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ORIGINAL RESEARCH

Rotavirus and Cryptosporidium infection in bovine calves in Southern Botswana

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SSP, conceived idea, designed study, collected samples & analysis, statistical analysis, preparation of manuscript; BM, collected samples, laboratory analysis

ABSTRACT

A cross-sectional study on prevalences of *Rotavirus* (*RV*) and *Cryptosporidium parvum* infections was carried out in dairy and beef calves in 31 farms over a 2-year period. Enzyme immunoassay (EIA) was to determine *RV* and *C. parvum* antigens in faecal samples. Cryptosporidial oocysts were detected in faecal smears stained by Modified Ziehl-Neelsen (MZN) technique. In dairy and beef animals, *RV* infections determined by EIA were 38.4% and 22.1% and *C. parvum* infection were 28.8% and 15.8%, respectively. MZN staining technique detected *Cryptosporidium* oocysts in 24.5% and 9.2% of the dairy and beef calves' samples, respectively. At herd level, at least one beef and dairy calf were positive for *RV* and *C. parvum* infection in 11 and 10 beef herds out of 18 (61.1% and 55.6%), respectively, and 12 of 13 dairy herds (92.3%) examined. Dairy calves were found to be equally susceptible to *RV* and *C. parvum* infections whether reared under semi-intensive or intensive managements, but differences in infections were significant ($P < 0.01$) among beef calves raised under semi-intensive versus extensive husbandry system. Forty-five calves (41 dairy and 4 beef) had combined *RV* and *C. parvum* infections. Younger dairy and beef calves aged ≤ 4 weeks and diarrheic animals showed significantly higher ($P < 0.01$) *C. parvum* infection than older (≥4 - ≤12 weeks) and non-diarrheic calves, but *RV* infection were not significantly different ($P > 0.05$) in these two age groups. Sex of the calf was not associated with shedding of *Cryptosporidium* oocysts and *RV* infections. Based on the above results, husbandry advice was given to farmers and adoption of good management practices and immunization of animals resulted in reduction in clinical cases of neonatal diarrhea and mortality rates among dairy calves. Greater understanding of *Cryptosporidium* species and molecular-based prevalence studies, good hygienic practices on farms and use of *RV* vaccine in pregnant animals will result in reducing infections. Livestock handlers need to be educated on zoonotic implications of *Cryptosporidium*, the possibilities of inter-species transmission abilities of *Rotaviruses*, and the importance of these pathogens in young animals.

Keywords : Beef calves, *Cryptosporidium parvum*, diarrhea, dairy calves, Enzyme immunoassay, *Rotavirus*

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INTRODUCTION

Rotaviruses (*RV*) and *Cryptosporidium* species infections are ubiquitous in nature and have been recognized as important etiological agents of gastroenteritis in young animals and children (Steele *et al*. 2004; Radostits *et al*. 2007). Both these organisms are distributed extensively in the intestinal tracts of diarrheic and clinically normal calves (Myers *et al*. 1984; Dupont, 1985). However, Trotz-Williams *et al*. (2005) demonstrated a three-time higher risk for calves shedding *Cryptosporidium* oocysts in diarrheic than non-infected calves. Hamnes *et al*. (2006) reported higher prevalences of both infections in large herds spreading over vast areas and among intensively reared animals since farmers were not able to pay proper attention to every calf and cleaning and elimination of pathogens from such farms proved to be difficult. In Botswana, an outbreak of diarrhea caused by *Cryptosporidium* and *Escherichia coli* organisms resulted into death of more than 500 children enrolled under HIV/AIDS's Prevention of Mother to Child Treatment program in 2006 (Anonymous, 2007). *Rotaviruses* are the most common enteropathogens detected in diarrheic calves (Snodgrass *et al*., 1986; Garcia *et al*., 2000). Cryptosporidiosis in calves is usually self-limiting, but its severity is enhanced by the presence of concurrent infections of *RV* and other pathogens (Holland, 1990). Transmission of both *RV* and *Cryptosporidium* species infections occur via the faecal-oral route by exposure of susceptible animals to faeces of infected individuals or to

fomites, but the usual mode of spread is from calf to calf. In a preliminary study (Sharma 2006) on bovine cryptosporidiosis, infection rates of 29.3% and 22.6% were recorded in dairy and beef calves, respectively in Botswana. The occurrences of *RV* infection have been recorded in children with gastroenteritis in Botswana (Kasule *et al*., 2003; Kebaabetswe *et al*., 2005), but similar information on animals is lacking. In the present study, faecal samples from both dairy and beef calves with or without diarrhea were examined to determine the presence of *RV* and *Cryptosporidium* species infections by enzyme immunoassay. Modified Ziehl-Neelsen technique was used to detect *Cryptosporidium* oocysts in faecal smears.

MATERIALS AND METHODS

Study Area and Sample Collection

Faecal sampling and data collection was done between September 2009 and February 2012. The study included 542 bovine calves (302 dairy and 240 beef) from 31 farms (13 dairy and 18 beef) located in four districts in south part of Botswana, namely Southern, South East, Kgatleng and Kweneng. Only calves up to 3 months of age were sampled. Of the13 dairy farms, A and B farms were large scale farms with 800 and 300 cows, respectively; C and D were medium scale farms with 30 and 60 cows and 9 were small-holders' farms with < 30 cows. Herd size in beef farms varied from 10 to70 animals with an exception of BCA farm with 180 cows. During the visit to each herd, a fresh rectal faecal sample weighing 10-15g was collected into screw capped plastic containers using disposable latex gloves for each calf and the sample stored under refrigeration (4-10°C). All the calves from the selected farms were sampled because of their relatively small numbers. Each animal was sampled once only during the study period. The clinical signs like coughing, general body condition, nasal and ocular discharges of the animals, the consistency of their faecal samples, the type of housing, the stocking densities and sanitary conditions in calf pens were recorded. Watery and loose faeces were considered to be excreted from diarrheic calves.

Farm Management

Of the thirty-one farms investigated, 163 and 99 calves sampled from 12 dairy and three beef herds, respectively were kept under semi-intensive management system in which the animals were allowed to graze on the premises of fenced farms. Only dairy farm A comprising of 139 calves practiced intensive husbandry system in which animals were stall-fed throughout the year. Fifteen of the 18 beef farms comprising of 141 calves used extensive or communal grazing system. Mortality rates among dairy and beef calves varied from 20 to 50% and 6 to10%, respectively. Dairy farms A and B reported very high mortality rates that ranged between 40% to 50% among young calves aged ≤ 1 month, largely due to acute gastroenteritis. At dairy farms, newborn calves were allowed to suckle colostrum from their dams for one to three days only; then these were moved to either conventional calf pens or mobile calf hutches where they were fed milk or milk replacer diets using bottles and buckets for about a month. After a month calves were transferred to open enclosures made of corrugated iron sheets or wire fence for about three months. Majority of beef farms wean calves after six weeks and then keep them in cluster groups.

Laboratory Analysis of Faecal Samples

Enzyme immunoassay (EIA)

RV and *Cryptosporidium* coproantigens were determined using commercial RIDASCREEN® Rotavirus (C 0901) and Cryptosporidium (C 1201) diagnostic kits (R-Biopharm AG, 64297 Daramstadt, Germany). EIA tests were conducted following the manufacturer's instructions. Photometric measurements were carried out at 450nm wavelength by a MULTISKAN microplate ELISA reader (Labsystems, Helsinki, Finland).

Modified Ziehl-Neelsen (MZN) technique

Faecal samples were directly smeared and then *Cryptosporidium* oocysts were detected microscopically using Ziehl-Neelsen stain following the procedure described by Garcia (2001) except that Malachite green instead of Methylene blue was used as counterstain. The smears were observed using a calibrated light microscope at x 1000 magnification under oil immersion objective. Light to bright red spherical and sub-spherical bodies measuring ~4.5 x 5 µm with refractile walls containing sporozoites were identified as *Cryptosporidium* oocysts.

Statistical analysis

The data was analyzed using Chi-square test for comparisons of the positive cases within groups and husbandry systems and significance considered at $P < 0.05$.

RESULTS AND DISCUSSION

This is the first cross-sectional investigation carried out in Botswana to study *RV* and *C. parvum* infections in dairy and beef calves. The results are presented in Figure 1, Tables 1 and 2. Of the 302 calves from 13 dairy farms, *RV* and *C. parvum* infections were observed in 38.4% and 28.8% calves, respectively. The prevalence of *RV* and *C. parvum* infections were 22.1% and 15.8% among 240 beef calves on 18 farms as determined by EIA (Figure 1).

This investigation suggests that both infections are enzootic on dairy and beef farms of southern Botswana. Studies worldwide (Fayer *et al*., 2000; El-Shazly *et al*., 2002; Santin *et al*., 2004; Kaushik *et al*., 2008) have

reported variability in infection rates which depend upon the procedure used for faecal screening, the frequency and seasons of sampling, the age, the clinical status of calves (diarrheic versus non-diarrheic) and farm management practices. A total of 45 animals (41 dairy and 4 beef) consisting of both diarrheic and asymptomatic calves were harbouring *RV* and *C. parvum* infections concurrently. Mixed infections of *Cryptosporidium*, *RV*, *Coronavirus*, *Escherichia coli* and *Salmonella* have been reported in one to 30-day- old diarrheic dairy calves from central Spain (de la Fuente *et al*., 1999; Garcia *et al*., 2000), Turkey (Emre and Fidanci, 1998), Sweden (Björkman *et al*., 2003) . According to Garcia *et al*. (2000), 43.6% neonatal diarrheic dairy calves were positive for *RV* infection in Spain with a concurrent infection in 58% of the *RV* infected calves, and the most common was *RV-Cryptosporidium* infection. The detection rates of other enteropathogens with *RV* infection were 85.2% for *Cryptosporidium*, 20.4% for *Coronavirus*, 16.7% for $F5^+$ *E. coli* and 1.8% for *Salmonella* (Garcia *et al*., 2000). Studies on the prevalences of other above mentioned enteropathogens could not be conducted in the present investigation.

In present study *RV* and *C. parvum* infections were significantly higher in dairy calves ($P < 0.01$, $\chi^2 = 15.9$) than beef calves ($P < 0.01$, $\chi^2 = 12$). This may possibly be due to greater stocking density, and therefore population at risk at any given time higher thereby favouring increased levels of environmental contamination in farm premises. This would be the case especially with the mobile hutches and calf pens for *RV* and *C. parvum* oocysts. Dairy Farm A was observed to be burdened with problems of gastroenteritis and heavy mortality of < 4-week-old calves and recorded the highest *Cryptosporidium* (29.5% ± 3.9) and *RV* (44.6% ± 4.2) infection rates largely due to the large size of the farm (>800 cows). Continual housing of calves in a limited area, allowing them to lick each others' body coats and perinea soiled with diarrheic faeces and poor hygienic conditions in calf pens and enclosures. There was marked reduction in the average calf mortality rate from an average of 45 % to less than 8% at dairy farms A and B within a period of two months on adoption of sanitary measures during housing and feeding of calves. Immunization of pregnant dairy cows against *Rotaviruses*, treatment of calves with halofuginone lactate and long-acting sulfa drugs and oral rehydration therapy were also suggested and partially implemented by the farmers.

MZN technique could detect *Cryptosporidium* oocysts in faecal samples of 24.5% dairy and 9% beef calves. Lower *C. parvum* infection rates recorded by MZN in comparison to EIA were due to less sensitivity of this technique in detecting oocysts especially when their excretions were low and intermittent. Similar observations have also been made by Scott *et al*. (1995). EIA has become widely accepted technique for screening stools for *C. parvum* infection in the past decade, because of its high sensitivity and specificity (Katanika *et al*., 2001). This technique was also found to be more sensitive than MZN in the present investigation. The present study might have underestimated the prevalence of *Cryptosporidium* infection due to oocyst detection limits as well as examining only one faecal specimen *per* animal. This is because a single sample may be negative as a result of intermittent oocyst excretion patterns.

Dairy and beef calves which were ≤ 4 weeks were found to be more susceptible to *Cryptosporidium* infection than those aged ≥ 4 weeks to 12 weeks (Table 1) and the differences were significant ($P < 0.01$, χ^2 6.8 and 8.1, respectively). This finding is in accordance with several other international studies (de la Fuente *et al*., 1999; Santin *et al*., 2004, 2008; Sharma, 2006; Geurden *et al*., 2006). Shedding of oocysts in pre-weaned dairy calves is often observed between 1 to 3 weeks which peaks in the second week, corresponding well with the life cycle of *C. parvum*. This age associated differences were not detected in *RV* infected calves in the current study and the results are in agreement with the findings of Garcia *et al*. (2000) from Spain.

Diarrheic dairy and beef calves were observed to have significantly higher *Cryptosporidium* and *RV* infection rates when compared to apparently healthy calves passing solid faeces (Table 2).

Table 1. Prevalence of *Cryptosporidium parvum* and *Rotavirus* infections in two age groups of dairy and beef calves by Enzyme Immunoassay

Age Groups	No of Animals Tested	No of Animals Positive		% Prevalence ± SE	
		C. parvum	*Rotavirus*	*C. parvum*	*Rotavirus*
Dairy ≤ 4 weeks	157	56	59	35.7 ± 3.8[a]	37.6 ± 3.9
Dairy >4 - ≤12 weeks	145	31	57	21.4 ± 3.4[b]	39.3 ± 4.1
Total	302	87	116	28.8 ± 2.6	38.4 ± 2.8
Beef ≤ 4weeks	87	21	22	24.1 ± 4.6 [c]	25.3 ± 4.7
Beef > 4 - ≤12 weeks	153	17	31	11.1 ± 2.5 [d]	20.3 ± 3.2
Total	240	38	53	15.8 ± 2.4	22.1± 2.7

*Differences between *Cryptosporidium* infection rates in two age groups of dairy calves[a & b] were significant ($P < 0.01$)
*Differences between *Cryptosporidium* infection rates in two age groups of beef calves[c & d] were significant ($P < 0.05$)
**Differences between *Rotavirus* infection rates in two age groups of dairy and beef calves were not significant

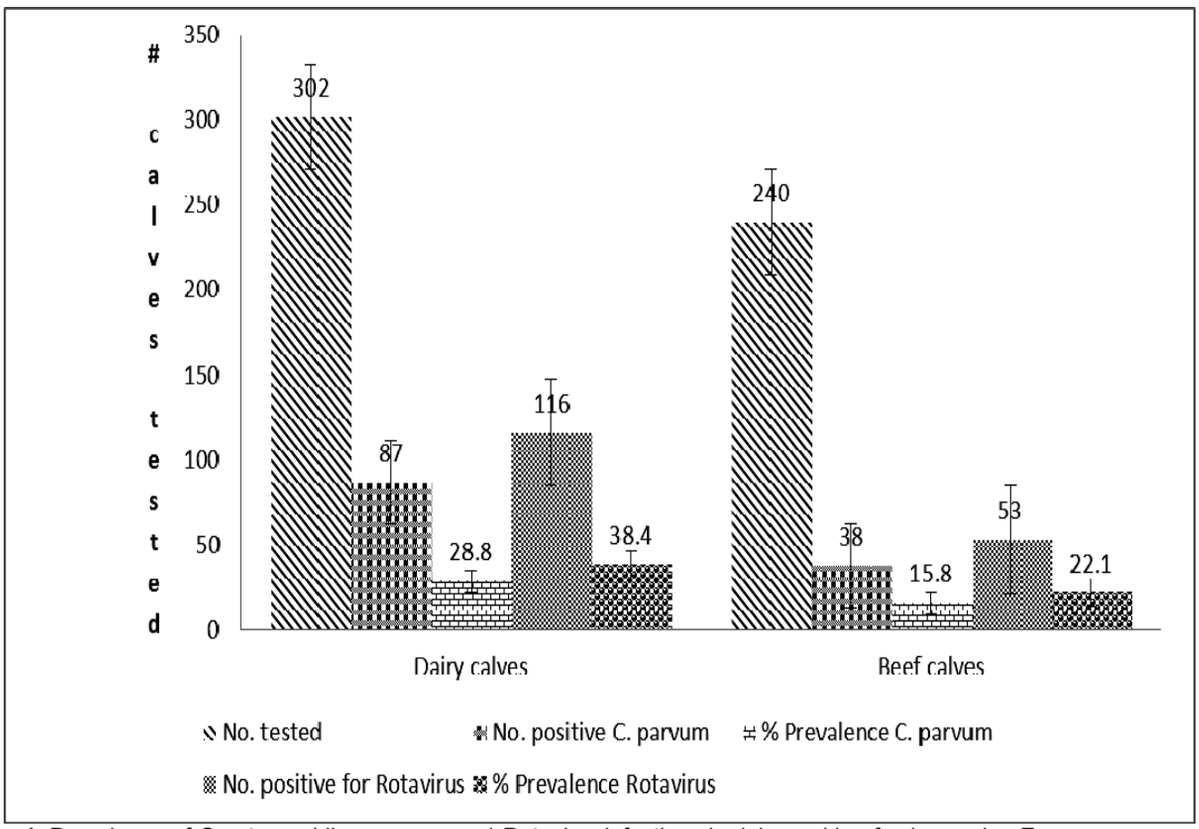

Figure 1. Prevalence of *Cryptosporidium parvum* and *Rotavirus* infections in dairy and beef calves using Enzyme immunoassay. The error bars are standard errors of the means

Table 2. Prevalence of *Cryptosporidium parvum* and *Rotavirus* infections in dairy and beef calves excreting liquid/soft and formed faeces by Enzyme Immunoassay

Types of Bovine Calves	Type of Faeces	No Animals Tested	No Animals Positive		% Prevalence ± SE	
			C. parvum	*Rotavirus*	*C. parvum**	*Rotavirus***
Dairy	Liquid/Soft	202	71	87	35.1± 3.4[a]	43.1±3.5[a]
Dairy	Formed	100	16	29	16 ± 3.7[b]	29 ± 4.5[b]
Beef	Liquid/Soft	96	22	28	29.2 ± 4.6[c]	32.8 ± 6.2[c]
Beef	Formed	144	16	25	17.4 ± 2.[d]	18.7 ± 3.2[d]

*Differences between *Cryptosporidium* infection rates in diarrheic and non-diarrheic dairy[a & b] ($P < 0.01$) and beef calves[c & d] ($P < 0.05$) were significant

**Significant differences in *Rotavirus* infection rates in diarrheic and non-diarrheic dairy[a& b] and beef calves[c & d] ($P < 0.05$)

These observations are consistent with those of others (Holland, 1990; Björkman *et al*., 2003; Steele *et al*., 2004; Sharma, 2006; Radostits *et al*., 2007). In this first-ever cross-sectional investigation, it is difficult to say whether *C. parvum* and *RV* infected animals passing faeces of normal consistency were in fact asymptomatic. It may be possible that some of these animals might have had bouts of gastroenteritis prior to our sampling or with subclinical infections or recovering from clinical disease.

The present study demonstrated a slightly higher *C. parvum* (41/139, 29.5% ± 3.9 and 46/163, 28.2% ± 3.5) and *RV* infection rates (62/139, 44.6% ± 4.2 and 54/163, 33.1± 3.7) among dairy animals reared under intensive management system as compared to those reared under semi-intensive management system, but the differences were not significant ($P > 0.05$). However, highly significant differences ($P < 0.01$, χ^2 7) in *Cryptosporidium* (14/141, 9.9% ± 2.5 and 24/99, 24. 2 % ± 4.3) and *RV* infection rates (40/141, 28.4% ± 3.8 and 13/99, 13.1% ± 3.4) were found between beef calves raised under extensive/communal and semi-intensive husbandry systems, respectively. Our results are in agreement with

those of Guerden *et al*. (2006) and Sharma (2006) who found significantly lower *C*. *parvum* infection rates in calves kept under traditional husbandry/communal systems compared to intensive and semi-intensive management systems in Zambia and Botswana, respectively. Higher infection rates recorded in the intensively and semi-intensively managed calves in comparison to those from animals under communal system are possibly due to group housing, housing in the previously contaminated calf pens and mobile hutches and calves' frequent nose- to- nose contacts.

In the present investigation, sex of the calf was not associated with shedding of *Cryptosporidium* oocysts and *RV* infections in both dairy and beef calves. Both male and female dairy and beef calves appear to be equally susceptible to *RV* and *C*. *parvum* infections. *Rotavirus* prevalence in male and female dairy and beef calves were 40.4% ± 4.2 versus 36.7% ± 3.7, and 22.2% ± 3.7, versus 21.9% ± 3.9, respectively. *Cryptosporidium* infection rates in male and female dairy and beef calves were 25% ± 3.7 versus 31.9% ± 3.6 and 11.9% ± 2.9 versus 20.2% ± 3.8, respectively. The present findings correspond well with those of Agunloye *et al*. (2001), Silverlås *et al*. (2009), Swai and Schoonman (2010) who found no difference due to sex.

The high prevalence of mixed *RV* and *C*. *parvum* infections observed in this study suggest that there is widespread distribution of these enteropathogens among bovine calves aged < 3-months in southern Botswana. This calls for urgent need for strict adherence to good calf husbandry practices since naturally infected calves are significant reservoirs of these organisms that have the potential of being transmitted to other mammalian species, including humans. Keeping in view the possibilities of animal rotaviruses crossing species barriers, the close proximity of people with their animals and zoonotic potential of cryptosporidiosis, intervention strategies targeting young calves at dairy and beef farms need to be implemented. These included adoption of hygienic practices during feeding and housing of young stock, careful management of manure, and immunization of pregnant cows with rotavirus vaccine. More surveillance studies on animal rotaviruses and molecular characterization of *Cryptosporidium* species in animals are required to understand the transmission dynamics and public health significance of these infections.

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Conflict of Interest: None

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