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Prevalence of *Cryptosporidium* spp. in asymptomatic small ruminants in Grenada, West Indies

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ABSTRACT

Cryptosporidium spp. is a protozoan parasite that causes enteric infection in a wide range of hosts, including livestock and humans. The aim of this cross-sectional study was to estimate the prevalence of *Cryptosporidium* spp. in small ruminants in Grenada, West Indies. Fecal samples were collected from 100 sheep and 202 goats from 32 farms. The fecal samples were tested using an Enzyme-linked immunosorbent assay (ELISA) for qualitative detection of antigens in feces (Diagnostic Automation Inc., USA). The overall prevalence of *Cryptosporidium* spp. was 19.5% [95% confidence interval (CI): 15.4% to 24.4%] in both sheep and goats. The prevalence of *Cryptosporidium* spp. in sheep and goats was 14% (95% CI: 8.4% to 22.3%) and 22.3% (95% CI: 17.1% to 28.5%), respectively. There was no significant difference in the prevalence of *Cryptosporidium* spp. infection between sheep and goats ($p = .42$, Fisher's exact test) in Grenada. Of the 32 farms visited, 19 (59.4%) had at least one *Cryptosporidium* spp. positive animal.

1. Introduction

Cryptosporidium spp. are protozoan parasites that cause enteric infection in a wide range of mammals, including humans (Quilez et al., 2008). Clinical symptoms of cryptosporidiosis manifest as diarrhea, dehydration and weight loss, especially in neonates and older animals with a compromised immune system (Fayer et al., 2000; Romero-Salas et al., 2016). Cryptosporidiosis is of economic importance to farmers because of the high morbidity, inefficient weight gain and occasionally mortality it can cause in livestock (Foreyt, 1990). In addition, some species such as *C. parvum* have been implicated in emerging zoonotic cryptosporidiosis (Fayer et al., 2000). Transmission among animals and humans is via infective oocysts through both water-borne and fecal-oral routes (Macpherson et al., 2000; Fayer et al., 2000).

Studies on the prevalence of *Cryptosporidium* spp. in farm animals have revealed that ruminants are an important reservoir host (Maurya et al., 2013). However, among the domestic animals, sheep and goats have received relatively little attention with regard to *Cryptosporidium* spp. infection. Despite this, the parasite is considered one of the major enteric pathogens associated with neonatal diarrhea and mortality in sheep and goats (Maurya et al., 2013).

Studies on cryptosporidiosis in goats and sheep have been performed worldwide. In the Caribbean region, studies on *Cryptosporidium* spp. in animals have mainly been undertaken in Trinidad and Tobago. A study by Kaminjolo et al. (1993) revealed a prevalence of 20% for *Cryptosporidium* spp. in lambs. A smaller longitudinal study performed involving various livestock species on selected farms did not find *Cryptosporidium* spp. in sheep (Adesiyun et al., 2001). In another cross-sectional study in foals in Trinidad and Tobago, a prevalence of 64.8% for *Cryptosporidium* spp. was reported (Harris et al., 2012). In Puerto Rico, there was evidence of contamination of water bodies by *Cryptosporidium* spp. and this was attributed to a farm animal source (Robinson et al., 2015). The aim of the present cross-sectional study was to estimate the prevalence of *Cryptosporidium* spp. in small ruminants in Grenada.

2. Materials and methods

2.1. Geographic location and climate of Grenada

Grenada is a small tri-island state located in the Caribbean. The island has a warm and humid climate which averages annual temperatures ranging from 24 °C to 31 °C. It is surrounded by the Caribbean Sea to the West and the Atlantic Ocean to the East.

2.2. Ethics statement

Institution for Animal Care and Use Committee (IACUC) approval

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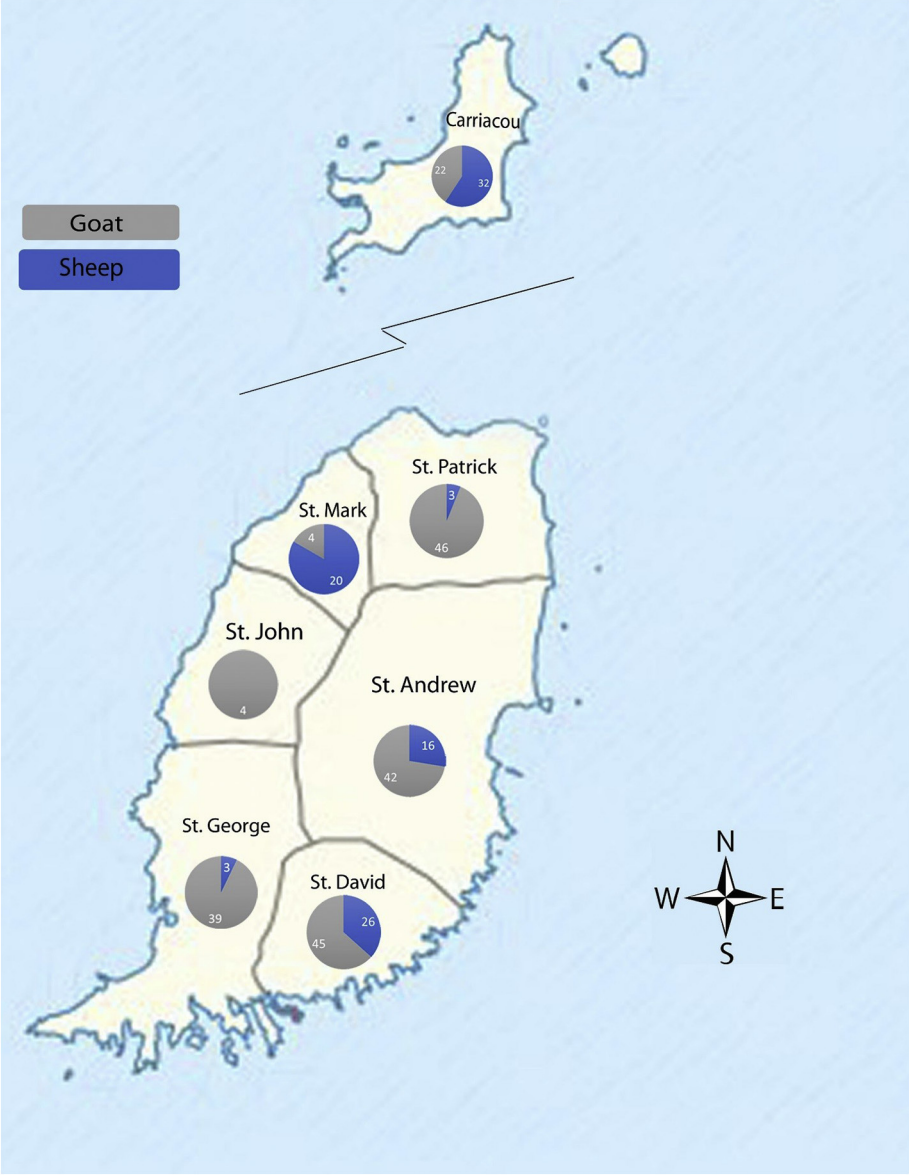

Fig. 1. Number of sheep and goats sampled from various locations in Grenada.

was not necessary for this study since the fecal samples that were used came from a previously approved study on *Campylobacter* spp. in small ruminants (IACUC No.11002 R).

2.3. Sampling and testing

From May 2011 to July 2011, fecal samples were collected from goat and sheep farms in the 6 parishes (St. David, St. Andrew, St. George, St. Mark, St. Patrick and St. John) of the main Island Grenada and from the dependent island of Carriacou (Fig. 1). All age groups of sheep and goats from each farm were sampled. Fecal samples were collected in plastic fecal containers, labeled appropriately and transported to the microbiology laboratory at the School of Veterinary Medicine, St. George's University. Fecal samples were stored at −20 °C until tested using an ELISA (Diagnostic Automation Inc., USA) according to the manufacturer's instructions.

Optical densities (OD) of the samples were read at 450 nm using an ELISA reader (BIOTEX; Model: ELx800, Biotex Instruments, USA). Based on the instructions from the *Cryptosporidium* spp. ELISA kit manual, any sample with an OD value of greater than or equal to 0.15 was considered positive whereas that with an OD value of < 0.15 was deemed negative.

3. Results

From the 100 sheep and 202 goat fecal samples tested, the overall prevalence for *Cryptosporidium* spp. in small ruminants was 19.5% [95% confidence interval (CI): 15.4% to 24.4%]. The prevalence of *Cryptosporidium* spp. in sheep and goats was 14% (95% CI: 8.4% to 22.3%) and 22.3% (95% CI: 17.1% to 28.5%), respectively (Table 1). There was no significant difference in the prevalence of *Cryptosporidium* spp. infection between sheep and goats (p = .42, Fisher's exact test).

Table 1
Prevalence of *Cryptosporidium* spp. in goats and sheep in Grenada.

Species	Number tested	Number copro-antigen positive	Proportion (%)
Caprine	202	45	22.3
Ovine	100	14	14.0
Total	302	59	19.5

Table 2
Proportion of *Cryptosporidium* spp. infection in small ruminants according to age group in Grenada.

Species	Age (yr)	No. tested	No. Positive (%)	Source and number positive*
Caprine	< 1	66	18 (27.3)	C (3), SG (3), SA (5), SP (6), SD (1)
	1–3	119	27(22.7)	SG (7), SA (10), SD (5), SP (5)
	4–6	17	3(17.6)	SA (2) SP (1)
Ovine	< 1	24	0 (0)	–
	1–3	67	11(16.4)	SG (1), SA (4), SP (2) C (4)
	4–6	9	2(22.2)	SD (1), SG (1)

Parish*: SG, St. George; SD, St. David; SA, St. Andrew; C, Carriacou; SP, St. Patrick.

Table 3
Optical density values according to age group in small ruminants in Grenada.

Species	Age (years)	Mean OD value	Standard deviation	Range
Caprine	< 1	0.29	± 0.13	0.17–0.62
	1–3	0.33	± 0.16	0.15–0.70
	4–6	0.23	± 0.01	0.22–0.24
Ovine	1–3	0.41	± 0.30	0.17–0.98
	4–6	0.20	± 0.01	0.19–0.20

Cryptosporidium spp. were detected on 19 of the 32 small ruminant farms visited, giving a flock prevalence of 59.4%.

The age group of < 1 to 3 years was more commonly affected than that of the age group of 4 to 6 years. There were no lambs infected with *Cryptosporidium* spp. (Table 2). Optical density values were generally higher in the < 1 to 3 years age group compared to the 4 to 6 years age group (Table 3).

4. Discussion

There are eight recognized species in the genus *Cryptosporidium* and among these, *Cryptosporidium parvum* is a widely reported zoonotic agent. It has been reported in seventy-nine species of mammals including small ruminants (de Graaf et al., 1999; Macpherson et al., 2000). Of all the species of *Cryptosporidium* known to be zoonotic, *C. parvum* was the only identified species in sheep and goats in Spain and Poland (Majewska et al., 2000; Quilez et al., 2008). In Zambia, *C. parvum* was diagnosed in several goat kids and lambs whereas *Cryptosporidium suis* was also found in one lamb (Goma et al., 2007). It is therefore likely that in the present study the *Cryptosporidium* spp. diagnosed belonged to *C. parvum*. A further study for molecular genotyping of *Cryptosporidium* spp. in small ruminants in Grenada is recommended.

The prevalence of *Cryptosporidium* spp. in sheep (14%) in the present study was comparable to the one (10.1%) reported from Poland (Majewska et al., 2000). However, it was different for goats since the same study in Poland did not find any goat positive to *Cryptosporidium* spp. (Majewska et al., 2000). The prevalence in Zambia (4.8% in lambs and 12.5% in goat kids) and India (1.8% in lambs and 3.5% in kids) was lower than the findings in the present study (Goma et al., 2007; Maurya et al., 2013). Conversely, the prevalence in Mexico in sheep (67.5%) and goats (72.5%) was higher than what we found in the present study (Romero-Salas et al., 2016).

It is widely believed that lambs are more affected by *Cryptosporidium* spp.; however, none of the lambs in our study tested positive to this parasite. The reason for this cannot not be completely explained, but it is suggestive of the ability of lambs to mount a strong immunity to intestinal parasites compared to goat kids (Gorski et al., 2004). Similarly, a study by Olson et al. (1997) found that the prevalence of *Cryptosporidium* spp. was higher in adult sheep than in lambs, though the difference was not statistically significant.

From the fecal consistency, all the small ruminant fecal samples in the present study were asymptomatic. It is possible that these animals were symptomatic at one point but had recovered by the time we were collecting the fecal samples. Similar to a study from Nigeria (Akinkuotu and Fagbemi, 2014) and France (Castro-Hermida et al., 2005), our findings suggest that small ruminants are potential reservoirs for *Cryptosporidium* spp. These animals can excrete oocysts resulting in contamination of the environment, potentially posing a risk for zoonotic transmission.

There are no studies on cryptosporidiosis in humans in Grenada. However, several studies on cryptosporidiosis in humans in the Caribbean and South America have reported evidence of types of *Cryptosporidium* spp. which normally infect domestic animals. Gatei et al. (2008) found *C. parvum, C. canis*, and *C. felis* in HIV-infected persons in Jamaica. Studies in Jamaica, Brazil and Argentina in a wide range of patients also revealed *C. parvum* (Lindo et al., 1998; Peralta et al., 2016). In Haiti *C. parvum* and *C. felis* were reported in HIV patients and children (Raccurt et al., 2006). Although the parasite was not characterized by molecular methods in Cuba, there was evidence of *Cryptoporidium* spp. in both HIV and paediatric patients (Escobedo and Núñez, 1999; Núñez et al., 2003).

5. Conclusion

The present study showed evidence of *Cryptosporidium* spp. in asymptomatic small ruminants in Grenada. Given the widespread and relatively high prevalence of *Cryptosporidium* spp. infection in small ruminants in Grenada, the potential for zoonosis exists in these animals.

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Conflict of interest statement

None.

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