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ORIGINAL RESEARCH

Prevalence of some gastrointestinal parasites of ruminants in southern Botswana

Sharma S.* and Busang, M.
Department of Animal Science & Production, Botswana College of Agriculture, P/Bag 0027, Gaborone, Botswana

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

ABSTRACT

The purpose of this study was to establish the prevalence of gastrointestinal parasites in cattle, sheep and goats by conducting a parasitological survey on 16 livestock farms. In all, a total of 465 faecal samples of 131 dairy calves, 94 beef calves,143 goat kids and 97 lambs aged ≤ 6 months were examined using Modified McMaster technique. Enzyme immunoassay (EIA) was performed for detection of *Cryptosporidium* coproantigen. The results of the parasitological survey showed helminthic and *Eimeria* species infection rates of 10.7 ± 2.7% and 13 ± 2.9% in dairy calves, 8.5 ± 2.9% and 10.6 ± 3.2% in beef calves, 21 ± 3.4% and 13.3 ± 2.8% in goat kids, 24.7 ± 4.4% and 11.3 ± 3.2% in lambs respectively. Animals excreting soft to liquid faeces showed 34.8 ± 4% of combined helminthic and *Eimeria* species infection rates compared to 24.4 ± 2.4% in animals with normal solid excreta and the differences were significant ($P < 0.05$). Animals aged ≤ 3 months and 3 to 6 months exhibited combined helminthic and coccidial infection rates of 12 ± 2.1% and 20.6 ± 2.6%, respectively. Percent *Cryptosporidium parvum* infection rates were 25.2 ± 3.8, 12.8 ± 3.4, 12.4 ± 3.3 and 10.5 ± 2.6 in dairy calves, beef calves, lambs and goat kids, respectively. Significantly greater *Cryptosporidium* infection rate ($P < 0.05$) was recorded in dairy calves than beef calves, goat kids and lambs. *C. parvum* infection rates were 28.4 ± 3.8% and 20.7± 2.7% in diarrhoeic and younger (≤3 months) animals in comparison to 9.9 ± 1.7% and 10.3± 2% in non-diarrhoeic and older animals (3-6 months) respectively and the differences were significant ($P < 0.01$). These findings suggest that gastrointestinal helminthic, *Eimeria* species and *C. parvum* infections are widely distributed in small stock and bovine calves and are characterized by gastroenteritis. These may lead to mortality in acute cases and/or sub-optimal performance in subclinical infections. Therefore livestock farmers need to be educated on the impact of gastrointestinal parasitism on animal productivity and the appropriate control strategies based on epidemiological observations. Farmers should be encouraged to adopt good animal husbandry practices. In addition animal handlers and immuno-deficient persons should be sensitized on the potential risks of acquisition of cryptosporidiosis when working with infected animals.

Keywords Beef calves, coccidial infection, *Cryptosporidium*, dairy calves, endoparasitism, gastrointestinal nematodes, goat kids, lambs

*Corresponding author E-mail: ssharma@bca.bw; Tel.: +267 3650222

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INTRODUCTION

Throughout the world, parasitic diseases constitute a major impediment to livestock production (Hoste *et al*., 2006). Cattle, sheep and goats of all ages are affected by a diversity of internal parasites. Faizal and Rajapakse (2001) recorded concurrent helminthic and coccidial infections rate of 78% in Sri Lanka. This concurrent infection increase clinical severity with weight loss and death (Abo-Sehaba and Abo-Farieha 2003; Agyei *et al* 2004) causing considerable economic losses. Clinical signs in the infected stock include gastroenteritis, anaemia and malabsorption sequel to gut damage by parasitic larvae and coccidial schizonts (Soulsby, 1982 Urquhart *et al*., 1996). According to Kagira and Kanyari (2001) parasitic diseases caused approximately 32% of all the ovine deaths during 1984-1994 in central Kenya. Helminthosis and heartwater transmitting tick accounted for 63% and 27% of all the parasitic cases, respectively. In a study conducted by Waruiru *et al*. (2001), *Haemonchus placei*, *Trichostrongylus axei*, *Cooperia* spp. and *Oesophagostomum radiatum* were responsible for parasitic gastroenteritis in Kenya. Sreedhar *et al.* (2009) reported helminthic infection in 42% of cattle and buffaloes in Andhra Pradesh in India. The highest frequency of 22.5% was of amphistomes followed by coccidia at 8.1%, strongyles at 7.6% and *Fasciola* at 3.8%. The incidence was higher in monsoon season as compared to summer and winter. Tsotetsi *et al*. (2013) recorded prevalence of helminthic parasites; 32% in sheep, 30% in goats and 1% in cattle for in Gauteng province of South Africa. Cryptosporidium species infection rates of 13%, 16.2%, 8.2% and 12.5% were recorded in goat kids, lambs, adult goats and sheep on 10 small stock farms in Gaborone of

Botswana (Sharma and Machete, 2009). In another study in Botswana, dairy and beef calves aged ≤ 4 weeks and diarrhoeic animals showed higher *Cryptosporidium parvum* infection than older (≥ 4- ≤ 12 weeks) and non-diarrhoeic calves (Sharma and Busang, 2012). Taking into consideration the major health implications caused by endoparasites, and zoonotic potential of *C. parvum*, this study was planned to record the prevalence of these parasitic infections in cattle, sheep and goats on farms in southern Botswana.

MATERIALS AND METHODS

Study Sites

The investigation was conducted in 16 randomly selected farms that included six dairy, five beef and five small stock farms located in Gaborone, Lobatse and Mochudi veterinary districts of south east Botswana. The climate in this area is mainly semi-arid to arid with annual rainfall and temperature range from 465 to 500 mm and 7°C to 35°C respectively. Most of the rainfall occurs during summer months of November to January and occasionally up to March (Mpapho *et al*., 2010; Batisani *et al*., 2012). Autumn season lasts from February to March; winter is from May to July, and spring is from August to October. The vegetation includes mixed type of grasslands and tree savannah with areas of woodland (De Wit and Bekker, 1990).

Sample Collection

Faecal samples were obtained from the recta of 131 dairy calves, 94 beef calves, 143 goat kids and 97 lambs, transported to Botswana College of Agriculture in ice-cooled containers and kept in the refrigerator until processing in Parasitology Laboratory within 3 to 4 days of their collection. All sampling in each herd was done only once between July 2010 to June 2011. The age, consistency of faeces, stocking densities and sanitary conditions on the farms were recorded. Animals excreting soft to liquid faeces and normal solid were considered as diarrhoeic and non-diarrhoeic, respectively. All 131 dairy calves, 59 calves from two beef farms, 28 lambs and 48 kids from two small stock farms were managed under semi-intensive management system in which they were allowed to graze on the premises of the fenced farms with occasional supplementary feeding. The remaining 45 beef calves, 49 lambs and 95 kids were reared under traditional communal management system where they were sent out to graze and confined to kraals without any shade at night with no supplementary feeding.

Laboratory Testing of Faecal Samples

Faecal samples were analyzed for helminthic eggs, worms and coccidial oocysts using a modified McMaster technique (MAAF, 1986). A sedimentation technique was used to detect trematode eggs in the samples. Using 87 faecal samples, nematode larvae were cultured in pooled faecal material (two grams of faeces from each of 10 animals) and incubating the samples at 26-30°C for a week. The moisture was maintained by addition of water. The resulting third stage nematode larvae were separated by standard Baerman method, identified and counted *per* gram of faeces (MAAF, 1986). Identification of characteristic helminthic eggs of *Trichuris* and *Moniezia* species, nematode larvae and coccidial oocysts was done at genus level only and was based on the morphological features as described by Soulsby (1982) and Urquhart *et al*. (1996). Tapeworm segments were recognized at the time of collection of faecal samples by the appearance of segments in the faeces. *Moniezia* species eggs were identified after grinding tapeworm segments in a few millilitres of water and examining the material. Nematode egg counts were recorded as high for ≥500 eggs *per* gram (epg) of cattle faeces and ≥1,000 in small ruminants (Soulsby, 1982; Grace *et al*., 2007). Coccidial oocyst counts of ≥5000 *per* gram (opg) in cattle and small stock were considered high. *Cryptosporidium* coproantigen was determined using Cryptosporidium (C 1201) diagnostic kit (R-Biopharm AG, 64297 Daramstadt, Germany). Enzyme immunoassay was conducted following manufacturer's guidelines. Photometric measurements were recorded at 450nm wavelength by a MULTISKAN microplate ELISA reader (Labsystems, Helsinki, Finland).

Statistical Analysis

Data obtained were analysed using standard statistical tools i.e. Mean, standard error (SE) and chi-square test for comparisons of the positive cases in different animal species using SAS for Windows, version 9.2 (SAS Institute, 2002-2008). The results were considered significant at P <0.05.

RESULTS

Prevalence of helminthic and *Eimeria* species infections

Table 1: Prevalence of helminthic and coccidial infections in calves, lambs and goat kids as determined by Modified McMaster technique

Animal species	No.of animals	Helminthic infection (% ±SE)	Coccidial infection (% ± SE)
Dairy calves	131	10.7 ± 2.7	13 ± 2.9
Beef calves	94	8.5 ± 2.9	10.6 ± 3.2
Lambs	97	24.7 ± 4.4	11.3 ± 3.2
Kids	143	21 ± 3.4	13.3 ± 2.8

The results of helminthic and coccidial infections in dairy and beef calves, goat kids and lambs are presented in Table 1. Small stock was relatively more susceptible to helminthosis (22.5%) than calves (9.8%). Worm egg counts and faecal culture revealed helminthic eggs and larvae of eight genera of strongylid nematodes, namely *Trichostrongylus*, *Haemonchus*, *Oesophagostomum*, *Cooperia*, *Chabertia*, *Bunostomum*, *Trichuris* and *Strongyloides* species, one species of trematode *Paramphistomum* and proglottids of *Moniezia* species, a cestode were identified. Strongyle infections caused by *Trichostrongylus*, *Haemonchus*, *Cooperia* and *Bunostomum* were observed in eight calves, whilst *Haemonchus*, *Trichostrongylus*, *Oesophagostomum*, *Chabertia*, and *Strongyloides* in 36 small stock. Segments of *Moniezia* species of tapeworm and adult paramphistomes were detected in one 5-month-old beef calf and two 6-month-old goat kids only. Mixed helminthic and coccidial infections were detected in two calves, three lambs and five goat kids. Pure coccidiosis was diagnosed in 8.6% (40/465) animals. Trichostrongylids eggs were more common (64.2%) than *Strongyloides* eggs (4.5%). Majority of the animals including calves, kids and lambs (45/76) were infected with one or two helminthic species, but multiple parasitic infections caused by *Haemonchus*, *Trichostrongylus*, *Bunostomum*, *Cooperia* and *Oesophagostomum* constituted 41% (31/76) of all parasitic cases. The dominant nematode genus infecting 39% animals was *Haemonchus*. Animals aged 3-6 months exhibited 20.6 ± 2.6% (28/232) helminthic infection in comparison to 12 ± 2.1% (48/233) in ≤ 3-month-old animals. A total of 23 out of 78 dairy and beef calves passing soft to liquid faeces were found positive either for helminthic eggs or coccidial oocysts or a combination of the two. Of 63 sheep and goats excreting liquid faeces, 26 harboured either helminthic or coccidial or mixed infection. Of 324 animals found excreting solid faeces, 79 (24.4%) showed mild helminthic infection (100 to 350 strongyle eggs/g) or coccidial (50 to 1950 oocysts/g) infections. Highest faecal egg count (FEC) of 6050 eggs with mixed strongyle infection caused by *Haemonchus*, *Trichostrongylus*, *Oesophagostomum*, *Bunostomum* and *Trichuris* species parasites was detected in a 6-month-old lamb from Oodi; whereas a higher count of *Eimeria* species oocyst of 4200/g was recorded in a 2-month-old kid from Kopong. Both animals with the highest number of helminthic eggs and oocyst counts were from two small stock farms managed under communal animal husbandry system. The intensity of helminthic infection in majority of animals was moderate and epg ranged from 500 to 1150 with an average 705 ± 49 in cattle and 1000 to 6050 with mean 1806 ± 174 in small stock.

Of the 465 faecal samples examined by EIA, the estimated prevalence of *Cryptosporidium* species infection in dairy and beef calves, lambs and goat kids were 25.2 ± 3.8%, 12.8 ± 3.4%, 12.4 ± 3.3% and 10.5± 2.6 %, respectively.

Table 2: Prevalence of *Cryptosporidium* species infections in calves and small stock determined by Enzyme immunoassay

Animal species	No. of animals	No. of positive animals	*Cryptosporidium* infection (% ±SE)
Dairy calves ≤ 3 months	68	21	30.9 ± 5.6
Dairy calves > 3-6months	63	12	19 ± 4.9
Significance			***
Beef calves ≤ 3 months	50	10	20 ± 5.7
Beef calves > 3-6 months	44	2	4.5 ±3.1
Significance			***
Lambs ≤ 3 months	36	6	16.7 ± 6.2
Lambs > 3-6 months	61	6	9.8 ± 3.8
Significance			***
Kids ≤ 3 months	78	11	14.1 ± 3.9
Kids > 3-6 months	65	4	6.2 ± 3
Significance			***

Differences in Cryptosporidium infection rates between animals ≤ 3months and 3-6 month-old were significant (P <0.001)

Table 3: Prevalence of helminthic, coccidial and *Cryptosporidium* species infections in diarrhoeic and non-diarrhoeic animals

Animal species	No of animals	% Mixed helminthic and coccidial infection ± SE	% *Cryptosporidium* infection ± SE
Dairy calves with diarrhoea	59	28.8 ± 5.9	35.6 ± 6.2
Dairy calves without diarrhoea	72	25 ± 5.1	16. 7 ± 4.4
Significance		NS[a]	***
Beef calves with diarrhoea	19	31.6 ± 10.7	36.8 ± 11
Beef calves without diarrhoea	76	23.7 ± 4.9	6.6 ± 2.8
Significance		NS	***
Kids with diarrhoea	37	37.8 ± 8	16.2 ± 6
Kids without diarrhoea	105	25.7 ± 4.3	8.6 ± 2.7
Significance		NS	***
Lambs with diarrhoea	26	46.2 ± 9.8	23 ± 8.3
Lambs without diarrhoea	71	22.5 ± 5	8.5 ± 3.3
Significance		NS	***

[a] NS = not significant; *** = (P < 0.001)

Infection rates in calves and small stock aged less than 3 months and 3 to 6 months old animals as well as in diarrhoeic and non-diarrhoeic animals are presented in Tables 2 and 3. Animals passing soft to liquid faeces considered as diarrhoeic showed higher infection rate of 28.4 ± 3.8% (40/141) when compared to those with normal solid faeces (9.9 ± 1.7%; 32/324). A total of 59 dairy and 19 beef calves had diarrhoeic faeces, of which 21 dairy (35.6%) and seven beef (36.8%) calves were found to be infected. Majority of diarrhoeic dairy calves i.e 34 out of 59 were from a dairy farm at Pitsane. The frequency of occurrences of *Cryptosporidium* infection was higher in younger animals aged 3 months or less compared to the older ones of 3 to 6 months (20.7 ± 2.7% versus 10.3 ± 2%).

DISCUSSION

Control of gastrointestinal parasitic infection in animals requires a comprehensive knowledge of epidemiology and an understanding of the agroclimatic conditions such to temperature, rainfall, pasture management and farm management practices. The number of parasitic eggs and coccidial oocysts varies according to species of worms, host species, body condition and immune status of the animal (Urquhart *et al*., 1996). However, FEC of 100 epg or more from *Haemonchus* or *Bunostomumm* species is likely to indicate severe damage, whereas a count of 500 epg of *Cooperia* species is expected to produce mild helminthosis in cattle (Soulsby, 1982). The results of the present study demonstrated the differences in helminthic infection rates between calves and small stock which stood at 9.8% and 22.5% in bovine calves and small stock, respectively. A similar study conducted by Maichomo *et al*., (2004) recorded nematodes and coccidial oocysts prevalence of 69.2%, 80%, 82% and 30%, 45% and 44% in calves, sheep and goats, respectively in Magadi division of south-western Kenya. Higher infection rates reported by Maichomo *et al*., (2004) were based simply on the detection of any nematode egg and coccidial oocyst in the faecal samples. Most of their animals were found infected with parasitic eggs ranging between 25 and 499 and only a few sheep and goats had epg above 1000. However, in the present study, faecal samples of calves with 500 epg or more and those of small stock with ≥1,000 epg were considered as positive (Grace *et al*., 2007).

In the current investigation, the age of the animals varied from 2 days to 6 months and might have also influenced the intensity of gastrointestinal parasitic infection. Increased helminthic infection rates in animals aged 3-6 months and in diarrhoeic animals were recorded in comparison to young ones (≤ 3 months) and non-diarrhoeic stock. This may probably be due to exposure of 3-6 months stock in their first grazing season immediately after weaning to grazing areas and water points with a heavy parasitic load. This would be exacerbated by lack of nutritional supplementation and/or lack of use of anthelmintic drugs by resource-poor small stock farmers. Traditionally, younger stock of less than 3 months are left in the kraals when older animals are turn out to pasture and these may reduce infection rates in younger animals. However, this is would be contrary to Kaufmann and Pfister (1990) whereby calves of 10 months of age carry low nematode burdens, while the maximum burdens are detected in 1.5 to 3-year-old animals. This may be suggesting differences in infection rates due to differences in production or management systems. In the current study lambs appeared to be more vulnerable to helminthosis than goat kids (24.7% vs 21%), though not statistically significant. Kanyari *et al*. (2009) in Kenya attributed this vulnerability of lambs to the fact that sheep graze closer to the ground, picking more larvae and hence harbouring more nematode larval stages compared to the goats. Goats are browsers and thus their exposure to gastrointestinal nematode infection is reduced (Hoste *et al*. 2010) as they graze at a higher height than sheep. However, Tsotetsi *et al*. (2013) did not find any significant difference in the nematode prevalence between the two species on small scale farms in Gauteng province of South Africa, possibly because of the diversity in ruminant management systems practised by the farmers. The two species co-grazed, where they could either graze or browse depending on the availability of either browse or grass. However, when sheep and goats graze grass pasture together, goats become significantly more infected than sheep; but in rangelands where goats follow their preferences for browsing, the reverse has been observed (Jackson *et al*; 2012).

Higher helminthic and coccidial infection rates recorded in 145 diarrhoeic animals in this study were caused by multiple gastrointestinal nematode and *Eimeria* species infections. Parasitic gastroenteritis caused by several nematode parasites, namely *Haemonchus*, *Trichostrongylus*, *Ostertagia*, *Cooperia*, *Bunostomum*, *Oesophagostomum*, *Chabertia*, *Trichuris*, *Nematodirus* and *Strongyloides* species and coccidial infection mostly by many pathogenic *Eimeria* species in small ruminants has long been established (Soulsby,1982; Urquhart *et al*., 1996; Kassai,1999). The predominance of *Haemonchus* species parasitic infection recorded in 39% animals in the present study corroborates observations of Kagira and Kanyari (2001) from Kenya. Allonby (1975). Mukhebi *et al*. (1985) attributed an annual loss of US$ 26 million in small stock to haemonchosis in Kenya, while returns could be increased by as much as 470% by controlling this parasitic infection.

Increased helminthic prevalence and egg counts during October to March in animals corresponded with annual rainfall of 450 mm and temperature range of 28 - 37°C in the south eastern Botswana during 2010 – 2011. However, higher temperatures and dry conditions lead to desiccation of nematode eggs and larvae (Maichomo *et al*. 2004; Nwosu *et al*. 2007). The increase in helminthic infection in the current study may be attributed to high level of contamination of grazing areas with infective L_3 in the rainy season. Cold dry weather prevailing during May to August appeared to be responsible for hypobiosis or inhibited/arrested larval development resulting into lower helminthic infections. Temperature and rainfall are the principal climatic factors influencing the incidence of internal parasites (Gibbs 1982) and can be used to predict the outbreaks of endoparasitism. Coccidial infection rates reported in this study were lower than those reported by Harper and Penzhorn (1999) from South Africa, and Kanyari (1993) and Kanyari *et al*. (2009) from Kenya.

High prevalence of coccidiosis in Kenyan livestock was probably due to favourable climatic conditions. Outbreaks of coccidiosis occur in young animals kept under poor sanitary conditions. The immune system in young animals is not fully developed to combat heavy parasitic infections. In Botswana, more than 95% small stock farmers keep their animals under extensive/communal management system (Statistics Botswana, 2013) and rarely use nutritional supplements, anthelmintics and coccidiostats. Another problem in managing nematode parasites among rural farmers is lack of expertise in identifying nematode infective species, often confusing them with tapeworm (Athanasiadou et al 2007). However, FAMACHA, a system developed in South Africa for identification of anaemic animals (Bath *et al*., 1996; Van Wyk and Bath, 2002) can be a valuable tool that farmers can learn and practice to identify infection. Alternate use of multiple dewormers and anticoccidial drugs in the anaemic animals is recommended to prevent economic losses inflicted by moderate to severe endoparasitism and reduce development of drug resistance by parasites.

In the present study, *Cryptosporidium* species infection was higher in dairy calves compared to beef calves, lambs and goat kids. Younger (< 3 months) and diarrhoeic animals exhibited increased infection rates than older (3 to 6 months) ones. Large number of the diarrhoeic calves (58%, 34/59) were from a Pitsane dairy farm with a history of 40% mortality as a result of gastroenteritis and dehydration among <1-month-old animals. These findings are similar to several other studies (Atwill *et al*., 1999; Nizeyi *et al*., 2002; Santin *et al*., 2004; Geurden *et al*., 2006; Sharma and Machete, 2009; Sharma and Busang, 2012). Infection in calves and small stock detected in the current study appears to have been caused by *C. parvum*. The organism is considered an important zoonotic species responsible for inducing neonatal diarrhoea in animals and humans. Though cryptosporidial infection in immuno-competent host is usually self-limiting, in the immuno-compromised human patients, livestock handlers and persons with close proximity to animals, especially young dairy calves, the parasite can cause life-threatening gastroenteritis (Xiao *et al*., 1999). This was demonstrated from an outbreak of cryptosporidiosis among children in Botswana in 2006, in which 531 children aged <5 years died as a result of diarrhoea caused by a mixed infection of *Cryptosporidium* and enteropathogenic *Escherichia coli*. Most of these children were enrolled under HIV/AID's Prevention to Mother Child Treatment program (Anonymous, 2007).

CONCLUSION

The overall results of the present study demonstrated that endoparasitism with gastrointestinal nematodes and *C. parvum* infection are endemic in small stock and dairy calves. These parasitic infections as recorded in the present study pose a great challenge and constraint in achieving increased productivity and sustainability of small ruminant production in southeastern Botswana. In order to limit the build-up of parasitic infections, livestock farmers need to be educated on the dangers of parasitic contaminated pastures and be advised on the strategic and tactical anthelmintic treatment of anaemic small stock. Animal handlers should be made aware of high prevalence of *C. parvum* infection in young dairy calves, and its zoonotic potential. Adoption of hygienic practices during feeding and housing of young animals in conjunction with use of antimicrobial drugs are recommended against cryptosporidiosis.

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Conflict of interest None

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