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Cryptosporidium infection in cattle in southern Botswana

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ABSTRACT

A parasitological survey was conducted to determine the prevalence of *Cryptosporidium* infection in cattle in Gaborone, Lobatse, Mochudi and Molepolole veterinary districts. Faecal samples from 137 dairy and beef calves < 3 months, 38 heifers and 36 cows were examined for oocysts of *Cryptosporidium* species in faecal smears stained with modified Ziehl Neelsen stain. The prevalence of *Cryptosporidium* infection was 29. 3% in dairy calves (n=106), 22. 6% in beef calves (n=31), 15. 8% in heifers (n=38) and 8. 3% (n= 36) in cows. The infection rate was significantly more in dairy calves in comparison to cows (χ^2 5. 4, P < 0. 05). Prevalence rates in dairy and beef calves < 4 weeks and 4 to 13 weeks old were 37. 8% of 74 and 15.9% of 63, respectively and the difference was statistically significant (χ^2 7. 1, P < 0. 01).The infection rate was significantly higher in diarrhoeic dairy and beef calves (64.3%) compared to that of non-diarrhoeic calves (23.6%) (χ^2 8.5, P < 0. 01) indicating role of *C. parvum* in neonatal diarrhoea. Morphologically, the oocysts observed in the faecal smears of calves were apparently indistinguishable from those of *C. parvum*, while in heifers and cows, the majority of oocysts were of *C. andersoni*. It is evident from this preliminary investigation that *Cryptosporidium* infection is fairly high in cattle especially calves which constitutes a serious risk of infection to animal handlers and HIV infected individuals who fail to wash their hands and they need to be educated on the possible dangers of their acquisition of this infection while working with these infected animals. Further studies are recommended by including more bovines and other livestock species from different parts of this country.

Keywords: Neonatal diarrhoea, *Cryptosporidium parvum*, prevalence, dairy calves, zoonosis.

INTRODUCTION

Bovine cryptosporidiosis caused mainly by *Cryptosporidium parvum* has recently emerged as an important aetiological agent of neonatal diarrhoea and a potential zoonosis. Cryptosporidial infection is a major fatal complication in HIV/AIDS infected and other immunocompromised individuals. It has become an increasingly important parasitic zoonosis and domestic animals have been implicated to transmit the disease to humans through direct contact and consumption of contaminated food and water with cow dung and human stool (Fayer *et al.*, 2000). Ruminants are infected mainly by two *Cryptosporidium* species. *Cryptosporidium parvum* affects mostly young calves, while *C. andersoni* is found in post weaned calves and adult cattle. The bovine genotype of *C. parvum* is the most common in ruminants, especially in cattle and sheep (Fayer *et al.*, 1997; Noordeen *et al.*, 2003) and it is zoonotic.

The prevalence of *Cryptosporidium* infection in animals has been reported frequently from Europe, North America, Australia and Asia with infection rates varying from 1 to 100% (Scott *et al.*, 1995; Lefay *et al.*, 2000; Santin *et al.*,

2004; Yu *et al.*, 2004). However, the information about its presence in livestock species from African countries is very scanty. Mtambo *et al.*, (1997), Agunloye *et al.*, (2001), Nizeyi *et al.*, (2002) and Goma (2005) recorded *C. parvum* infection rates of 5.3, 25.4%, 38 and 42.8% in dairy calves from Tanzania, Nigeria, Uganda and Zambia, respectively.

Though there has been no published report about the occurrence of cryptosporidiosis in human and animals in Botswana, yet nearly 18% children demonstrated mild to heavy *C. parvum* infection on coprological examination of more than 400 stool samples during the period between 2000-2003 at National Health Laboratory (NHL), Gaborone (Thakur, 2005: Personal Communication). Botswana, being one of the hardest hit countries by HIV/AIDS pandemic (CSO, 2005) and the recognition of *Cryptosporidium* infection as a potential fatal complication in HIV infected persons world-wide, it was considered necessary to establish the existence of *Cryptosporidium* infection in animals in this country. The present preliminary study reports the prevalence of *Cryptosporidium* infection in dairy and beef calves, heifers and cows at eight selected farms located in southern Botswana.

MATERIALS AND METHODS

Study area

Between February and October, 2005, a parasitological survey was carried out at eight farms, namely: Notwane Farm of Botswana College of Agriculture and Department of Agricultural Research (DAR) Farm at Sebele; Crossly Park Farm, Lobatse; Matshipa Farm, Kopong; Diphring Farm, Gabane; Prison and Mosenki Farms, Molepolole and Boswela Kgosi cattle-post, Bokka. These farms are located in Gaborone, Lobatse, Molepolole and Mochudi veterinary districts. Dairy animals at Notwane, DAR, Diphring and Crossly Park Farms were intensively managed. Mosenki and Matshipa Farms reared both dairy and beef animals under semi-intensively management system. Dairy and beef animals of Prison Farm and Boswela Kgosi cattle post grazed on communal native pastures under traditional husbandry system.

Sample collection

Faecal samples were collected directly from the rectum, using disposable latex gloves from 211 bovines comprising of 106 dairy and 31 beef calves (< 3 months), 38 heifers and 36 cows. Freshly expelled faeces which had not been contaminated by soil and dirt were also used. Samples were collected twice after an interval of 20-25 days, transported on ice and were kept refrigerated in Laboratory till their processing. The consistency of the faecal samples (watery, loose and formed) collected from calves was recorded. All animals of the selected farms were sampled because of relatively smaller herd size at these farms.

Oocyst detection

Two methods were used for detection of oocysts of *C. parvum* and *C. andersoni*. Thin faecal smears of approximately 2 cm were made in duplicate for each sample and stained with Modified Ziehl Neelsen (MZN) stain following the technique described by Garcia (2001) except that Malachite green instead of Methylene blue was used as counterstain. Stained faecal smears were examined using a calibrated

light microscope at 1000x magnification under oil immersion. Red spherical and sub-spherical bodies with refractile walls containing one to four dark granules were identified as *Cryptosporidium* oocysts.

Morphologically, smaller (~ 4. 5 x 5_m) and larger oocysts (~ 8 x 6 _m) were noted as oocysts of *C. parvum* and *C. andersoni*, respectively. The faecal samples found negative by direct faecal smear method were further subjected to Sheather's sugar concentration method. Smears were made from the supernatant, stained with MZN and examined microscopically as described earlier. Faecal samples found positive for *Cryptosporidium* oocysts by either one of the above methods were screened for helminthic eggs and coccidian oocysts by faecal floatation method as well as these were streaked on 5% Brilliant green and MacConkey agar plates for isolation of *Escherichia coli* and *Salmonella* species bacteria. Enzyme immunoassay was performed to detect rotavirus antigen by using Pathfinder™ Rotavirus kit supplied by Bio-Rad Laboratories, Redmond, WA, USA.

Data analysis

The data were analyzed using standard statistical tools i.e. Mean, standard error (SE) and Chi square test for comparisons of positive cases. The results were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The present study has demonstrated the occurrence of *Cryptosporidium* infection in bovine species of Botswana. The results of this study showed prevalence rates of 29. 2%, 22. 6%, 15. 8% and 8. 3% in dairy calves, beef calves, heifers and cows, respectively. The infection rate was significantly higher in dairy calves (χ^2 5. 4, $P < 0.05$) in comparison to that in cows (Table 1). *C. parvum* oocysts were detected in 31 of 106 dairy and seven of 31 beef calves examined, while *C. andersoni* (syn. *C. muris*) oocysts were observed in six of 38 heifers and three of 36 cows. However, further studies are warranted as our sample size pertaining to beef calves, heifers and cows was small. The infection rate was 37. 8% of 74 dairy and beef calves < 4 weeks compared to 15. 9% of 63 calves > 4 to 13 weeks old and the difference between these two age groups was statistically significant (χ^2 7. 1, $P < 0.01$) (Fig. 1).

Table 1. Prevalence of *Cryptosporidium* infection in cattle in southern Botswana.

Animal	*Number Tested*	*Number Positive*	*Per cent Prevalence ± S.E*
Dairy calves	106	31	29.2 ± 4.4[a]
Beef calves	31	7	22.6 ± 7.5
Heifers	38	6	15.8 ± 5.9
Cows	36	3	8.3 ± 4.6[b]

Difference between infection rates in dairy calves [a] and cows [b] was significant ($P < 0.05$).

Development of this age related resistance against this infection and other findings in this study are consistent with several other studies reported worldwide. Atwill *et al.* (1999a) detected *C. parvum* in 3. 9% of 915 calves 1-11 months of age and 0. 6% of 484 adult cattle >12 months of age. Fayer *et al.* (2000) reported *C. parvum* in 20.7% of 184 cattle over 6 months of age, including 4.7% of 43 cattle over 1 year of age. Agunloye *et al.* (2001) and

Nizeyi *et al.* (2002) recorded 38% and 25.4% infection rates among 50 and 130 calves in Nigeria and Uganda, respectively. Of 971 calves, 345 were found infected with *Cryptosporidium* (35.5%), but more pre-weaned calves (253 of 503; 50.3%) than post-weaned (92 of 468; 19.7%) were found infected (Santin *et al.*, 2004). In Zambia, Goma (2005) reported infection rate of 42.8% of 250 dairy calves < 3 months. Lower infection rate in dairy calves in the present investigation in comparison to those reported from Uganda and Zambia as well as from European countries may probably be due to prolonged summer season and severe drought conditions which prevailed throughout southern Botswana during the last few years. Temperature extremes and dry weather have been reported to have adverse effect on the viability of *Cryptosporidium* oocysts (Tzipori, 1983; Anderson, 1985; Walker *et al.*, 2001). A few asymptomatic heifers and cows found excreting oocysts in this study may act as potential reservoirs of infection for highly susceptible newborn calves and other susceptible animals. Appropriate management of livestock manure may be helpful in reducing infection in young bovines and contamination of the environment.

On microscopic examination of stained faecal smears, the average size of the *Cryptosporidium* oocysts was 4.7 × 5 _m in calves and 8.5 × 6.3 _m in heifers and cows. The small and large size oocysts of *C. parvum* and *C. andersoni* were morphologically indistinguishable as per described by Upton and Current (1985), Chermette and Boufassa-Ouzrout (1988). However, Santin *et al.* (2004) suggested that the identification of oocysts based solely on morphology must be reassessed using molecular methods to validate species of *Cryptosporidium*.

The prevalence rates based on the consistency of faeces in calves are presented in Table 2. A significantly higher proportion of diarrhoeic dairy and beef calves excreting watery faeces were found positive for *Cryptosporidium* oocysts (9 of 14 ; 64.3%) compared to that of non-diarrhoeic calves with loose to formed faeces (29 of 123; 23.6 %) (χ^2 8. 5, P < 0. 01) indicating possible role of *C. parvum* in inducing neonatal diarrhoea..

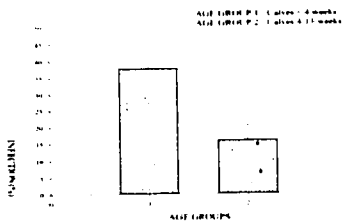

Fig. 1: *Cryptosporidium* infection in two age groups of calves.

It is possible that some of the animals with loose and formed faeces that were found excreting oocysts might have manifested diarrhoea earlier prior to our sampling. On further investigation of *C. parvum* positive calves with diarrhoeic faeces, four, two and one were positive for *Escherichia coli*, rotavirus antigen and *Eimeria bovis* oocysts, respectively. Lower parasitic burden and *E.coli* infection may be due to regular deworming and antibiotic medication being practiced in majority of these farms. Under field conditions *Cryptosporidium* infections in calves were reported to be commonly linked to concurrent infections with other enteropathogens like rotavirus, coronavirus, *E.* coli, *Salmonella*,

Eimeria and *Giardia* species microorganisms (Krogh and Henriksen, 1985; Reynolds *et al.*, 1986; de Graff *et al.*, 1999). Animals under intensively and semi-3.3%) compared to those kept under traditional management system (8 of 43; 18.6 ±.5.9%) but the difference was not significant (Fig 2)

Table 2. Prevalence of *Cryptosporidium* species infection in calves based on the consistency of faeces.

Animals	Dairy calves			Beef calves		
	Watery	Loose	Formed	Watery	Loose	Formed
Number Tested	11	16	79	3	7	21
Number Positive	7	6	18	2	2	3
%positi s ± S.E	63.6 ±14.5	37.5 ±12.1	22.8 ±4.7	66.7 ±27.2	28.6 ±17.1	14.3 ±7.6

Great stocking densities often observed under intensive and semi-intensively managed farms in this study probably contributed towards the increased levels of environmental contamination by *Cryptosporidium* infected animals excreting large number of oocysts. This is in conformity with the findings of Garber *et al.* (1994) and Atwill *et al.* (1999b). However, Castro-Hermida *et al.* (2002) did not report any difference in the prevalence of *C. parvum* in calves with intensive and semi-extensive management systems in Galicia, Spain.

The present study has demonstrated the presence of *Cryptosporidium* infection among cattle for the first time in Botswana. Of these, a fairly high percentage of dairy and beef calves < 13 weeks (38 of 137, 27.7%) were found excreting oocysts of *C. parvum* which may probably be acting as potential reservoirs for human cryptosporidiosis especially for children, animal handlers, HIV/AIDS patients and other immune compromised persons of this country as evidenced from the parasitological investigations at NHL, Gaborone (Thakur, 2005: Personal Communication).

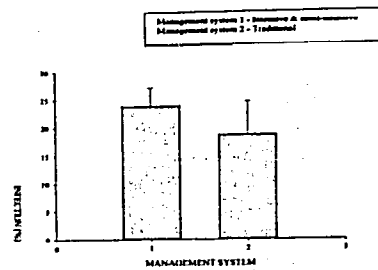

Fig. 2: *Cryptosporidium* infection in cattle under different management System.

Control measures involving good management and hygienic practices should be targeted against this age group of animals to combat cryptosporidiosis. It is suggested that the animal handlers be educated on the possible dangers of contracting the infection from calves at dairy farms and beef ranches. Also adequate care must be taken by individuals to protect themselves and from becoming a source of infection. Further studies are warranted by including more bovines and other livestock species from different parts of the country to improve the understanding about the epidemiology of cryptosporidiosis in Botswana.

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