technique (Casemore, 1991) and quantified as described by Noordeen *et al.* (2000).

Samples determined to be positive for *Cryptosporidium* by staining were pooled separately according to the host species. The DNA extraction from these pooled *Cryptosporidium* positive samples were done by simple freeze-thaw procedure using *DNAzol*® (Life Technologies, USA) and then amplification was done by PCR using a set of primers (5' CCTGATCCTGTACCACCTCC 3' and 5' GTCATTTCTGATGAGCACGG 3') which were designed from β-tubulin gene of *C. parvum* to identify the species (Widmer *et al.*, 1998). The PCR thermal cycle consisted with initial denaturation for 2 min at 95°C, 40 cycle amplification at 94°C for 30 seconds, 61°C for 1 min, and 72°C for 1 min with a 5 min final extension at 72°C. The PCR products were subjected to electrophoretic separation in 1% agarose gel stained with Ethidium Bromide. A molecular mass ladder (100 bp) and a negative control were used in batch run.

## RESULTS AND DISCUSSION

*Cryptosporidium* oocysts were identified from faeces of 65% cattle, 25% buffaloes and 0% goats examined. All cattle and buffaloes shedding oocysts in their faeces were asymptomatic. The intensity of *Cryptosporidium* and the mean oocyst excretion in cattle and buffaloes were 1900±770 and 190±123 oocysts per gram of faeces, respectively. Though there was a very high variation in the number of oocysts present within the host species, the indication of the presence of oocysts confirm the *Cryptosporidium* infection. The DNA of cattle and buffalo *Cryptosporidium* oocysts was identified as *C. parvum*. Agarose gel visualization of PCR products is shown in Fig. 1.

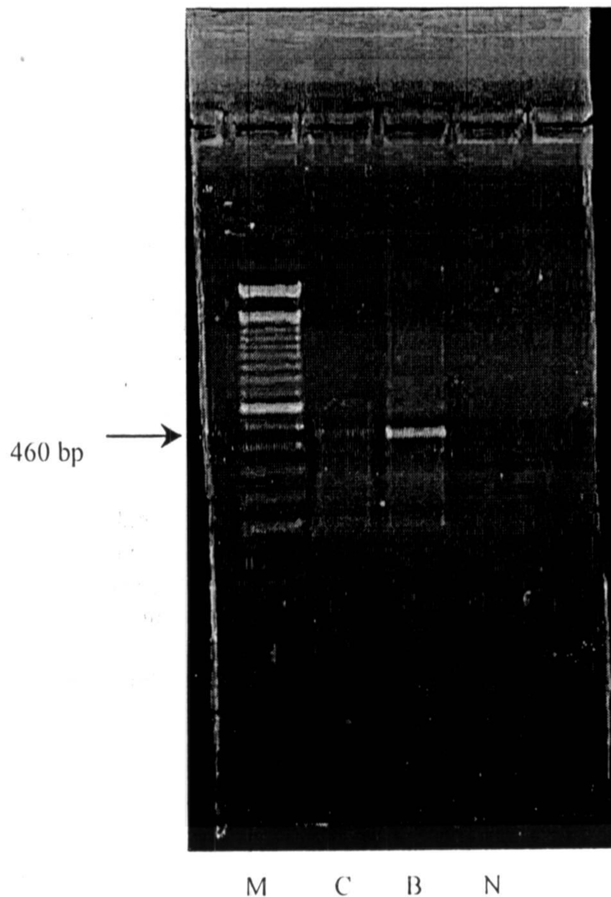


**Fig. 1. Agarose gel analysis of PCR amplification of *Cryptosporidium parvum* DNA.** [Note: *Cryptosporidium* oocyst isolates originated from cattle (C) and buffalo (B). Lane 'N' shows the negative control. Expected *C. parvum* specific band size 460 base pairs. Lane 'M' represents the molecular size marker (100 bp). Number in left side shows DNA fragment size in base pairs]

The results of the study provided evidence that *C. parvum* infection occurred only in buffaloes and cattle in the farm and not in the goats. This was not surprising since the goats in the farm were raised more hygienically on wooden slatted floors and there was very minimal contact between the goats and the infected cattle and buffaloes which in turn would have prevented faecal oral transmission of *C. parvum* oocysts. However, a considerable prevalence of *C. parvum* in goats in the dry areas of Sri Lanka has been recorded (Noordeen *et al.*, 2000).

Though *Cryptosporidium* oocysts have been identified from diarrhoeic children in Sri Lanka (Perera, 1988; Perera and Lucas, 1990), and which species and genotype (Genotype 1 or 2) were involved in these human infections were not confirmed yet. Therefore, the source of the infection (zoonotic or anthroponotic) for these human infections were not clear. However, the facts that cattle and buffaloes harbour the zoonotic *C. parvum* as observed in the present study, the close association of children and these animals in rural agricultural community and the use of common surface water sources by animals and human being, increase the likelihood of transmission of this possible Genotype 2 *C. parvum* from animals to susceptible human being. Therefore, attention should be given to improve the hygienic practices in the farm and to educate the farmer to prevent the spread of this infection to human and other animal species.

It was reported that a single infected cow can shed up to 720 million *C. parvum* oocysts daily (Scott *et al.*, 1995) that are resistant in the environment (Robertson *et al.*, 1992). In the present study, it was observed that the infected cattle were excreting an average of 1900 oocysts per gram of faeces. Since an adult cattle excretes 30–40 kg of faeces every day, it is possible to excrete 57–76 million of oocysts to the environment by an adult cow. This is a high level of environmental contamination and a different source of infection for man and animals.

Contamination of water sources by infective oocysts can play a major role in disease outbreaks in human being and animals. Such incidence has been reported elsewhere (Widmer *et al.*, 1996; Smith and Rose, 1998) and could be possible in the dry zone of the country as human being and animals frequently utilize common water sources. Wallowing behaviour of infected buffaloes in surface water sources may increase the likelihood of contamination of water sources. Thus, the transmission of *Cryptosporidium* oocysts to susceptible human being can be possible since most of the agricultural communities also use surface water sources at least for bathing and laundry purposes.

## CONCLUSIONS

A portion of the observed cattle and buffaloes were excreting *Cryptosporidium* oocysts whereas tested goats in the farm were not excreting *Cryptosporidium* oocysts. The *Cryptosporidium* oocysts isolated from both cattle and buffaloes represent zoonotic *C. parvum* since these animal species are known to harbour only the Genotype 2. However, further investigations should be carried out on genotypic evaluation of the isolates from man and animals. Such studies indeed would give more information on the exact source for the *C. parvum* infection in susceptible human being in Sri Lanka.

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