APPLIED BIO-SYSTEMS TECHNOLOGY

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Human Health Importance Zoonotic Gastrointestinal Helminths and Ectoparasites among Stray Dogs in Galle District, Sri Lanka

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Abstract

Background: A notably high stray and semi domesticated dog population exist in close proximity to humans in Sri Lanka. However, the prevalence of gastrointestinal and ecto-parasites among these stray dog population has been limitedly studied. Therefore, the present investigation focused on zoonotic potential among stray dog community in Sri Lanka.

Methods: A total of 110 stray dogs randomly captured from the Galle District of Sri Lanka during May to July 2018 was considered for the study. Freshly voided fecal samples were obtained. Ectoparasites were collected using a lose comb and stored in an alcohol solution. Presence of major gastrointestinal and ectoparasitic species were investigated using standard microscopic methods. The Chi-square test of independence was used for statistical analysis.

Results: The highest egg count as Eggs per Gram (EPG) were detected from *A. caninum* (264.65 $\pm$ 86.02 EPG), followed by *T. canis* (58.38 $\pm$ 7.22 EPG) and *E. vermicularis* (22.70 $\pm$ 5.70 EPG). Approximately one third of the stray dog population (29.1%; n= 32) indicated ectoparasitic infestations, dominated by *Rhipicephalus sanguineus* (40.9%; n=45), *Ctenocephalides canis* (23.6%; n=26) and *Ixodes scapularis* (19.1%; n=21). The results of the Chi-square test of independence denoted that there was a significant difference on the prevalence of helminthic parasites, among male and female stray dog populations (χ^2= 15.19, *df* = 7, *P* = 0.03).

Conclusions: The present study revealed the potential risk on human health by intestinal helminthic and ectoparasites among stray dog populations. Hence, better understanding of such diseases and their control is essential.

Keywords: Dog, Ectoparasites, Gastointentinal, Helminthic, Infection, Prevalence

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INTRODUCTION

Human-animal link is a beneficial dynamic relationship that is influenced by behaviours, which may useful for health and well-being of both animals and humans. This bond is advantageous in emotional, psychological, and physical interactions of people, animals, and the environment [1]. According to some research findings, presence of a friendly dog, moderates blood pressure reactivity to a social stressor in children [2], young adults [1-4]. Although there are some beneficial effects to humans from animals, animal pets are also considered as important sources of zoonotic infections. There are several reported health hazards associated with owning a pet animal. Among them, animal bites and allergies have been identified as the commonest and predominant health hazards. In addition, a diverse range of infections such as parasitic, bacterial, fungal and viral diseases are possible to be transmitted into humans from domestic pets [5-6].

Some studies have emphasized that the potential health risk by enteric parasites to the humans, which were harboured from pet dogs and cats, remains as a significant problem in the world [7]. Immuno-compromised individuals are highly vulnerable for acquiring parasitic infections from their pets [8]. In addition, there are some risk groups such as young children, elderly, pregnant women, veterinarians or animal nurses, who remain at a greater risk, due to either their immune system, behaviour or occupation [9].

The larval stages of several animal parasites can infect humans and produce severe diseases. Visceral and ocular larval migrans caused by common dog roundworm and *Toxocara canis* are two well-recognized clinical syndromes [10]. However, with the adoption of good hygiene and a thorough knowledge of the transmission of these parasites, people who are at risk should be able to continue enjoying the significant benefits of pet ownership. Although this aspect is well recognized and studied in developed countries, canine parasitic zoonoses pose a lowly prioritized public health problem in developing countries such as Sri Lanka, where conditions are conducive for transmission.

As a developing country, Sri Lanka's populations of stray and semi domesticated dogs exist in close proximity to increasing densities of human populations in urban environments. However, records on the prevalence of gastrointestinal and ectoparasites among stray dog population has been limitedly documented in Sri Lanka. Therefore, the objective of the present study was to investigate the occurrence of gastrointestinal and ectoparasitic species of human health importance, within a randomly selected stray dog population in Galle District of Sri Lanka.

METHODOLOGY

Study Area

The present study was conducted in the Galle District, Southern Province of Sri Lanka (6° 3' 12.6684'' N; 80° 13' 15.5208'' E). It covers an area of 1,652 km^2 of which 35 km^2 is covered by water bodies. This District contains a total of 1,058,771 human population [11]. "Dog Care Clinic E.V" is a Non-Governmental Organization (NGO) in Sri Lanka, which conducts animal welfare activities as a free service. This free service screens randomly captured stay dogs and treat for disease conditions, if necessary. The treated stay animals are usually returned to their original population, after proper treatment and follow-up. Stray dogs captured from four areas namely; Dalawella, Harumalgoda West, Heenatigala South and Thalpe South) in Habaraduwa District Secretariat Division, Galle District were selected for the study (Figure 1).

Collection of Samples and Examination for Ecto and Endo Parasites

A total of 110 randomly captured stray dogs, which were taken to the animal welfare clinic (for sterilization), during May to July 2018 were selected for the study.

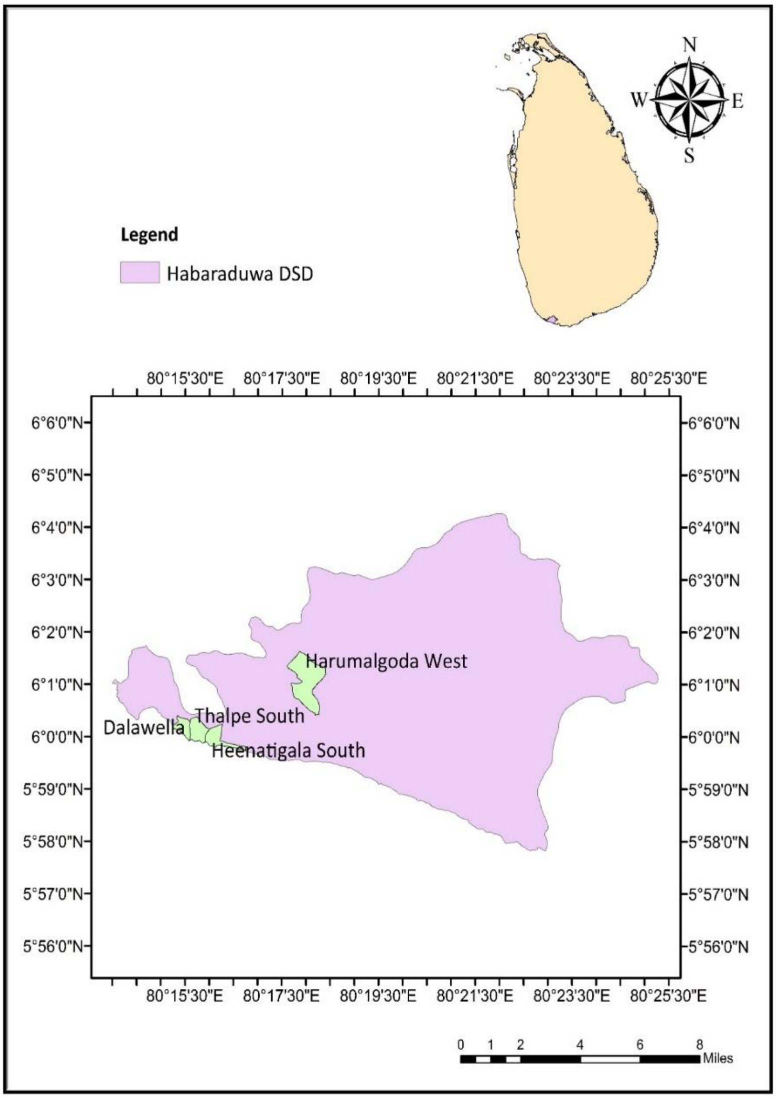

Figure 1: Spatial Location of the Habaraduwa District Secretariat Division

Collection of Stool Samples to Screen Gastrointestinal Parasites

Freshly voided fecal samples (30 g) were collected in to separate vials containing 10% formaldehyde and transported to the laboratory at the Department of Parasitology, Faculty of Medicine, University of Kelaniya, Ragama.

Screening for Ecto-Parasites

Each animal was rubbed with an ether soaked cotton wool. The dogs were combed on to a white paper. The collected ecto-parasites were preserved in 70% alcohol for morphological identification. All collected specimens were transported to the laboratory at the Department of Parasitology, Faculty of Medicine, University of Kelaniya, Ragama, Sri Lanka.

Processing of Samples at the Laboratory

Ecto-Parasite Specimens

Collected ecto-parasites (Fleas, ticks and lice) were macerated overnight with 10% potassium hydroxide (KOH) solution followed by dehydration with a series of alcohol solution. The specimens were mounted in Berlese medium and observed at 40X magnification under a binocular dissecting microscope.

Stool Samples for Gastrointestinal Parasites

The stool samples were processed by Kato-Katz thick smears using the modified Kato-

Katz technique [12]. Fifty mg of the sediments delivered by Kato-Katz template was taken onto a degreased glass slide.

It was covered with a cellophane strip soaked overnight in 50% solution of glycerol-malachite green. Slides were examined for helminthic eggs under a light microscope, immediately after preparation. Parasite eggs were identified based on the morphological characteristics. Density of infection, as expressed by Eggs per Gram (EPG) of faeces, was calculated by multiplying each slide count by 20 [13]. Each sample was duplicated to maintain the accuracy.

Data Interpretation

The prevalence, intensity, mean intensity and mean abundance were calculated for different gastrointestinal parasites as mentioned below [14].

Prevalence (P)

The number of infected animals with one or more individuals of a particular parasite species, divided by the number of hosts examined.

Intensity

The number of individuals of a particular parasite species in a single infected host (expressed as a numerical range).

Mean Intensity

The average intensity, expressed as the total number of parasites of a particular species found in a sample, divided by the number of infected hosts.

Mean Abundance (A)

The total number of individuals of a particular parasite species in a sample of a particular host species, divided by the total number of hosts of that species examined, including both infected and uninfected hosts.

Statistical Analysis

The chi-square test of independence was used to evaluate the significance in the GI parasitic prevalence, in terms of gender and spatial locality. Significance of the effect of the spatial location and gender on the intensity of GI parasitic infections among stray dogs, was statistically evaluated by using the General Linear Model followed by the Tukey's pair-wise comparison in IBM SPSS Statistics (version 23 copyright IBM Corporation). The 95% confidence levels (CI) of the EPG in the study populations were generated by using the one sample t test.

In addition, the Bray Curtis similarity based Cluster analysis followed by Analysis of Similarities (ANOSIM) was used to evaluate the overall clustering status of dog communities from different study areas, based on the intensity of GI parasites. In addition, Distance-Based Redundancy Analysis (dbRDA) was also performed to recognize the underlying segregation patterns of the study populations based on the intensity of GI parasite assemblages using the Plymouth Routines in Multivariate Ecological Research version 6 (PRIMER 6).

RESULTS AND DISCUSSION

Prevalence of Ecto-Parasites

A total of 110 dogs, consisting of 50 (45.5%) males and 60 (55.5%) females were examined in the current study. Of them, 65 dogs (59.1%) had ectoparasitic infestations, dominated by *Rhipicephalus sanguineus, Ctenocephalides canis*, and *Ixodes scapularis* (Figure 2). *R. sanguineus* was the most prevalent, with a prevalence of 40.9% (n=45), followed by *C. canis* (23.6%; n=26). Meanwhile, *I. scapularis* denoted the lowest prevalence rate of 19.1% (n=21). It was interesting to note that nearly one third of the surveyed population (29.1%; n= 32) had more than one ectoparasitic infestations (Figure 2).

Prevalence of Gastrointestinal Helminthic Parasites

Examination of stool smears revealed the occurrence of seven nematodes, namely *Ancylostoma caninum, Trichuris vulpis, Toxocara canis, Enterobius vermicularis, Eucoleus aerophilus, Uncineria stenocephala* and

Physaloptera rara, along with one parasite belonging to the phylum Platyhelminthes (*Paragonimus kellicotti*) within the studied dog population.

Out of the total surveyed, 33.6% (n=37) had GI helminthic infections of eight parasitic species. *A. caninum* was the most prevalent parasite, accounting for 27.3% (n=30) infections, while *E. vermicularis*, *E. aerophilus*, *P. rara* and *P. kellicotti* indicated the lowest prevalence rates (0.91%) in the total investigated dog population (Figure 4). Even among the infected dogs detected for GI helminthic parasites, *A. caninum* infection was the most predominant recording 81.1% (n=30) of the total infections. Interestingly, 13.5% of them had co-infections mostly with *A. caninum* and *T. canis (Figure 3).*

When the prevalence of eggs within infected dogs is considered, *A. caninum* advocated the highest prevalence of 264.6 $\pm$ 86.0 EPG, followed by *T. canis* (58.4 $\pm$ 7.2 EPG) and *E. vermicularis* (22.7 $\pm$ 5.7 EPG). The lowest egg count of 0.7 $\pm$ 0.3 EPG was denoted by *E. aerophilus* (Table 1).

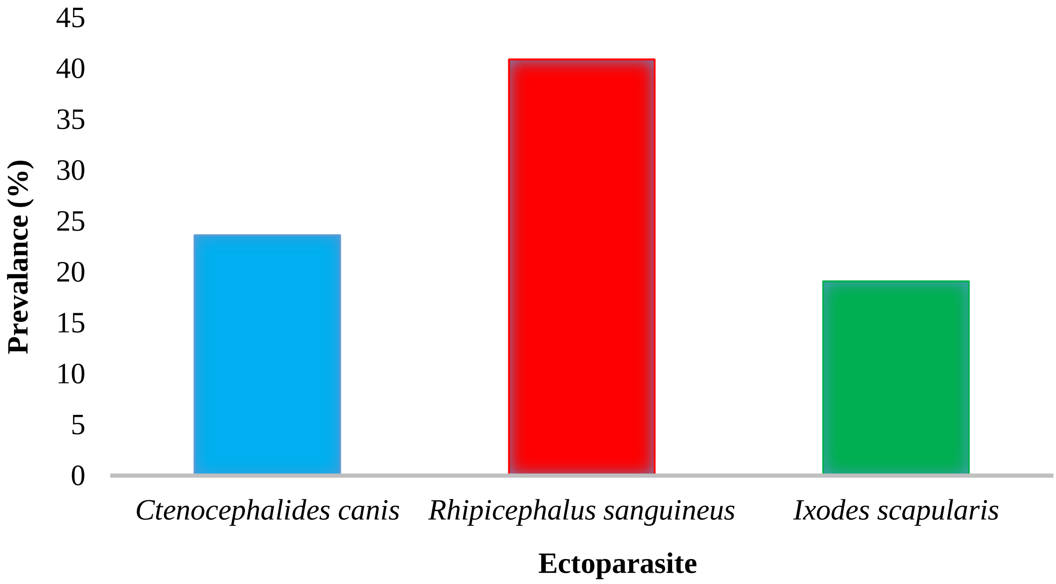

Figure 2: Prevalence Rates of Ectoparasitic Infestations in the Studied Dog Community

Table 1. Intensity of Gastrointestinal Helminths as Eggs per Gram in Stool

Phylum	Species	Mean	Standard Error	95% CI
Nematoda	*Ancylostoma caninum*	264.65	86.02	(90.20 - 439.10)
	Trichuris vulpis	17.51	5.64	(4.14 - 33.10)
	Toxocara canis	58.38	7.22	(17.10 - 108.52)
	Enterobius vermicularis	22.70	5.70	(9.45 - 68.7)
	Eucoleus aerophilus	0.65	0.31	(0 - 1.96)
	Uncineria stenocephala	2.59	1.74	(0.53 - 6.72)
	Physaloptera rara	3.89	1.89	(0.40 - 10.57)
Platyhelminthes	*Paragonimus kellicotti*	2.59	1.54	(0.67 - 5.86)

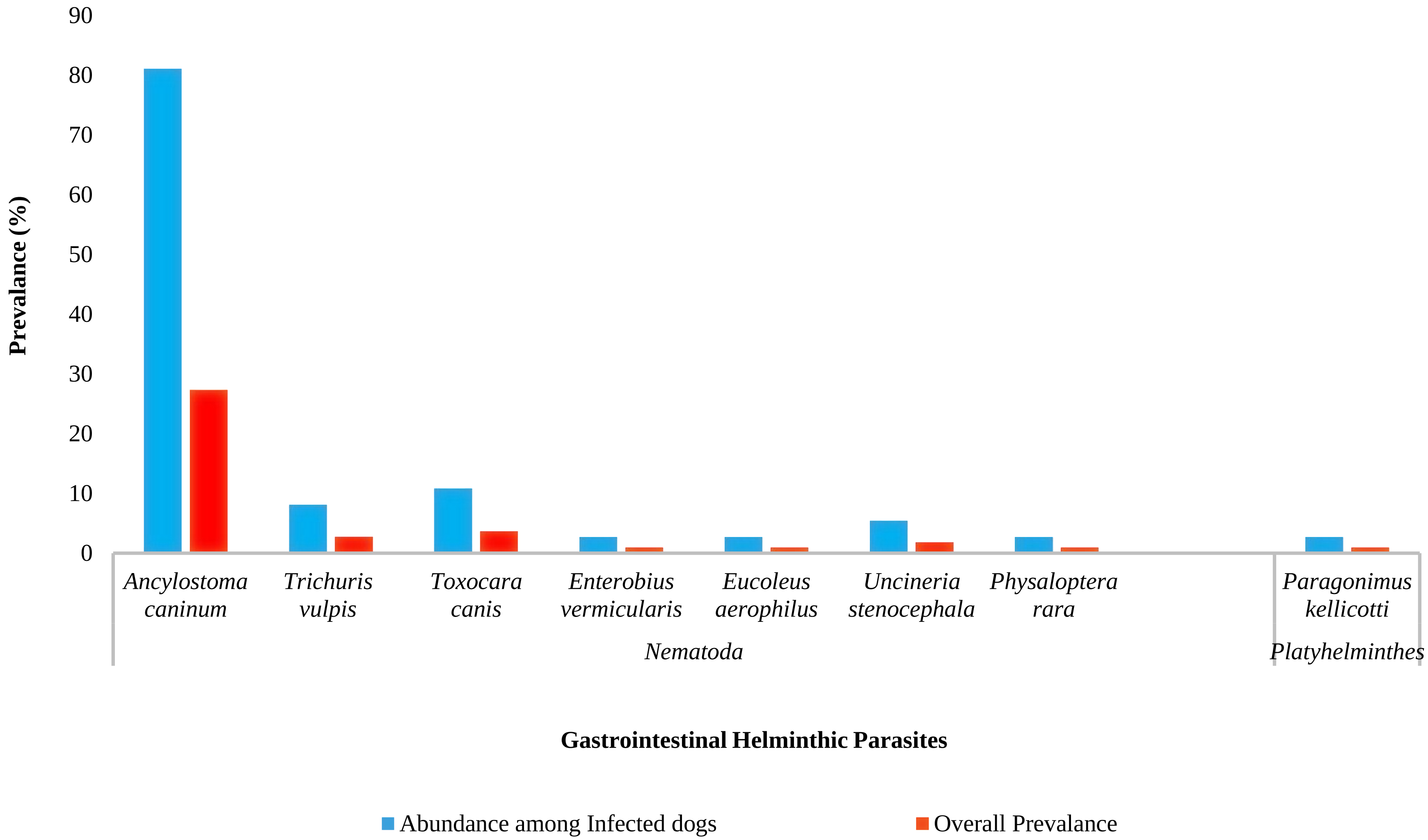

Figure 3: Prevalence Rates of Gastrointestinal Helminthic Infections among Stray Dogs

Gender Precise Prevalence of Gastrointestinal Helminthic Parasites

Among 60 female dogs, 26 dogs had GI helminthic parasites indicating an overall prevalence of 43.3%, while males showed an infection rate of 22.0% (n=11/60). A higher prevalence rate of *A. caninum, T. canis* and *U. stenocephala* were observed among female dogs (56.8%, 8.1% and 8.1%, respectively) than the in males (24.3%, 5.4% and 5.4%, respectively). Interestingly, few parasites such as *E. vermicularis*, *E. aerophilus*, *P. rara* and *P. kellicottiall* were identified only from male stray dogs (Table 2). According to the results of the Chi-square test of independence, the prevalence of infections among stray dogs denoted a significant difference among males and females (χ^2= 15.19, *df* =7, *P*=0.03). However, it was noted that the intensity of the infections (EPG) were not significantly different among the male and female dogs based on the GLM (*P*>*0.05*).

Spatial Distribution of Gastrointestinal Helminthic Parasites

The prevalence rates indicated significant spatial variations in the spatial distribution of gastrointestinal helminthic parasites between the studied GNDs (χ^2= 12.11, *df* = 3, *P*=0.007). All the GI parasites identified during the study were reported from Dalawella GND at significantly higher prevalence rates.

On the other hand, the stray dogs screened in Heenatigala South and Harumalgoda West GND areas had *A. caninum* and *T. canis* infections (Figure 4). In case of the intensity of parasitic infections, the EPG counts of parasites advocated significant spatial variations as indicated by the GLM (*P*=0.017). Heenatigala South GND denoted the highest parasitic load of all recorded parasite intensities, except for *E. vermicularis* and *T. vulpis*. However, the highest parasite intensity of *E. vermicularis* and *T. vulpis* were observed among the stray dogs screened from Dalawella area.

As depicted in the dendrogram of the cluster analysis, the gastrointestinal parasitic assemblages formed two major clusters sharing a 60% similarity, in terms of the EPG (Figure 5). Thalpe South GND formed one major cluster, while the remaining three GND areas, namely, Dalawella, Heenatigala South and Harumalgoda West formed the second cluster, which was proven to be significant through the Analysis of Similarities (ANOSIM) at 95% level of confidence (Global R=0.97). The dbRDA plot also confirmed the above clustering status. The relatively lower intensities of all parasites (except for *T. Vulpis*) could be the contributing factor for the formation of a single cluster by Thalpe South GND, as depicted by the radiating axils in the dbRDA plot (Figure 6).

Table 2: Gender Precise Prevalence of Gastrointestinal Helminthic Parasites among Stray Dogs

Phylum	Species	Prevalence (%)	
		Male	Female
Nematoda	*Ancylostoma caninum*	24.3	56.8
	Trichuris vulpis	2.7	2.7
	Toxocara canis	5.4	8.1
	Enterobius vermicularis	2.7	0.0
	Eucoleus aerophilus	2.7	0.0
	Uncineria stenocephala	5.4	8.1
	Physaloptera rara	2.7	0.0
Platyhelminthes	*Paragonimus kellicotti*	2.7	0.0

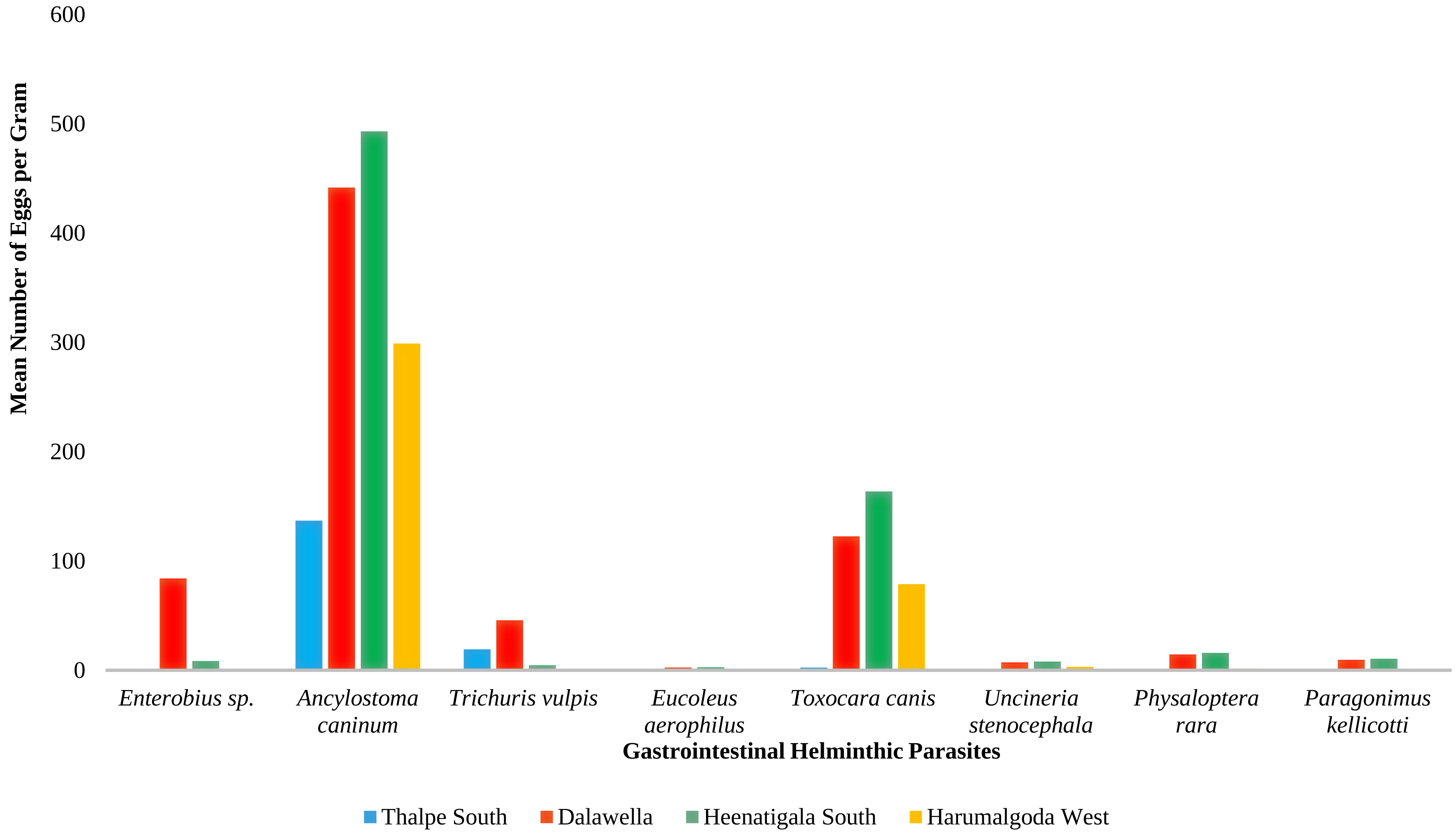

Figure 4: Parasitic Load of Gastrointestinal Helminths in Terms of in Eggs Per Gram Among Stray Dogs

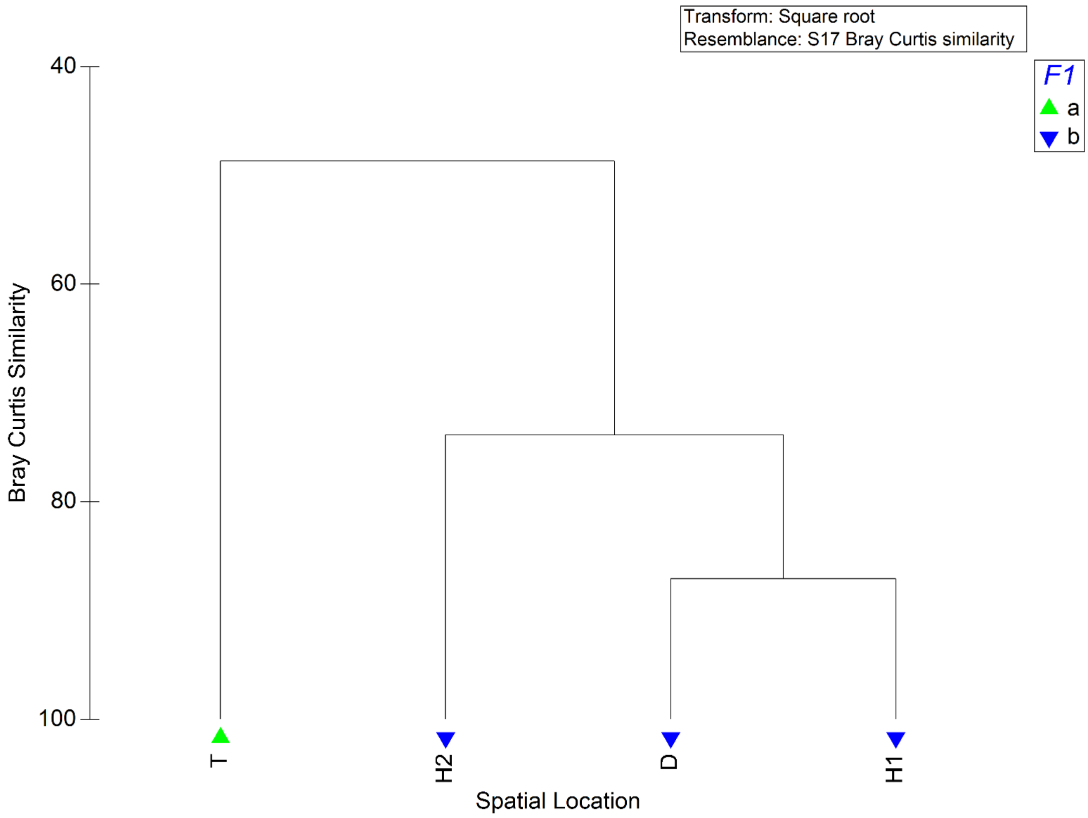

Figure 5: Dendrogram of the Cluster Analysis of Studied Dog Communities in the 4 GND Areas in terms of Eggs per Gram (EPG) of Gastrointestinal Helminthic Parasites

Note: D: Dalawella, H1: Heenatigala South, H2: Harumalgoda West and T: Thalpe South

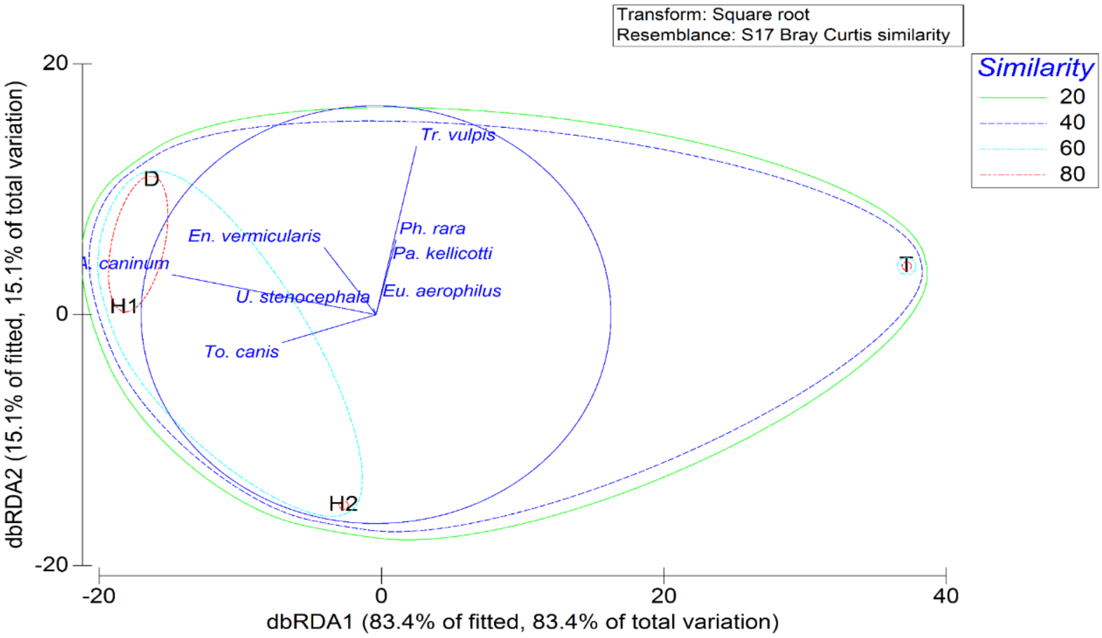

Figure 6: dbRDA Plot of Studied GND Areas in terms of Eggs Per Gram (EPG) of Gastrointestinal Helminthic Parasites in Stray Dogs

Note: D: Dalawella, H1: Heenatigala South, H2: Harumalgoda West and T: Thalpe South

Different pathogens have been identified to cause health impacts to humans through more than 250 zoonoses [15-16]. The reservoirs for these zoonoses are domestic livestock, carnivores and rodents [16]. Among these, dogs contribute majorly as definitive or reservoir hosts for many zoonotic parasites, especially in low economic and developing countries [17-18]. In Sri Lanka, dog population is estimated at three million in 2008 and has reduced up to 2.2 million as a result of the successful Catch-Neuter-Vaccinate-Release (CNVR) programme [19]. It is reported that about 500,000 of these are stray dogs. This amount increases day by day and local Government Authorities receive a considerable number of complaints from the general public [20].

There is a potential to transmit zoonotic infections to humans by swallowing or inhaling pathogens from dogs as the animal reservoir hosts, eating the hosts or via bites. Parasites of human health importance may also be transmitted from dogs to humans by vectors, such as fleas, ticks and mosquitoes [21]. Parasitic helminths are among the most commonly encountered disease causing agents in dogs all over the world [22]. A study conducted in Brazil has also evidenced that the helminth and ectoparasite species were highly prevalent among stray dogs [16].

According to literature, typical helminthic parasites of dogs are *Echinococcus granulosus*, *Dipylidium caninum*, *Toxocara canis*, *Dirofilaria immitis* and *Ancylostoma caninum* [16]. Studies conducted in Sri Lanka has identified some GI parasites with zoonotic potential namely; *T. canis*, *Strongyloides* sp, *E. coli*, *Trichuris* sp, hookworm, *G. duodenalis*, *S. lupi*, *Toxascaris* sp, and *Taenia* sp [23]. Meanwhile, some earlier records have highlighted the occurrence of *Toxoplasma gondii*, *Echinococcus granuloses*, *Ancylostoma caninum*, *A. braziliense*, *Diphylobothrium latum, T. canis* [24-26], *Isospora* sp, *Cyclospora* sp and *Capillaria aerophyla* among dogs [27]. The present study revealed the presence of *A. caninum*, *T. canis*, *E. vermicularis*, *T. vulpis*, *P. rara*, *P. kellicotti*, *U. stenocephala* and *E. aerophilus*. However, *E. vermicularis* has not been reported in dogs. In this case the dogs could have eaten any other animal with oxyurids.

It is well known that the close and frequent contact between dogs and people increases the risk for the transmission of zoonotic diseases. The high prevalence rates of *T. canis* may cause visceral and ocular larva migrans to humans, which lead to blindness and *A. caninum* associated with hookworm related cutaneous larva migrans [16, 28-30]. In addition, the present study recorded the presence of other hook worm species namely; *U. stenocephala,* which may also cause cutaneous larva migrans [16]. Cutaneous larva migrans is caused by the penetration of third stage L3 larvae of the hook worm into the human skin. This has been reported mainly in tropical areas, where climate and other abiotic factors favour for the development of nematode life cycle [28]. Therefore, Sri Lanka, being a tropical country with all favorable conditions to facilitate the nematode life cycle, high prevalence of hookworms among stray dog population may indicate the potential risk for human health. In addition, some studies have indicated that *E. aerophilus* has a potential to cause lung diseases in humans [31].

Overall, the highest prevalence rates were observed with *A. caninum* (80%) followed by *T. canis* and *T. vulpis*. Presence of *A. caninum* with highest percentage of prevalence, is in agreement with previous studies conducted among stray dogs in Sri Lanka (around 80%) [27] and India (72 – 89%) [32]. However, a study conducted in Brazil has reported a prevalence rate of 95% [16]. Some studies have highlighted that the presence of one parasitic species may enhance the occurrence of another species, since dogs with high parasitaemia generally persist with low immunity levels [33]. It is reported that a strong positive association has been identified between *Ancylostoma* sp. and *T. vulpis* [33-34]. Therefore, co- occurrence of *T. vulpis* as the third highest parasite with compared to *A. caninum* in the present study, agrees with the above finding.

Trichuris vulpis is distributed all over the world. However, it is mostly predominant under warm and humid climatic conditions. Even though, *T. vulpis* infection is rare, humans may acquire this infection when they accidentally ingest embryonated eggs, through contaminated soil, food or fomites [35]. The present study highlighted a prevalence rate of 9% for *T. vulpis*, which remained similar to the findings from Spain, Southern Brazil that have reported prevalence rates of 10% and 9.3%, respectively [36-37]. However, this rate was clearly lower than a previous study conducted in Sri Lanka, (36.7%) among a stray dog population in Hantana area, Kandy District, Central province [27]. The zoonotic potential of *T. vulpis* is questionable [38]. Although dog whipworms are generally not considered as an intestinal nematode of zoonotic importance, there are some records on *T. vulpis* causing visceral larva migrans syndrome and intestinal infections in humans [39-42].

The occurrence of *T. canis* was higher among females (8.1%) than males (5.4%) in the present study. This also lies in agreement with the previous studies, which have reported prevalence rates ranging from 8.7% to 5.5% [16, 37, 43]. A previous study conducted in Sri Lanka has also reported the presence of *T. canis* as the most dangerous zoonotic disease, since it may cause both visceral larvae migrans and ocular larvae migrans in humans [27].

Among the ectoparasites, *Rhipicephalus sanguineus* remained predominant, followed by *Ctenocephalides canis* and *Ixodes scapularis.* Ticks and tick-borne diseases have drawn a wider attention due to its increasing trend in global burden [44]. Different tick species have been detected from dogs. Species belonging to the family Ixodidae are important vectors of various parasites, in terms of both veterinary and public health aspects [45]. *Rhipicephalus sanguineus* is the most diverse tick species that has been recorded, especially in tropical countries [46]. This tick species can act as a vector for a wide range of pathogens, comprising the genera *Babesia*, *Hepatozoon*, *Ehrlichia*, *Rickettsia* and *Mycoplasma* [16, 45]. In addition, the prevalence of *I. scapularis* has a potential medical importance as *Ixodes* ticks in Europe (*I. ricinus* and *I. persulcatus*) has reported the ability of transmitting tick-borne encephalitis (TBE) virus, a flavivirus that can cause fatal brain infection among humans [47-49]. In addition, *I. scapularis* is a major vector of pathogens in North America that cause diseases in humans including Lyme disease, human babesiosis and granulocytic anaplasmosis [44-50].

Ctenocephalides canis is an intermediate for the transmission of *Dipylidium caninum* to humans [51-52]. In the life cycle of *D. caninum*, dogs and wild carnivores are the final host, while the human is considered as an occasional host. *Ctenocephalides canis* is regarded as the most abundant ectoparasite among dogs worldwide [45]. The present study identified *C. canis* at a prevalence rate of 23.6%, which stands contract to some other previous studies that have reported prevalence levels of 45.7% in Brazil [16] and Nigeria [53]. Therefore, the presence of *C. canis* may indicate a transmission potential of *Dipylidium caninum* to humans as an occasional host.

Globalization and urbanization are contributing factors that tend to increase the risk of zoonoses for humans in the coastal region of the island. There is a considerable influx of tourists in the coastal region from December to March annually. The availability of stray dogs in these areas may be a new or first ever experience to many of the tourists travel in these areas who have arrived from developed countries. Hence, this implicate some potential impacts on tourist and local communities via intestinal parasites and ectoparasitic infections, even though there are no published evidences to quote. It is also important to the note that the abundance of stray dogs and feces bestowed on public and private properties are a recurrent irritant and an important public health issue, due the occurrence of parasites with Zoonotic potential [23].

The present study revealed that the intestinal helminthosis is common among stray dog populations in the Galle District of Sri Lanka, some of which may have zoonotic potential. In addition, presence of ectoparasites, which can act as vectors for diseases, can cause serious impacts on human health. Therefore, screening of stray and domestic dongs is vital in order to minimize the potential risk for human health. On the other hand, raising awareness on animal welfare and potential risks associated with zoonotic parasites among general public is of paramount importance. Furthermore, health authorities should focus on implementing or strengthening current CNVR and animal welfare programmes catering to the current need.

CONCLUSION

The present study revealed that the intestinal helminthosis is common among stray dog populations in the Galle District of Sri Lanka, some of which may have zoonotic potential. In addition, presence of ectoparasitses which can act as vectors for diseases can cause serious impacts on human health. Therefore, it is recommended that public education on the proper care of dogs, including veterinary care, and potential risk of these parasites on human health.

CONFLICT OF INTEREST

The authors would like to declare that there are no conflicts of interest.

AUTHORS' CONTRIBUTIONS

SR: Data collection, samples identification and reviewed the manuscript. NG: Designed the research and wrote the manuscript. LU: Performed the statistical analysis and wrote the manuscript. All authors read and approved the manuscript.

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