Research article

Prevalence of canine tick-borne haemoparasites in three Divisional Secretariat Divisions (Rambewa, Tirappane, and Galenbidunuwewa) in the Anuradhapura district, Sri Lanka

D Weerathunga[1], A Amarasinghe[1], D Iddawela[1], S Wickramasinghe[1]

Abstract

Introduction and Objectives: Ticks act as the vector for transmission of many parasitic pathogens. Several tick-borne haemoparasite infections are widespread among canines in tropical countries. However, information is scarce regarding canine tick-borne infections in Sri Lanka. The present study was therefore carried out to identify the canine tick-borne haemoparasite species and thereby assess their prevalence in three Divisional Secretariat divisions in the Anuradhapura district.

Materials and methods: Blood samples were collected from dogs in three Divisional Secretariat (DS) divisions namely, Rambewa, Tirappane, and Galenbindunuwewa in the Anuradhapura district, Sri Lanka. From each blood sample, two thin smears were prepared and stained with Giemsa. Stained slides were subsequently examined with a light microscope to detect haemoparasites.

Results: Out of 319 blood samples, 139 were positive for haemoparasites. Of the positives, 51.61% were from Rambewa, 40.47% were from Galenbindunuwewa and 38.88% were from Tirappane. Three haemoprotozoan species, *Hepatozoon canis*, *Babesia gibsoni, Babesia canis* and one bacterial species, *Ehrlichia canis* were morphologically identified in the present study.

Conclusions: Comparatively, the prevalence of ehrlichiosis (20.06%) and babesiosis (16.29%) were high in three DS divisions compared to the prevalence of *H. canis* infection (1.56%). The prevalence of the haemoprotozoan infections was significantly different among thee DS divisions

[1]*Department of Parasitology, Faculty of Medicine, University of Peradeniya, Sri Lanka*

Address for correspondence: Dr S Wickramasinghe, Department of Parasitology, Faculty of Medicine, University of Peradeniya Telephone: +94770074544 Email: susijijp@yahoo.co.jp

https://orcid.org/0000-0003-2046-1093

(P<0.01). Co-infections among dogs suggest that the same vector may be involved in transmitting different infections.

Introduction

Canine tick-borne diseases are a major health problem among dogs in tropical and subtropical countries. Ticks have the capability to successfully transmit disease agents such as viruses, bacteria andprotozoa.[1] Babesiosis, ehrlichiosis and hepatozoonosis are some common tick-borne parasitic infections in dogs.[2,3] These infections can occur in dogs due to the transmission of haemoparasites by several tick species or by the same species.[4] For example, the main vector of *B. canisvogeli, H. canis,* and *E. canis* is the brown dog tick (*Rhipicephalus sanguineus*).[5]

Babesiosis is caused by intraerythrocytic piroplasms of the genus *Babesia.* It is a common disease among domestic and wild canines.[6] *Ehrlichia* is an alphaproteobacterium belonging to the family Ehrlichiaceae. *E. canis* and *E. ewingii* cause tropical canine pancytopenia and canine granulocytic ehrlichiosis respectively.[7] *E. canis* causes severe clinical signs in dogs compared to the other species. *E. chaffeensis* causes infections in both humans and dogs.[9] Canine hepatozoonosis is a systemic disease caused by the protozoan *H. canis* (Apicomplexa, Hepatozoidae). It is transmitted by the dog tick *R. sanguineus*. *H. canis* is found in Africa, Southern Europe, Asia, Australia and America.[7] Ticks acquire the pathogen by feeding on an infected host. Transstadial transmission from the nymph to the adult stage can occur.

Previous studies have determined the prevalence of canine haemoparasites in Sri Lanka. *H. canis* was first identified in 1961 in Sri Lanka.[9] In 2017, cases of acute hepatozoonosis, characterized by neurological symptoms, ataxia or paresis, emaciation and anaemia was detected.[10] An early study has determined the prevalence of canine ehrlichiosis in Sri Lanka based on conventional examination of stained blood smears.[11] A study carried out to determine the prevalence of canine vector-borne diseases in the Western Province identified the prevalence of both single and mixed infections among dogs.[12] However, there is a relative paucity of studies on the prevalence of canine tick-borne infections in Sri Lanka. In the present study, our objectives were to identify the canine tick-borne haemoparasite species and assess their prevalence in three Divisional Secretariat divisions in the Anuradhapura district.

Materials and Methods

Study area
This study was conducted in three Divisional Secretariat (DS) divisions: Rambewa, Tirappane, and Galenbindunuwewa in the Anuradhapura district, Sri Lanka (Figure 1).

Figure 1: Map of Anuradhapura District Divisional Secretariat (DS) Divisions.
The three sampling sites; Rambewa, Galenbindunuwewa and Tirappane DS divisions are highlighted.

Sample collection

The sample size was calculated using the Creative Research Systems survey software (*http://www.surveysystem.com/sscalc.htm*). The calculated sample size was 375 (dog population was about 14,698 and the confidence interval was 95%). Blood samples were collected from April 2014 to July 2014 at the prophylactic anti-rabies vaccination and sterilization centers. Blood samples (1-2 ml) were collected into ethylenediaminetetraacetic acid (EDTA) tubes. Samples were stored at 4 °C and dispatched to the Department of Parasitology, Faculty of Medicine, University of Peradeniya, Sri Lanka to carry out the laboratory investigations.

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Microscopic examination

From each blood sample, 2-3 thin smears were prepared and stained with Giemsa according to the method described in a previous study.[13] Smears were examined with a light microscope (Carl Zeiss™ PrimoStar™, Germany) under oil immersion (×1000) to identify the haemoparasites. *H. canis* was identified by observing the gamonts with an ellipsoidal, brick-like shape inside the cytoplasm of the neutrophils.[14] In the erythrocytes, *B. canis* was identified by the morphology of merozoites described in a previous study.[15] Detection of *B. gibsoni* in the infected erythrocytes was carried out according to the morphological features described by Lempereur et al.(2014).[16] *E. canis* was identified by the round shape and purple colour morulae in the cytoplasm of lymphocytes.[17]

Statistical analysis

Chi square test was conducted in R[18] to identify whether the prevalence of the haemoprotozoan infections was significantly different among the three DS divisions.

Results

Sample collection

All the dogs included in this study were females with an average age of 3 years and were asymptomatic. A total of 319 blood samples were collected from Rambewa (124), Tirappane (108) and Galenbindunuwewa (87) DS divisions. Of those, 312 were obtained from stray dogs and 7 were from pet dogs.

Microscopic examination

Four haemoparasites namely *E. canis, H. canis, B. canis* and *B. gibsoni* were identified by microscopic examination (Figure 2).

Prevalence in the three DS divisions

The prevalence of infection differed significantly between the divisions. (χ^2=18.47, df=6, P<0.01). In Rambewa DS division, the number of mixed infections was high (Table 1). There were no *H. canis* single infections. In Galenbindunuwewa, *H. canis* with *B. gibsoni, B. gibsoni* with *E. canis* were the only mixed infections detected. In Tirappane the only mixed infection detected was *B. gibsoni* with *E. canis*. However, no *B. canis* infections were identified in Tirappane DS division.

Overall prevalence of the haemoparasites

When considering the percentage overall prevalence in the three DS divisions, of the positive samples, the highest prevalence (51.61%) was from Rambewa and the lowest (38.88%) from Tirappane (Table 1). Among haemoparasites, Rambewa DS division had a significantly high number of *E. canis* infections. The highest number of *B. gibsoni* infections was found in the Tirappane DS division. *H. canis* was present in all three DS divisions. *B. canis* presented in Rambewa and Galenbindunuwewa DS divisions but was not present in the Tirappane DS division (Table 1).
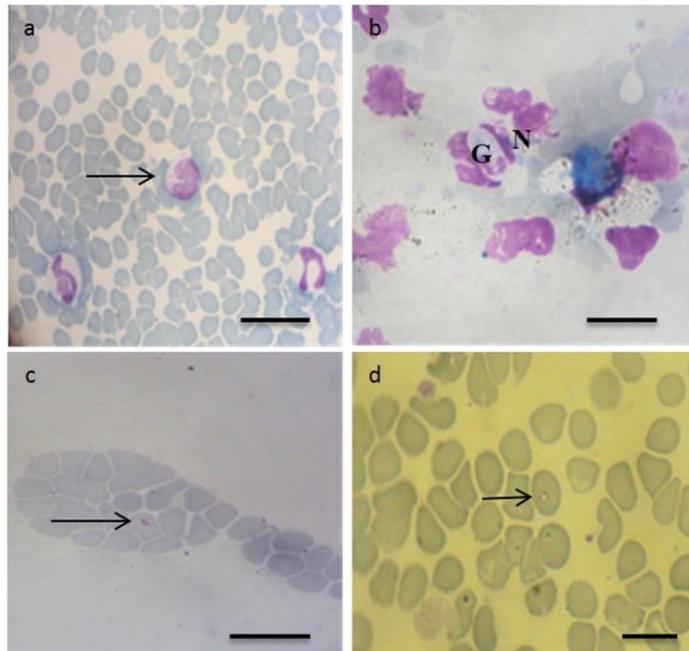
Figure 2: Haemoparasite species identified in the canine blood samples.

a: Morulae (arrow) of *E. canis* in the lymphocytes.
b: Gamont of *H. canis* in the neutrophil (G-Gamont and N-Nucleus of the neutrophil).
c: Pear shaped *B. canis* merozoite (arrow) in the red blood cell.
d: Signet ring shaped *B. gibsoni* merozoite (arrow) in the red blood cell
(Scale bar in each figure represents 20 µm).

The prevalence of each species was determined by examining all the positive samples. *H. canis* was present in 1.56% of samples as a single infection. Another 1.56% had mixed infections of *H. canis* with both *E. canis* and *B. gibsoni*. In 15.04% of the samples, *B. gibsoni* was detected as a single infection. *B. gibsoni* showed mixed infections in 5.05% of samples either with *E. canis*, *B. canis* or *H. canis*. In 1.25% of samples *B. canis* was observed as a single infection. Another 1.25% had mixed infections of *B. canis* and *E. canis*. In 20.05% of samples, *E. canis* was detected as a single infection. Furthermore, 4.38% had *E. canis* mixed infections with one or more of the other three species.

Table 1: Prevalence of single and mixed infections of canine haemoparasites in three Divisional Secretarial divisions in the Anuradhapura district

Type of infection	Rambewa n=124	Galenbindunuwewa n= 87	Tirappane n=108	Overall prevalence n=319
Single infections				
B. canis	2 (1.61%)	2 (2.29%)	0 (0.00%)	4 (1.25%)
B. gibsoni	13 (10.48%)	11 (12.64%)	24 (22.22%)	48 (15.04%)
E. canis	35 (28.22%)	16 (18.39%)	13 (12.03%)	64 (20.06%)
H. canis	0(0.00%)	2 (2.29%)	3 (2.77%)	5 (1.56%)
Mixed infections				
H. canis+ E. canis	1 (0.80%)	0(0.00%)	0 (0.00%)	1 (0.31%)
H. canis+ B. gibsoni	1 (0.80%)	2(2.24%)	0 (0.00%)	3 (0.94%)
H. canis+ E. canis + B. gibsoni	1 (0.80%)	0(0.00%)	0 (0.00%)	1 (0.31%)
B. canis+ B. gibsoni	1 (0.80%)	0(0.00%)	0 (0.00%)	1 (0.31%)
B. gibsoni+ E. canis	7 (5.64%)	1(1.14%)	1 (0.92%)	9 (2.82%)
B. canis+ E. canis	3 (2.41%)	0(0.00%)	0 (0.00%)	3 (0.94%)
Total prevalence	64 (51.61%)	34 (39.08%)	41 (38.88%)	139 (43.57%)

n: number of samples collected in each DS division

Discussion

In this study, the prevalence and distribution of canine vector-borne infections in three DS divisions in the Anuradhapura district were investigated. There were 43.88% dogs positive for haemoparasites indicating infections caused by this group of parasites are common among dogs in the localities examined. Single infections were found in 37.93% of dogs. This is higher than a previous report on dogs in the Western Province (29.15%).[11] Mixed infections were detected in 5.95% of dogs examined which agrees with the previous study done by Kumara et al. in 2013.[12] Mixed infections with only two pathogens were observed in 5.6% dogs. Similar results have been documented in the Western Province of Sri Lanka.[11]

This study reports a high prevalence of *E. canis* infections (20.06%) compared to the previously reported prevalence of 14% in dogs in the Western Province.[12] The prevalence of *B. canis* (1.25%) and *B. gibsoni* (15.04%) reported in this study were also higher than previously reported in the Western Province.[11] The prevalence of *H. canis* (1.56%) was lower than in the Western Province (8.57%).[12]

A study conducted in Nigeria has shown a high rate of mixed infection (37%) that was caused by the same haemoparasite species identified in this sutdy.[19] *E. canis* infection can predispose dogs to opportunistic pathogens, such as *B. canis* and *H. canis*.[5] Having multiple tick-transmitted infections in dogs is associated with severe and fatal conditions.[5] The Brown dog tick (*R. sanguineus*) is the main vector transmitting the pathogens found in mixed infections.[20] The prevalence of this tick species is common among domestic animals in the dry zone.[21] It can therefore be suggested that any control measures taken to eradicate this tick species can reduce more than one type of haemoparasitic infection found in dogs. All the haemoparasite infections identified are known to be asymptomatic.[22-25] However, in some dog breeds, *B. gibsoni* infection can be symptomatic.[26]

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Differences in the distribution of vector-borne diseases are largely determined by the geographical distribution and local density of their arthropod vectors. Furthermore, environmental changes have a direct impact on the geographical distribution of arthropods, size of their population and their ability to act as a vector to transmit diseases.[27,28] Future studies are required to examine the putative tick vectors and their population characteristics. Results obtained during this study would be useful for the accurate design of such investigations. We would suggest implementation of coordinated control and prevention programs of canine tick-borne diseases with the help of veterinary and public authorities. Prophylactic measures, including the use of acaricides and insecticides can be used to control the vectors.[29] These control measures are necessary to prevent any possible zoonotic transmission.

Limitations of the study
Blood samples were collected only at the prophylactic anti-rabies vaccination and sterilization centers. However, the expected numbers of dogs were not brought to these centers. As a result, the number of samples collected are less than the calculated sample size. Any background information about these dogs was not considered. Haemoparasite species were identified using morphological characteristics. Species identities should be further confirmed using molecular techniques.

Conclusions

This study was carried out on tick-borne canine haemoparasitic infections in three DS divisions in Anuradhapura district, Sri Lanka. Comparatively, the prevalence of ehrlichiosis and babesiosis was high in the three DS divisions. The prevalence of *H. canis* was low. Co-infections among dogs suggest that the same vector may be involved in transmitting different infections.

Conflict of interests: The authors declare that they have no conflicts of interests.

Ethical statement: This study was approved by the Institutional Animal Ethics Committee of the Postgraduate Institute of Science (PGIS), University of Peradeniya, Sri Lanka.

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