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RESEARCH ARTICLE

Gastrointestinal and blood parasites of a free grazing flock of sheep in Kaithady farm in the Jaffna District

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Abstract: Parasitism is one of the major economic and health problems affecting sheep industry. The present study determined the prevalence, intensity and the types of gastrointestinal (GI) and blood parasites of sheep at Kaithady farm in the Jaffna District. Fresh faecal samples (~ 5 g) from sheep belonging to a breed known as 'Jaffna Locals' were collected in September 2010 (during the dry season) and March 2011 (after the rainy season) and processed by salt floatation, direct saline and iodine smears and sedimentation method. Geimsa stained smears were prepared from the blood samples. Identification of the parasites was based on light microscopic morphology and faecal culture was done to identify strongyles. Seven genera of GI parasites including *Haemonchus contortus*, *Toxocara*, *Trichuris*, *Moniezia, Eimeria, Giardia,* amphistome eggs and an unidentified strongyle type eggs were recorded. Strongyle infection was the most common (78 %) followed by *Eimeria* spp. (76 %). Concurrent infections of *Eimeria* spp. and GI nematodes were common. Although there was no difference in the overall prevalence of GI parasites between the rainy and dry seasons (chi square test; $p > 0.05$), the intensity of infection was significantly higher in the rainy season (Mann Whitney U test; $p < 0.05$). Seven types of GI parasites were recorded after the rainy season while only five were found in the dry season. Infections of *Moniezia, Giardia* and *Trichuris* were recorded only after the rainy season. A tick-borne haemoparasite, *Theileria* sp. was found with a very high prevalence of 87 %. Sheep in the Kaithady farm harboured many parasites. Regular and proper administration of anthelmintics would provide an effective strategy in controlling these infections.

Keywords: Helminths, Jaffna Local, lambs, protozoans, sheep.

INTRODUCTION

Sheep are primarily useful for meat and wool production. Infection of sheep by gastrointestinal (GI) parasites is one of the major economic and health problems affecting sheep industry. Loss of plasma protein, alteration in protein metabolism, diarrhoea and decrease in weight gain are the common abnormalities in infected animals (Hadid & Lotfy, 2007). Others include decreased production, costs for treatment and prophylaxis as well as occasional mortality (Hadid & Lotfy, 2007). Moreover, due to parasitism the animals become susceptible to other health problems (Sutar *et al*., 2010). Lambs are more susceptible to parasitic infections than adults (Soulsby, 1982). The immunity gradually develops as lambs are exposed to parasites and is fully developed by 10 – 12 months of age. Once immunity has developed, parasite burdens are restricted except during disease, malnutrition and stress (Fleming, 2005).

The livestock sector is an important component of the farming system in the Jaffna District. Up to 1950, only local cattle and goats were reared for milk and meat. From 1950 to 1984, livestock enterprise developed very fast and it was a single or supplementary source of income for nearly 30 % of the district's population. Backyard poultry and rearing cows and goats for milk and meat generate additional income for the farmers. The livestock population reduced to a considerable extent during the last two decades due to the civil war (GMSL, 2005). Although there is no demand for wool in Sri Lanka, sheep could play an important role in livestock production as an important source of animal protein. They also produce a considerable amount of manure, which is of special importance in those areas where cattle are of lesser importance (Sutar *et al*., 2010). However, there is no organized programme for sheep industry in

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Sri Lanka. The indigenous sheep found in the Jaffna District are called 'Jaffna Local', which is confined to the Northern Peninsula (Rajaguru, 1991). These sheep are reared under a traditional management system unique to the specific cultural practices in the Jaffna area, which could be described as a mixed crop-livestock system operated predominantly on a large scale under free-range arrangement (FAO, 2006). Genetic studies have shown that the Jaffna Local sheep is a unique population with a high diversity (Ranathunga & Silva, 2009).

In Kaithady farm, sheep are reared for using the faecal material as a biomass in an agricultural land and also for meat. People in Jaffna believe that sheep meat cause allergies and hence they rarely consume it (*Personal communication*). Thus, it is usually marketed to other parts of the country. In addition, one or two sheep are reared as pets because they believe that rearing sheep brings fortune. There is no published information available on the GI and blood parasites of sheep in Sri Lanka. The prevalence of GI parasites and the severity of infection vary considerably depending on the genera of parasites involved, animal species, local environmental conditions such as humidity, temperature, rainfall, vegetation and the management practices (Ghanem *et al*., 2009). The present study was the first attempt to determine the prevalence, intensity and types of GI and blood parasites of sheep in Sri Lanka, which are predominantly found in the Jaffna District.

METHODS AND MATERIALS

Study site

The Jaffna District is located on a flat peninsula at the Northern tip of Sri Lanka in close proximity to the sub-continent of India. Most of the area is dry and sandy and the climate is tropical with a seasonal rhythm of rainfall. Annual temperature in the Jaffna District ranges from 24 to 31°C with an annual precipitation of 1231 mm evenly spread over the area. The dry season is from March to September and the rainy season is from December to February. Kaithady is a village 11.2 km away from Jaffna city (Figure 1) with an elevation of 10.5 m above the sea level.

Study animals

The sheep flock used in this study consisted of about 500 animals housed in an agricultural land of about 2 ha during the dry season in Kaithady (Figure 2A). When the rains begin they are temporarily shifted to Navatkuli area (Figures 1 & 2B), which is located in a slightly higher elevation as Kaithady area gets flooded. These sheep are free grazers during both dry and rainy seasons but fed with harvested fodder when all their grazing lands are flooded. According to the veterinary surgeon of the area, routine deworming is done every five months.

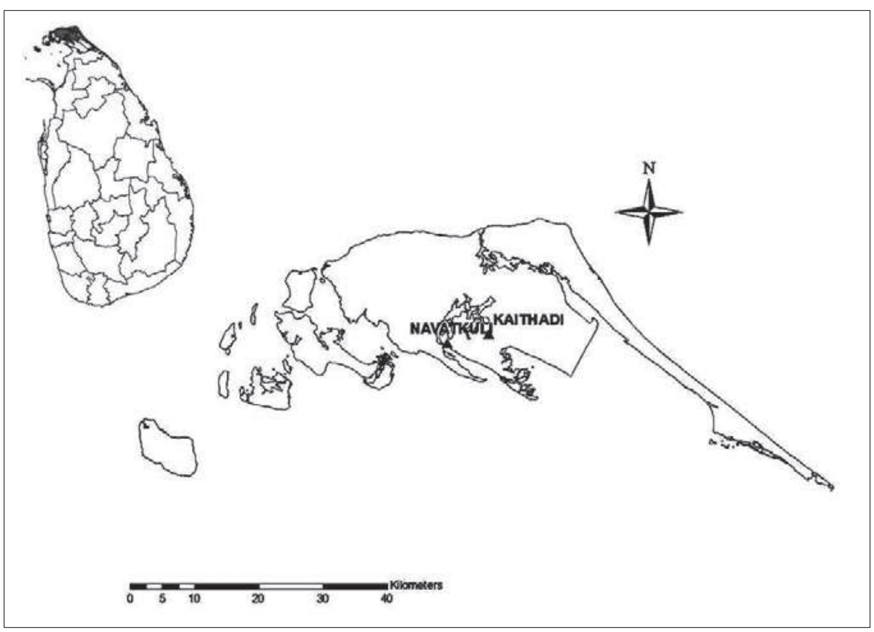

Figure 1: Map of study area in the Jaffna District showing the two locations where the sheep flock is kept during the dry (Kaithady) and rainy seasons (Navatkuli)
▲ Sampling sites

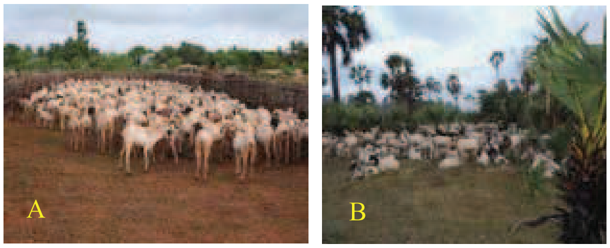

Figure 2: During the dry season and most part of the year, the sheep flock is kept in a farmland in the Kaithady area (A) and the flock is transferred to the Navatkuli area located in a higher elevation during the rainy season (B)

Collection of samples

Fresh faecal samples were collected into 50 mL plastic containers from the rectum of the animals or freshly dropped faeces from the ground. Fifty samples (~ 10 % of the flock) were collected in each season and sampling was carried out twice in September 2010 (at the end of the dry season) and in February 2011 (at the end of the rainy season). Blood samples (~ 5 mL) were collected from 15 randomly selected animals from the jugular vein. Blood was collected only once in September 2010. Samples were brought to the laboratory in an ice box and were immediately transferred to a refrigerator at 4˚C until processed. Information, such as age and sex of the sheep, management, and deworming practices of the farm were collected by interviewing the farmer and from the area veterinary surgeon.

Analysis of faecal samples

Faecal samples were analyzed using simple salt floatation technique, direct saline and iodine smears and sedimentation techniques. Eggs of different parasite species were identified using light microscopy. Faecal cultures were prepared for nematode identifications. Larvae were examined after adding Lugol's iodine to the larval suspension.

Simple salt floatation technique

Approximately 3 g of faeces was measured and mixed with 50 mL saturated sodium chloride solution. This mixture was ground with a pestle and mortar, and sieved through a tea strainer. The filtered faecal suspension was poured into a 15 mL tube. The tube was gently toped with the suspension, leaving a convex meniscus at the top of the tube and a coverslip was carefully placed on top. Test tube was allowed to stand for 20 min. The coverslip was carefully lifted from the tube together with the drop of fluid adhering it, and was immediately placed on a microscopic slide. It was observed under the compound light microscope for parasite eggs and cysts.

Direct saline and iodine mounts

A drop of saline and a drop of iodine solution were placed separately on a glass microscope slide. Using a toothpick, a small portion (size of a match head) of the faecal sample was picked up and was mixed with the drop of saline. This was repeated with the drop of iodine. The two smears were covered with separate cover slips and observed under the light microscope. Eggs and cysts were identified and their relative numbers in a sample size of a match head was estimated.

Sedimentation technique

The faecal suspension from the above simple salt floatation technique was centrifuged under 3000 g for 10 min and the resultant sediment was examined for the presence of trematode eggs. This technique is used for detecting trematode eggs as they are heavier than the other eggs. The eggs get deposited at the bottom of the tube after centrifugation with sodium chloride solution.

Nematode cultures

The faeces were broken up finely using pestle and mortar and mixed with dried dung dust in 1 : 1 ratio. Distilled water was added until the mixture was moist and crumbly. This mixture was kept in a wide-mouthed jar and incubated at room temperature (27 ˚C) for 14 days. The cultures were aerated daily and a few drops of distilled water were added to maintain the moisture level. After 14 days, the cultures were Baermannized and infective larvae were isolated.

Identification of larvae, eggs and cysts

A drop of Lugol's iodine was added directly to a drop of larval suspension placed on a microscopic slide. Species identification of infective larvae, eggs and cysts were done according to the morphology keys described Zajac and Conboy (2006). Species identification of *Eimeria* oocysts were done according to their size, shape and the presence or absence of micropylar caps described by Bhatia *et al*. (2004).

Analysis of blood samples

Thin blood smears were prepared, air dried, stained with Giemsa stain and observed under the light microscope using an oil immersion lens for the presence of blood parasites. Ticks were collected from sheep, preserved in 95 % ethanol and identified using the keys described by Seneviratna (1965).

Data analysis

The differences in the prevalence of GI infections during dry and rainy seasons, between males and females and between lamb and adults were compared using a chi square test. A quantitative analysis of EPG (eggs per gram) and OPG (oocyst per gram) counts was done using a Mann Whitney U test. Data were analysed using the Minitab software (version 14).

RESULTS

Gastrointestinal parasites

Sheep were infected with both helminth and protozoan parasites. A total of seven genera of GI parasites were found including *Haemonchus*, *Toxocara*, *Trichuris*, *Moniezia,* amphistome eggs*, Eimeria* and *Giardia* and an unidentified strongyle type eggs. The most common type of infection was strongyle type eggs followed by oocysts of *Eimeria* (Table 1). From the faecal cultures, some of the strongyle type eggs were identified as *Haemonchus contortus*. Four species of *Eimeria* were identified of which *E. ovinoidalis* was the most common type followed by *E. ovina*, *E. intricata* and *E. parva*. Concurrent infections of *Eimeria* spp. and GI nematodes were common. Seven genera of GI parasites were recorded in the samples collected after the rainy season while only five were found in the dry season. Amphistomes were recorded only in the dry season while low infections of *Moniezia* sp.*, Giardia* sp. and *Trichuris* sp. were recorded only after the rainy season (Table 1).

Of the sheep that were sampled, a higher percentage of individuals was infected during the rainy season (92 %) compared to the dry season (84 %). However, this difference in the prevalence of infection was not statistically significant (χ^2 = 2.9536; p = 0.0857). Moreover, there was no significant difference in the prevalence of GI parasitic infections between females (85 %) and males (83 %) or between lambs (94 %)

Table 1: Prevalence of gastrointestinal parasites in a free grazing flock of sheep at the Kaithady farm in the Jaffna District (n = 50 per season)

Parasite	Prevalence (%)					
	Age		Gender		Season	
	Lamb	Adult	Female	Male	Dry	Rainy
Nematodes	75.0	81.3	81.0	83.3	75.9	85.7
Strongyle	75.0	79.7	81.0	83.3	75.9	85.7
Toxocara sp.	0.0	3.1	1.3	5.0	2.0	2.0
Trichuris sp.	2.8	1.6	2.5	0.0	0.0	4.0
Protozoans	83.3	71.9	72.4	66.7	58.6	85.7
Eimeria spp.	83.3	71.9	72.4	66.7	58.6	85.7
Giardia sp.	2.8	0.0	0.0	5.0	0.0	2.0
Cestodes	16.7	4.7	7.5	15.0	0.0	18.0
Moniezia sp.						
Trematodes	2.8	3.1	2.5	5.0	6.0	0.0
Paramphistomum spp.						
Total GI parasite	94.4	84.4	84.5	83.3	84.0	92.0
Chi value (p)	0.799 (0.371)		0.999 (0.317)		2.953 (0.086)	
Strongyle + *Eimeria* mixed infections	24.0	43.0	53.0	14.0	27.0	40.0

and adults (84 %; chi square test $p > 0.05$). When the intensity of infection was considered, the mean EPG of strongyle type eggs and mean OPG of *Eimeria* spp. were significantly higher during the rainy season compared to the dry season (Mann Whitney U test, $p < 0.05$). However, there was no significant difference in the intensity of infection between adults and lambs or between males and females (Mann Whitney U test, $p > 0.05$).

Blood Parasite

A haemoprotozoan parasite *Theileria* sp. (Phylum: Apicomplexa; Family: Theileriidae) was found in 87 % of the 15 animals screened. After observing *Theileria* sp. in the blood smears, ticks (36) were collected from the animals to confirm the presence of the vector. Three species of ticks were identified, with *Haemophysalis intermedia* (73 %) being the most common species followed by *Rhipicephalus haemaphysaloides* (16 %) and *Rhipicephalus sanguineus* (11 %) (Figure 3).

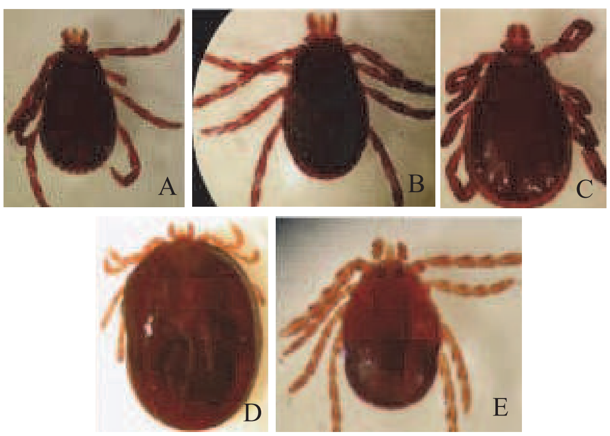

Figure 3: Tick species collected from the sheep at the Kaithady farm (A) male; (B) female *Rhipicephalus sanguineus*; (C) male *Rhipicephalus haemaphysaloides*; (D) engorged female and (E) male of *Haemophysalis intermedia*

DISCUSSION

The sheep herd at Kaithady in the Jaffna District was infected with seven identified genera of GI parasites and one blood parasite. Although the overall prevalence of parasitic infections was high during the rainy season than during the dry season, this difference was not statistically significant. However, the parasite burden was significantly higher during the rainy season compared to the dry season. High intensity infection of nematodes during the rainy season specifically with *Haemonchus contortus* was seen. It might be due to the re-infection of these free grazing animals, because it provides a more suitable environment for the survival and dispersal of infective larvae of nematodes on pasture. The flock was shifted to Nawatkuli, which has a higher elevation and kept there for about four months during the rainy season due to flooding at Kaithady. Animals were all housed together at night, and were weak and reported to be suffering from fever during the rainy season (*Personal communication*). Heavy rainfall lowers the resistance of animals and this is taken advantage of by the infective larvae to establish a heavy infection (Miller & Horohov, 2005).

Strongyle eggs, predominantly *Haemonchus contortus* were the most common type of infection. *Haemonchus contortus* is a blood-sucking parasite found in the abomasum and causes severe blood plasma and protein loss. Haemonchosis is primarily a disease of sheep in warm climates. However, since high humidity, at least in the microclimate of the faeces and the herbage is also essential for larval development and survival, the frequency and severity of outbreaks of the disease is largely dependent on the rainfall in any particular area (Urquhart *et al*., 1996). Symptoms of acute haemonchosis include dark coloured faeces with blood and sudden death of the affected animals (Bhatia *et al*., 2004). Presence of such symptoms had been observed in the studied animals during the study period (*Personal communication*). Moreover, *Haemonchus contortus* causes immunosuppression, which probably predisposed the animal towards secondary infections.

The second most prevalent GI parasitic infection among the sheep at Kaithady farm was with *Eimeria* spp. Infection with *Eimeria* causing coccidiosis is one of the most economically important diseases of sheep and lambs (Bhatia *et al*., 2004). Many *Eimeria* spp. were recorded in goats in Sri Lanka including those that were recorded in the sheep (Faizal *et al*., 1999; Faizal & Rajapakse, 2001; Bandara *et al*., 2007). Of the four *Eimeria* spp. identified, *E. ovinoidalis,* was the most common followed by *E. ovina*, *E. ovinoidalis* and *E. ahsata*. All these species are pathogenic, especially for lambs aged between 1 − 6 months; *E ovina* appears to be somewhat less pathogenic (Bhatia *et al*., 2004). Older sheep serve as sources of infection for the young.

Mixed infections of both strongyle and *Eimeria* spp. were common in the present study. Concurrent infections of *Eimeria* spp. and GI nematode infections were recorded in goats in the Dry Zone of Sri Lanka

(Fernando, 1957; Faizal *et al*., 1999; Rajapakse *et al*., 2000; Faizal & Rajapakse, 2001) and also in sheep in other countries (Kanyari, 1993; Vercruysse, 1982; Reginsson & Richter 1997, Skirnisson, 2007; Yakhchali & Rezaei, 2010). Of the strongyle infections, 88 % were mixed infections either singly or in mixed with species of *Eimeria* spp., *Moniezia* sp., *Paramphistomum* spp., *Trichuris* sp., *Toxocara* sp. and *Giardia* sp. of various combinations.

Low infection of helminths such as *Toxocara* sp., *Trichuris* sp., *Moneizia* sp. and amphistome eggs was found. Presence of *Toxocara* eggs in sheep faeces could be a spurious or false infection. The eggs that were observed during faecal examination could be from sheep eating pasteur contaminated with the dung of cattle in the same grazing land. Recently, Khan *et al*. (2010) recorded 15.4 % infection with *T. vitulorum* in sheep in Pakistan. Infection might be due to the common grazing behaviour of sheep with cattle. In the present study, infection was found only in lambs in both seasons; it may be due to the susceptibility and/or low immunity caused by low nutrition or due to any disease of the animals. *Trichuris* sp. was found with low infection only in females during the rainy season. *Moneizia expansa* is the sheep tapeworm and usually considered harmless except when present in large numbers. Eggs of *Moniezia* spp. were found only during the rainy season.

A tick-borne haemoparasite *Theileria* spp. was found with a high prevalence in the sheep at Kaithady farm. Of the three *Theileria* species occurring in sheep, *T. lestoquardi* (previously known as *Theileria hirci*; Razmi *et al*., 2003) is considered to be the only one that is highly pathogenic. *Theileria ovis* and *T. seperata* are less pathogenic. *Theileria lestoquardi* has been recorded from sheep in Sudan (Salih *et al*., 2003). Although a large number of sheep at the Kaithady farm were infected (87 %), they did not show any severe clinical symptoms of theileriosis other than occasional fevers during the rainy season and hence may be harbouring a non-pathogenic species. Tick species such as *Rhipicephalus bursa* and *Hyalomma anatolicum* act as vectors of *Theileria*. In the present study *R. sanguineus*, *R. haemaphysaloides* and *H. intermedia* were collected from the sheep. These ticks may act as potential vectors of theileriosis. Acaricides are rarely used by farmers. Crows remove the ticks from the ear and body of the animals (*Personal communication*).

Anthelmintics are still an important part of parasite control. However, they must be used properly to ensure effectiveness of the treatment and slow down the rate at which worms develop drug resistance. Due to transportation difficulties during the civil war, deworming treatments and acaricides were not available to the farmers and they were unable to send blood samples for proper diagnosis of infections *(Personal communication*). For example, cattle in the area had been treated for tick borne infection, babesiosis only by considering the clinical signs (*Personal communication*). Since blood tests were not done to diagnose the parasite, treatment was prescribed based on only the clinical symptoms. Although the farmers claimed that animals were dewormed every six months, all the animals of the flock were not dewormed simultaneously due to the high cost of anthelminthic drugs. Deworming is done mostly to the animals with clinical signs of the diseases. Results of the present study show that many GI parasites were prevalent at low level without causing any disease condition especially during the dry season but the intensity of infection increased when the weather conditions were favourable. Farmers should be educated on the proper use of anthelminthics and the importance of a proper method of deworming. Frequent uses or misuses of anthelmintics could increase the incidence of anthelmintic resistance of GI nematodes (Kumara *et al*., 2010).

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