

RESEARCH ARTICLE

Potentially zoonotic gastrointestinal parasites of dogs in Lunugala Tea estate community in Central Sri Lanka

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Abstract: Coprological examination of gastrointestinal (GI) parasites and their life stages in humans and dogs and in soil was carried out in a low income tea estate community in the Central Province. This community has limited access to public health facilities and veterinary services and lives in close contact with free roaming dogs. Parasites in faeces were isolated and identified morphologically and morphometrically using microscopical methods, followed by molecular confirmation of selected protozoans. Soil samples collected from the neighbourhood were analyzed for soil inhabiting parasitic stages. Of the 50 dogs examined, 86.0% was infected with one or more parasites with a significantly higher number of dogs having mixed infections than single infections. Dogs harboured 13 GI parasites, of which nine were known zoonotic species: *Toxocara canis*, *Strongyloides* sp., *Entamoeba coli*, hookworm, *Trichuris* sp., *Giardia duodenalis*, *Spirocerca lupi*, *Toxascaris* sp., and *Taenia* sp. Additionally *Entamoeba histolytica*, coccidia, unidentified trematodes and cestodes were also found in dogs. Six types of GI parasites were identified in humans, of these four types, *E. coli*, *G. duodenalis*, *Strongyloides* sp. and *Blastocystis* sp. were potentially acquired from animals. A total of 16 soil samples were analyzed, of which 44.4% were carrying infective nematode L₃ larvae and eggs, cysts of *E. coli* and eggs of *T. canis* all of which were zoonotic. High prevalence of zoonotic infections in dog population and in soil poses a serious health threat to the community. Results highlight the importance of regular deworming of both humans and dogs and reducing environmental contamination, a One Health approach incorporating veterinary and public health interventions in the surveillance and management of zoonoses.

Keywords: zoonoses, gastrointestinal parasites, dogs, humans.

INTRODUCTION

Gastrointestinal (GI) parasites of companion animals, dogs in particular, can cause serious illness in human as they facilitate zoonotic transmission by acting as a source of infection for people and as a bridge between wildlife and humans. In developing countries, abundance of stray dogs and canine faeces deposited on public and private properties are a recurrent irritant and an important public health issue due to the GI parasites with zoonotic potential (Rai *et al.*,

2000; Sarvi *et al.*, 2014). Many canine GI parasites exclude their eggs, larvae or oocysts with the dog faeces (Rinaldi *et al.*, 2009). These parasites thrive in conditions with warm temperatures and high humidity especially in communities with low socio-economical standards with overcrowded housing, low sanitation and free roaming dogs with close human-dog interaction. Surveillance of dogs can play a critical role in preventing human illness serving as sentinels for infection.

Zoonotic possibility of dog-inhabiting parasites as *Ascaris* sp., *Trichuris*, *Toxocara canis*, *Cryptosporidium*, *Giardia intestinalis* and hymenolepids has been studied in Asia, Australia (Provic and Croese, 1996; Traub *et al.*, 2002; Chattha *et al.*, 2009; Khante *et al.*, 2009; Shalaby *et al.*, 2010;^a Ngui *et al.*, 2014), Ethiopia (Degefu *et al.*, 2011) and in Italy (Rinaldi *et al.*, 2009). Residents of estate communities might experience higher exposure to some zoonotic parasites than the population in general. A study done by Traub *et al.*, (2005) in a tea growing community in India disclosed the high possibility of dogs to transmit *Ascaris* sp., *Trichuris* sp., *Giardia duodenalis* and hookworm to human. Some of the possible zoonotic GI parasites in Sri Lanka include helminths as *Ascaris* sp., *T. canis* and *Echinococcus granulosus* (Dissanaike, 1993; de Silva *et al.*, 1994; Iddawela *et al.*, 2003) and protozoans like *G. duodenalis*, *Cryptosporidium*, *Entamoeba* sp. (de Silva *et al.*, 1994) and coccidians (Wijesundara, 1995). A study carried out by Sorensen *et al.* (1994) in a plantation community in Sri Lanka reported a high *Ascaris* infection in women and children highlighting the fact that a higher risk in places with less sanitary facilities and with congested living conditions. Furthermore, they suggest that, by increasing sanitary facilities, the risk of soil transmitting helminths to the human population can be reduced (Sorensen *et al.*, 1994). Another study reported high prevalence (50.0%) of *Ascaris* sp. in a low country tea plantation in Sri Lanka (Gunawardena *et al.*, 2004) and they point out the risk of *Ascaris* infection increases with congested living conditions and poor sanitary facilities due to the high level of faecal contamination of such environments. Moreover, these authors highlight that good

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personal hygienic conditions and the boiling of drinking water reduce the risks of *Ascaris* infection even if the environment is highly contaminated (Gunawardena *et al.*, 2004). A study carried out in a tea plantation in Kandy district reveals the presence of 13 canine GI parasites 11 of which have a zoonotic potential (Perera *et al.*, 2013). Later, in the same tea plantation, high prevalence of *Ascaris lumbricoides* and a low prevalence of *Enterobius vermicularis* in the human population was reported with a higher worm burden in children (Galgamuwa *et al.*, 2014). These studies have examined the GI parasites in dogs and humans separately. Here we take a holistic approach to address the connection between health of humans, dogs and the environment of a low income estate community at Lunugala Tea estate in relation to the GI parasites.

MATERIALS AND METHODS

Study site

Lunugala tea estate is located in an elevation of about 1,046 m with temperature between 5.5-35°C and an average annual precipitation is between 3000 mm – 5000 mm. The population is 663 comprising mainly of estate community with a low socio-economic status. Almost all the workers are tea pluckers and rarely employed elsewhere. They live mostly in line houses or small blocks of houses connected together. Both dog and the human population have low sanitation, improper and haphazard waste disposal and overcrowded housing. Large number of stray and owned dogs is found in the locality. According to the Teldeniya Divisional Secretariat reports in 2010, the dog to human ratio was one to five.

Study group

Faecal samples were collected from 50 dogs (representing one third of the dog population) and 50 human (representing 7% of the human population) in the selected study area using a convenient sampling technique. Information about the human participants and dogs were collected using a questionnaire. Dogs were either owned or stray. Owned dogs were vaccinated against rabies and may be de-wormed at least once in lifetime. The stray dogs are the street dogs that are not owned, not vaccinated against rabies, not been dewormed and usually fed on garbage. Mostly, the conditions of the owned dogs remain the same beside the vaccination against rabies and mingled with the stray dogs in the neighborhood. Human participants were initially informed about the research and faecal samples were collected from volunteered participants after verbal consent.

Sample Collection

Fresh faecal samples were collected from 50 dogs and 50 villagers in Lunugala estate in the month of May during a rainy period. Dog samples were collected following the dog until they defecate. Human participants were instructed to place a faecal sample into a vial soon after the defecation. Information about the dog and the human participant such as sex, age, deworming practices was recorded after collecting samples. Later, soil samples were

collected from the localities where the parasitic infections were high in order to find out the soil inhabiting stages of parasites. All the samples were brought to the laboratory in a cooler within two hours of collection and were kept at 4 °C until process.

Faecal analysis

Faecal samples were analyzed using Sheather's sucrose floatation method and direct iodine smears. Morphological identification was done according to the photo guide and text book "*Clinical Parasitology of Dogs*" by Dunsmore and Shaw (1990). Length and the width of eggs and cysts were measured under high power (10×40) for morphometric identification of eggs, cysts and larvae using a calibrated eyepiece. For *Giardia duodenalis* Trichrome staining and molecular methods were used for confirmation.

Sheather's sucrose floatation (Blagburn and Butler, 2006)

Approximately, 3 g of the faecal sample was weighed using an electrical balance and taken into a 50 ml centrifuge tube filled with 45 ml of distilled water. Faeces was stirred well using an applicator stick. Then the suspension was centrifuged at 3000 rpm for 20 min after capping the centrifuge tubes tightly. The supernatant was discarded using a Pasteur pipet. Again the pellet was washed with distilled water followed by two centrifugations to obtain a clear solution. After removing the supernatant, the Sheather's sucrose solution (specific gravity 1.27) was added up to the 45 ml level and then followed by a centrifugation at 3000 rpm for 20 min. Approximately, 5 ml of the top meniscus was aspirated into a 15 ml centrifuge tube. The total volume was made to 14 ml level by adding distilled water and the tube was centrifuged at 3000 rpm for 10 min at 16 °C. The supernatant was pipetted out leaving approximately 1 ml of supernatant in the tube. The sediment was thoroughly mixed with remaining supernatant and then transferred to a 1.5 ml Eppendorf® microfuge tube using a Pasteur pipette. Distilled water was added up to the 1.5 ml level and was centrifuged for 10 min at 3500 rpm. The supernatant was decant leaving only 0.5 ml in the Eppendorf® microfuge tube and then the pellet was thoroughly mixed and the whole volume in the tube was used to make a microscope slide. Eggs and cysts were identified using the ×10 and ×40 objective lenses, length and width were measured and photographed for further identification.

Iodine smear (Garcia and Bruckner, 1988)

A drop of Lugols' iodine was laced on a microscopic slide. A small amount of the faecal sample was picked up using a wooden applicator stick and mixed well. For each sample, three smears were prepared and observed under the light microscope using ×10 and ×40 objective lenses. Parasitic eggs and cysts were measured and photographed under x40 objective for further identification.

Trichrome staining (ThermoFisher® catalog, n.d)

Trichrome staining was performed for positive samples for *Giardia* sp. isolated delete resulted from coprological methods. A thin faecal smear was prepared and half dried.

The smear was fixed using freshly prepared HgCl₂ fixative. Then the smear was fixed using 70% ethanol, 70% ethanol in Lugol's iodine, 70% ethanol for 2 min, 5 min and 2 min, respectively. Smear was stained using the Trichrome stain. Excess stain was removed using acid alcohol. The smear was rinsed 2-3 times using 95% ethanol and fixed using absolute ethanol for 3 min. A drop of depex was placed on the prepared smear and a cover slip was placed. Smear was dried for couple of minutes and observed under light microscope using high power ($\times 40$ objective lens) for protozoans.

Molecular identification

Samples positive for *Giardia* sp. by coprological methods were processed for molecular confirmation. Genomic DNA of the parasite was extracted using MO BIO soil extraction kit and amplified by nested Polymerase Chain Reaction (PCR) using specific primers for SSU rRNA gene. For outer PCR Giar RH11 and Giar RH 4 primers were used and then for the nested PCR Giar F and Giar R primers were used (Table 1). Appropriate temperature conditions used in the PCR are given in Table 2. The amplified DNA was visualized by agarose gel electrophoresis. An agarose gel (0.8%) was ran under 40 volts for 45 min with Ethidium Bromide as the visualizing dye.

Soil analysis

Sixteen soil samples were collected from localities where a high incident of infections of human and dog population was found. Soil samples were collected, processed and analyzed according to Horiuchi *et al.*, 2013 and further analysed using Sheather's sucrose floatation method after filtering the larger soil particles out. Larvae and eggs were extracted and identified.

Statistical analysis

Comparisons between protozoan and helminth infections and differences among the risk groups were carried out using a Chi square test and the analyses were performed using MINITAB Version 17.

Ethical clearance

Protocols for faecal sample collection and processing was reviewed and approved by the Ethical Review Committee, Faculty of Medicine, University of Peradeniya.

RESULTS

Prevalence of parasites

Of the 50 dogs examined, 43 (86.0%) were positive for one or more GI parasites. There was no difference in the prevalence of infection between stray and owned dogs or between male and female dogs (Chi square test, $p > 0.05$). Adult dogs however, had a significantly higher infection than puppies ($\chi^2 = 5.081$; $p = 0.024$). Helminth infections were significantly common than protozoan infections ($\chi^2 = 21.182$; $p < 0.001$). Polyparasitism was significantly higher (62.8%) than monoparasitism ($\chi^2 = 4.937$; $p = 0.026$). None of the dogs had protozoan-protozoan mixed infections. Mixed infections were either helminth-protozoan or helminth-helminth, with no significant difference between the two groups ($\chi^2 = 0.486$; $p = 0.486$). A total of 13 GI parasite species were found of which, *Toxocara canis* had the highest prevalence (28.0%) followed by *Strongyloides* sp. (26.0%), and *Entamoeba coli* (24.0%; Table 3). In addition, *Trichuris* sp., Hookworm, *Giardia duodenalis*, *Spirocerca lupi*, *Entamoeba histolytica*, *Toxascaris* sp., *Taenia* sp. and *Blastocystis* sp. were also recorded in dogs (Table 3, Figure 1).

Of the 50 human samples examined 31 (62.0%) carried one or more GI parasites. There was no difference in the prevalence of infection between males and females ($\chi^2 = 0.764$; $p = 0.382$) or between adults and children (below 12 years of age; $\chi^2 = 2.266$; $p = 0.132$). Monoparasitism was significant than polyparasitism ($\chi^2 = 10.519$; $p = 0.001$). None of the humans had helminth-helminth mixed infections but protozoan-protozoan mixed infection (37.5%) and protozoan-helminth infection (62.5%) were found. A total of six GI parasites were found in humans with *E. coli* recording the highest prevalence (32.0%) followed by *G. duodenalis* (26.0%). Other parasites include *Ascaris lumbricoides*, *Strongyloides* sp., *Balantidium* sp. and *Blastocystis* sp. (Table 3, Figure 1).

In the soil samples, 44.4% were positive for life stages of GI parasites such as infective L₃ of nematodes (11.1%),

Table 1: Primer sequences and melting temperatures used to isolate and amplify DNA of *Giardia duodenalis*.

| Gene Locus | Sequence from 5'-3' | Melting temperature (°C) |
|------------|---------------------|--------------------------|
| Giar RH 11 | CATCCGGTCGATCCTGCC | 58.3 |
| Giar RH 4 | GTCGAACCTGATTCTCCG | 55.7 |
| Giar F | GACGCTCTCCCAAGGAC | 57.9 |
| Giar R | CTGCGTCACGTGCTCG | 59.8 |

Table 2: PCR cycling conditions used during the DNA amplification of *Giardia duodenalis*.

| | Outer PCR | Nested PCR |
|------------------------|------------------|-----------------|
| Initial denaturation | 94 °C for 5 min | 94 °C for 5 min |
| Denaturation | 94 °C for 30 s | 94 °C for 30 s |
| Annealing primers | 53 °C for 1 min | 56 °C for 1 min |
| Extension of strands | 72 °C for 30 s | 72 °C for 30 s |
| Completion of reaction | 72 °C for 10 min | 72 °C for 7 min |

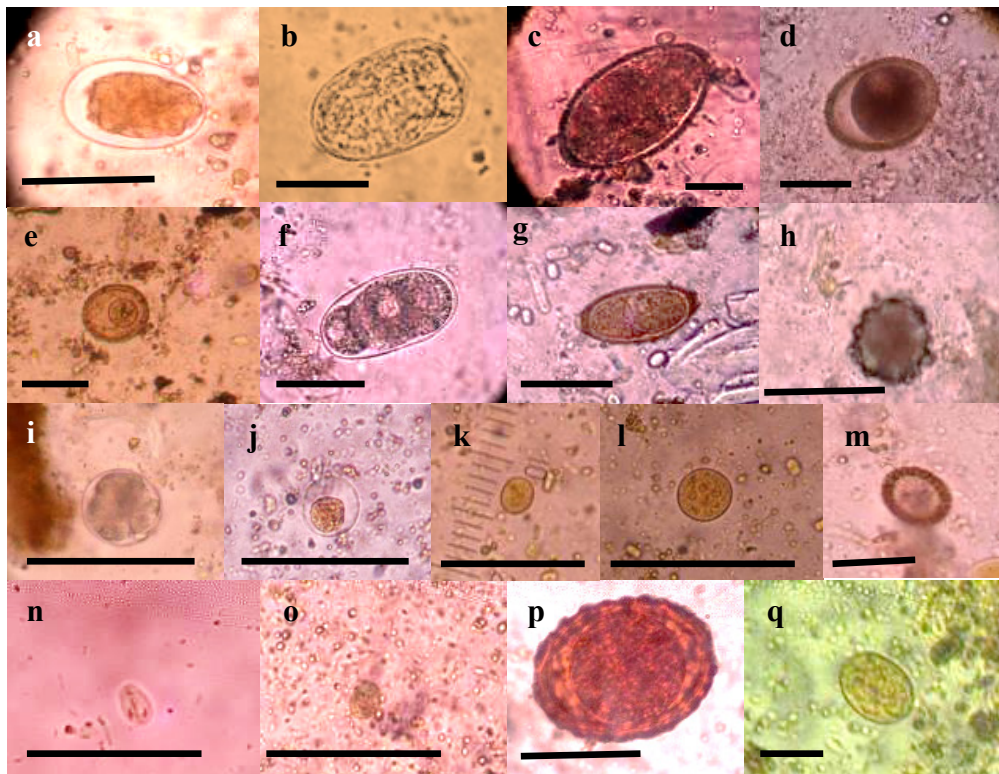


Figure 1: Parasitic stages found in dogs (a, c, d, e, f, g, h, i, k, m and n) and human (b, j, l, o, p and q). a, b- *Strongyloides sp.*, c- unidentified Trematode egg, d- *Toxocara canis*, e- *Toxascaris sp.*, f- Hookworm, g- *Trichuris sp.*, h- unidentified Cestode egg, i, j - *Blastocystis sp.*, k, l- *Entamoeba coli*, m- *Taenia sp.*, n, o- *Giardia duodenalis*, p- *Ascaris sp.*, and q- *Balantidium sp.* (Scale bar represent 50 μ m). All images are taken under iodine wet mount under high power.

Table 3: Diversity and prevalence of gastrointestinal parasites in stray and owned dogs.

| Parasitic species | Prevalence of infection (n) | | | | | | |
|-------------------------------------|-----------------------------|------------|------------|-----------|-------------|-----------------------|-----------------------|
| | Overall (50) | Stray (15) | Owned (35) | Male (34) | Female (16) | Adults (33) | Puppies (17) |
| <i>Toxocara canis</i> * | 28.0 (14) | 0.0 | 40.0 (14) | 35.3 (12) | 12.5 (2) | 27.2 (9) | 29.4 (5) |
| <i>Stroglyoides sp.</i> * | 26.0 (13) | 20.0 (3) | 28.6 (10) | 26.5 (9) | 25.0 (4) | 21.2 (7) | 35.3 (6) |
| <i>Trichuris sp.</i> * | 20.0 (10) | 20.0 (3) | 20.0 (7) | 26.5 (9) | 6.3 (1) | 30.3 (10) | 0.0 |
| Hookworm* | 16.0 (8) | 13.3 (2) | 17.1 (6) | 14.7 (5) | 18.8 (3) | 18.2 (6) | 11.8 (2) |
| Helminths <i>Spirocerca lupi</i> * | 16.0 (8) | 20.0 (3) | 14.3 (5) | 8.8 (3) | 31.3 (5) | 21.2 (7) | 5.9 (1) |
| <i>Toxascaris</i> * | 4.0 (2) | 6.7 (1) | 2.8 (1) | 5.9 (2) | 0.0 | 6.1(2) | 0.0 |
| Un. trematodes | 2.0 (1) | 0.0 | 2.8 (1) | 0.0 | 3.0 (1) | 6.1 (1) | 0.0 |
| Un. cestodes | 2.0 (1) | 0.0 | 2.8 (1) | 2.9 (1) | 0.0 | 6.1 (1) | 0.0 |
| <i>Taenia sp.</i> * | 2.0 (1) | 6.7 (1) | 0.0 (0) | 0.0 | 6.3 (1) | 0.0 | 5.9 (1) |
| Prevalence of helminths | 90.7 (39) ^b | 80.0 (12) | 77.1 (27) | 79.4 (27) | 75.0 (12) | 87.9 (29) | 57.8 (10) |
| Protozoans <i>Entamoeba coli</i> * | 22.0 (11) | 33.3 (5) | 17.1 (6) | 20.6 (7) | 25.0 (4) | 27.2 (9) | 11.8 (2) |
| <i>G. duodenalis</i> * | 16.0 (8) | 13.3 (2) | 17.1 (6) | 11.8 (4) | 25.0 (4) | 18.2 (6) | 11.8 (2) |
| <i>E. histolytica</i> | 6.0 (3) | 6.7 (1) | 5.7 (2) | 5.9 (2) | 6.3 (1) | 3.0 (1) | 11.8 (2) |
| <i>Blastocystis sp.</i> | 2.0 (1) | 6.7 (1) | 0.0 | 2.9 (1) | 0.0 | 3.0 (1) | 0.0 |
| Prevalence of protozoans | 44.2 (19) ^b | 53.3 (8) | 5.7 (2) | 29.4 (10) | 50.0 (8) | 42.4 (14) | 29.4 (5) |
| Overall infection Single infections | 37.2 (16) ^a | 46.7 (7) | 25.7 (9) | 26.5 (9) | 43.8 (7) | 36.4 (12) | 23.5 (4) |
| Mixed infections | 62.8 (27) ^a | 46.7 (7) | 57.1 (20) | 55.9 (19) | 50.0(8) | 57.6 (19) | 47.1 (8) |
| Helminth/protozoan mixed infections | 34.8 (15) | 40.0 (6) | 25.7 (9) | 32.4 (11) | 31.3 (5) | 36.4 (12) | 17.6 (3) |
| Total prevalence of GI infections | 86.0 (43) | 93.9 (14) | 82.9 (29) | 82.4 (28) | 93.8(15) | 93.9(31) ^c | 70.6(12) ^c |

^{a, b, c} denote $p < 005$ Chi square test; Un. = Unidentified; n= number of samples * potential zoonoses

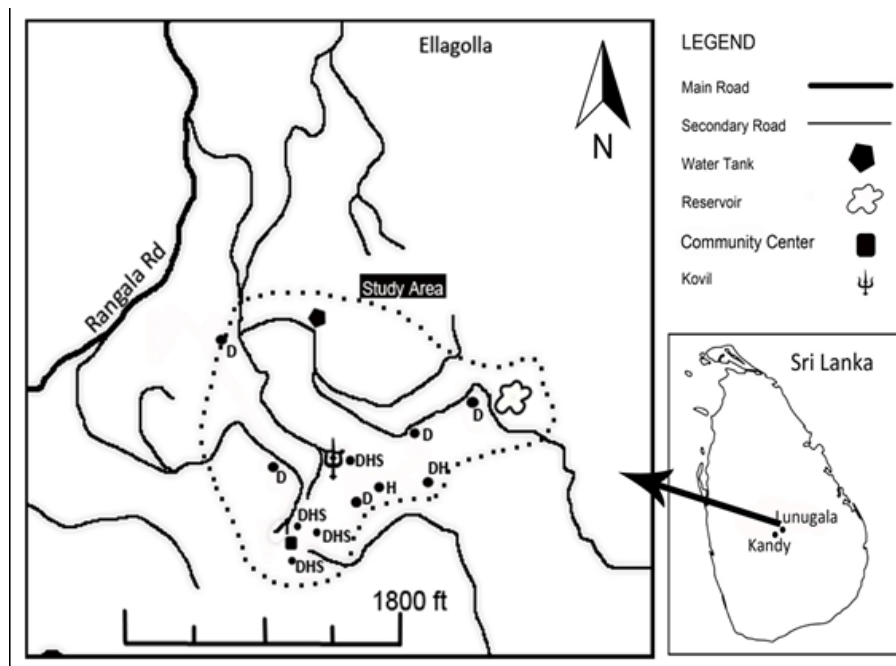


Figure 2: Map showing the study area, “S” represents the localities where the positive soil samples were collected, “H” represents the localities where human faecal samples were positive with parasitic stages and “D” represents the localities where dog faecal samples were positive with parasitic stages.

Table 4: Prevalence of gastrointestinal parasites in human samples.

| Parasitic species | Prevalence of infection (n) | | | | |
|-------------------------------------|-----------------------------|------------------|------------------|------------------|------------------|
| | Overall | Male (25) | Female (25) | Adults (14) | Children (36) |
| Helminths | | | | | |
| <i>A. lumbricoides</i> | 18.0 (9) | 12.0 (3) | 24.0 (6) | 28.6 (4) | 11.1 (4) |
| <i>Strongyloides</i> * | 2.0 (1) | 0.0 | 4.0 (1) | 0.0 | 2.8 (1) |
| Helminth infections | 35.5 (11) | 12.0 (3) | 32.0 (8) | 35.7 (5) | 16.7 (6) |
| Protozoans | | | | | |
| <i>E. coli</i> * | 32.0 (16) | 24.0 (6) | 40.0 (10) | 57.1 (8) | 22.2 (8) |
| <i>G. duodenalis</i> * | 26.0 (13) | 28.0 (7) | 24.0 (6) | 35.7 (5) | 22.2 (8) |
| <i>Balantidium</i> sp. | 2.0 (1) | 4.0 (1) | 0.0 | 0.0 | 2.8 (1) |
| <i>Blastocystis</i> sp.* | 2.0 (1) | 0.0 | 4.0 (1) | 0.0 | 2.8 (1) |
| Protozoan infections | 80.6 (25) | 48.0 (12) | 52.0 (13) | 64.3 (9) | 44.4 (16) |
| Single infections | 74.2 (23) ^a | 48.0 (12) | 44.0 (11) | 42.8 (6) | 47.2 (17) |
| Overall infection | | | | | |
| Mixed infections | 25.8 (8) ^a | 8.0 (2) | 24.0 (6) | 35.7 (5) | 8.3 (3) |
| Helminth/protozoan mixed infections | 16.1 (5) | 4.0 (1) | 16.0 (4) | 21.4 (3) | 5.6 (1) |
| Overall | 62.0 (31) | 56.0 (14) | 68.0 (17) | 78.6 (11) | 55.6 (20) |

^a denotes $p < 0.05$ Chi square test; n = number of samples * potential zoonoses

cysts of *E. coli* (22.2%) and eggs of *T. canis* (11.1%), all of which were zoonotic. Soil samples were collected (denote “S” in Figure 2) from the localities where the human and dog had positive samples (denote “H” and “D” in Figure 2).

Zoonotic potential

Of the 13 GI parasites in dogs, nine were zoonotic: *T. canis*, *Strongyloides* sp., hookworm, *E. coli*, *Trichuris* sp., *G. duodenalis*, *Spirocerca lupi*, *Toxascaris* sp. and *Taenia* sp. and of the six GI parasites in humans four: (*E. coli*, *G. duodenalis*, *Strongyloides* sp. and *Blastocystis*) were potentially zoonotic (Table 3 and 4).

DISCUSSION

Results show that the majority of the dogs in the Lunugala

tea estate were infected with one or more GI parasites. Veterinary care facilities of state sector were extremely limited and people do not seek any accessible private facilities due to their low economic level, thus none of these dogs had been treated for worm infections. Infections of GI parasites in dogs are higher in developing countries and especially in communities with low socio-economic conditions like tea estate communities where living standards are not up to the accepted levels (Sherman, 2010; IFAD, 2014). A study carried out in Hantana tea estate area in Kandy district, in the Central Sri Lanka reported 90.0% of the dogs are infected with GI parasites (Perera et al., 2013) and a tea estate community in India recorded 99.0% of the dogs examined are infected with GI parasites (Traub, 2013). There was no difference in the prevalence

of GI infections between stray and owned dogs. All the dogs faced similar hygienic and health conditions, whether owned or stray, lived outdoors and were permitted to mingle and roam freely. Lack of deworming, and scavenging on improperly disposed garbage are likely routes of parasitic infection. There was no difference in the prevalence of GI parasites between male and female dogs, but the adults carried significantly more infections than puppies, this is however, on the contrary to the general consensus that the prevalence of GI parasites is higher in puppies than in adults as puppies are at higher risk of trans-placental and trans-mammary transmission (Schantz, 1999) and lack of acquired immunity after repeated exposures (Ramirez-Barrios *et al.*, 2004). However, Fontanarrosa *et al.* (2006) argue that higher infection rates in older dogs could be caused by parasites that are not transmitted to dogs at early age or by parasites that do not elicit an immune response.

In regards to species composition, a total of 13 GI parasites were found in dogs and of these, nine were zoonotic: *Toxocara canis*, *Strongyloides* sp., *E. coli*, *Trichuris* sp., hookworm, *G. duodenalis*, *S. lupi*, *Toxascaris* sp., and *Taenia* sp. In Sri Lanka, early records indicate zoonoses of *Toxoplasma gondii*, *Echinococcus granulosus*, *Ancylostoma caninum*, *A. braziliense*, *Diphyllobothrium latum* and *T. canis* (Dissanaike, 1961; Senadhira, 1967; Dissanaike, 1995) and more recently *Isospora* sp, *Cyclospora* sp and *Capillaria aerophyla*, (Perera *et al.*, 2013) were recorded in dogs. Moreover, adult tapeworm of *Echinococcus granulosus* has been recorded in a dog in Kandy district (Dissanaike 1957; 1961). Although the present study reports the hookworm infections in dogs, the species had not been identified. In a study more than five decades ago Dissanaike (1957; 1961) reported the existence different hookworm species like *A. caninum*, *A. ceylanicum* and *A. lumbricoides* in dogs and other studies report they can also be found to a lesser extent in humans as well (Rinaldi *et al.*, 2009; Bowman *et al.*, 2010; Shalaby *et al.*, 2010; Inpankaew *et al.*, 2012; Traub, 2013; Ngui *et al.*, 2014 a,b).

In the tea estate community, 72.0% was infected with one or more GI parasites and of which 28.0% had mixed infections and protozoan infections were more common than helminths. This could be due to administration of anthelmintic drugs especially to children, although not regular and proper. The estate management together with the Ministry of Health conducts mobile clinics for vaccinations and deworming once in every six months. However, proper administration of the recommended dosage is questionable as the education level of the community is low (Personal communication with Estate Management). Protozoans are important etiological agents of waterborne diarrheal diseases (Shortt *et al.*, 2006) and Southeast Asian countries diarrheal diseases is a major reason of morbidity and mortality, especially among children (Perera *et al.*, 1999; Traub *et al.*, 2005; WHO, 2005). In Sri Lanka, there are records of increased contamination of drinking water with protozoan cysts like *G. duodenalis* and *Cryptosporidium* sp. (Shortt *et al.*, 2006). Studies around the world have shown variable occurrences of human infection by *Toxocara* sp. (Itoh *et al.*, 2009; Sharif *et al.*, 2010). For instance, in

USA alone 10,000 cases are recorded annually (Sarvi *et al.*, 2014). In Sri Lanka, 43% seroprevalence for *Toxocara canis* has been recorded by Iddawela *et al.*, (2003) with a higher prevalence in children.

Soil samples had different life stages of GI parasites such as infective L₃ of nematodes, cysts of *E. coli* and eggs of *T. canis*. In Lunugala estate, 11.1% of the soil samples were infected with *T. canis* eggs. Presence of *T. canis* eggs in the soil in public parks in Great Britain have been reported (Chiodo *et al.*, 2006; Talaizadeh *et al.*, 2007). In addition, many other studies report the presence of *T. canis* in soil, together with other infections such as *A. lumbricoides*, *T. vulpis*, *Ancylostoma* sp., *Giardia* sp. and *Cryptosporidium* sp. in soil (Rubel and Wisnivesky, 2005; Itoh *et al.*, 2009; Stojcevic *et al.*, 2010).

A very important, widely present zoonotic parasite, *Cryptosporidium* was not encountered in any of the samples. This could be because the coprological examinations done were not specifically designed to extract or identify *Cryptosporidium*, and therefore the *Cryptosporidium* cases might be seriously undermined during the study. Studies done on human and dog faecal and drinking water of Sri Lanka indicates high prevalence of this zoonotic protozoan (de Silva *et al.*, 1994; Iddawela *et al.*, 2003; Shortt *et al.*, 2006; Perera *et al.*, 2013).

This study involving animal, human and environment with a One Health approach offers information on the potentially zoonotic parasites in dog faeces and environment of the Lunugala tea estate community. Dogs provide a constant reservoir of parasites to the human community. Since the houses were clustered and crowded, animals were kept in close proximity to human who defecate in the compound makes household members posing a high risk to the community. Traub *et al.*, (2013) in a similar community discloses the need in health education and risk management practices in order to lessen the infection ratio among dogs and human.

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APPENDIX

Appendix 1: Morphometric analysis of parasite eggs and cysts in dog and human faeces.

| Parasitic Species | Dogs | | | N | Humans | |
|------------------------------|------|------------------|-----------------|----|------------------|-----------------|
| | n | Length (Mean±SD) | Width (Mean±SD) | | Length (Mean±SD) | Width (Mean±SD) |
| <i>Toxocara canis</i> | 14 | 80.5±3.0 | 71.9±1.7 | - | - | - |
| <i>Stroglyoides sp.</i> | 13 | 52.5±8.2 | 36.8±6.2 | 1 | 100.3±2.1 | 52.3±2.1 |
| <i>Trichuris sp.</i> | 10 | 53.7±2.4 | 23.9±2.3 | - | - | - |
| Hookworm | 8 | 64.1±2.8 | 36.4±2.3 | - | - | - |
| <i>Spirocerca lupi</i> | 8 | 33.4±3.0 | 12.5±1.9 | - | - | - |
| <i>Toxascaris</i> | 2 | 74.5±2.3 | 64.2±2.3 | - | - | - |
| Unidentified Trematodes | 1 | 158.0±1.4 | 92.5±6.4 | - | - | - |
| Unidentified Cestodes | 1 | 44.3±3.1 | 35.3±0.6 | - | - | - |
| <i>Taenia sp.</i> | 1 | 36.3±1.5 | 30.0±1.0 | - | - | - |
| <i>Entamoeba coli</i> | 11 | 12.1±1.9 | - | 16 | 11.5±2.7 | - |
| <i>Giardia duodenalis</i> | 8 | 13.8±1.0 | 11.1±16.4 | 13 | 14.9±0.8 | 8.4±0.7 |
| <i>Entamoeba histolytica</i> | 3 | 21.7±1.4 | 12.4±1.1 | - | - | - |
| <i>Blastocystis sp.</i> | 1 | 10.0±1.0 | 7.7±0.6 | 1 | 12.0 | 9.0 |
| <i>A. lumbricoides</i> | - | - | - | 9 | 73.1±1.6 | 43.9±2.9 |
| <i>Balantidium sp.</i> | - | - | - | 1 | 70.5±2.1 | 52.0±1.4 |

n = Number of positive samples