Original Research Paper

Understanding the Epidemiology of *Trypanosoma Evansi* Infection in Dogs (*Canis lupus familiaris*) from Urban Areas of Colombia

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Abstract: *Trypanosoma evansi* is a protozoan parasite that is the causative agent of the animal disease known as "surra", which affects a wide variety of wild and domestic mammals including humans. This study aimed to evaluate the molecular point prevalence and clinic-epidemiological traits of *T. evansi* infection in dogs from municipalities of the Metropolitan Area of Bucaramanga (MAB), Santander, Colombia. From 2019 and 2020, a cross-sectional study in four municipalities of MAB was carried out to evaluate the above features in 213 healthy dogs from these cities. Molecular analysis using the primer ESAG/6 and ESAG/7, showed a *T. evansi* frequency of 1.8% (CI 95% = 0.04-3.68%), while Woo test didn't detect positive animals. A high prevalence of infection was detected in Piedecuesta (10.53%) and Bucaramanga (1.65%), apparently negative animals were detected in Giron and Florida Blanca. Clinical characteristics revealed that all positive animals (4/4) showed low Mean Corpuscular Hemoglobin (MCH) and (3/4) low Mean Corpuscular Volume (MCV), and one animal had a moderate fever with prolonged capillary refill time. The epidemiological characteristics showed that all animals received mixed food, (3/4) came from low socio-economic areas, and (2/4) from neighborhoods without weekly garbage collection. In conclusion, molecular methods detected a considerable prevalence of animals chronically infected by *T. evansi* in MAB, with more frequency in neighborhoods of a low socio-economic level where animals have more risk of oral transmission. These results should be considered during urban control programs of surra in MAB.

Keywords: Protozoan, Epidemiology, Pets, Disease Reservoirs, South America

Introduction

Canine trypanosomiasis is a disease complex caused by two main *Trypanosoma* species: *Trypanosoma* (*Schizotrypanum*) *cruzi* producing the Chagas disease (American form) and *Trypanosoma* (*Trypanozoon*) *evansi* producing the surra (African form) (Desquesnes *et al*., 2013). In America, dogs are considered the main reservoir for the transmission of *T. cruzi* infection in domestic areas, with most animals presenting a subclinical infection, with few cases developing hematological alterations and significant cardiac abnormalities that rarely cause fatality (Crisante *et al*., 2006; Jaimes-Dueñez *et al*., 2020). In contrast, dogs naturally infected with *T. evansi* show acute and severe clinical signs (case-fatality rate

> 90%) (Echeverria *et al*., 2019; Filgueiras *et al*., 2019; Jaimes-Dueñez *et al*., 2017a), that discard the role of this host as an important reservoir of the parasite.

T. evansi infections in dogs exhibit acute manifestations such as apathy, weight loss, hyperthermia, corneal opacity, hepatosplenomegaly, anorexia, emesis, diarrhea, anemia, thrombocytopenia, liver damage, and death within two or three weeks, even after specific treatment with diminazene diaceturate (Echeverria *et al*., 2019; Filgueiras *et al*., 2019; Greif *et al*., 2018; Jaimes-Dueñez *et al*., 2017b). In South America, trypanosomiasis caused by *T. evansi* in dogs is often associated with rural areas where animals are exposed to vectors such as *Stomoxys calcitrans* and

Tabanidae, and reservoirs such as capybaras (*Hydrochoerus hydrochaeris*) and rodents. Nevertheless, oral transmission through the consumption of domestic animals such as cattle, horses, and pigs, has also been associated with the infection (Desquesnes *et al.*, 2013).

Although some studies have evaluated the dynamics of transmission of *T. evansi* in dogs from rural areas (Echeverria *et al.*, 2019; Franke *et al.*, 1994; Jaimes-Dueñez *et al.*, 2017a), little is known about it in urban ones where sporadic cases are reported (Coelho *et al.*, 2013; Filgueiras *et al.*, 2019). In the last years an increase in canine trypanosomiasis cases has been recorded by parasitology methods in veterinary diagnostic centers of the Metropolitan Area of Bucaramanga Santander (2018, 0.059% (1/1689) of the diagnosis; 2019, 0.10% (2/1845) of the diagnosis; 2020 0.12% (2/1564) of the diagnosis; 2021, 0.0.21% (4/1875) of the diagnosis, ignoring the presence of *T. evansi* in these cases (Biovet, 2021). Considering the little knowledge about *T. evansi* infection in dogs from urban areas of Colombia, this study evaluated the molecular prevalence, and clinical and epidemiological traits associated with natural infection of *T. evansi* in dogs from the Metropolitan Area of Bucaramanga (AMB) Santander, Colombia.

Materials and Methods

Animal Enrollment

Blood samples derived from 213 dogs originating from the AMB and collected under the frame of previous studies carried out in our research group were screened for the presence of *T. evansi* DNA (Jaimes-Dueñez *et al.*, 2020). The inclusion criteria for pets, epidemiological information, and collection and processing of samples were carried out following Jaimes-Dueñez *et al.*, (2020). AMB is a Colombian conurbation, located in the department of Santander, in the Río de Oro valley. Its main nucleus is Bucaramanga, and its satellite municipalities are Girón, Piedecuesta, and Floridablanca (DANE, 2018). Its human population is around 1,341,694 inhabitants, and the proportion of dogs corresponds to a dog by every 4.7 people (Florez and Solano, 2019). The study area consists of tropical dry forest covered by crops, and patches of native forest, with an average annual temperature of 23.4°C and a rainfall average of 1159 mm/year (IDEAM, 2021). In the area, high infestation rates by *Ctenocephalides canis*, *Rhipicephalus sanguineus*, *Stomoxys calcitrans*, *Culex* spp., *Aedes* spp., and other arthropods were recorded in pets throughout the year (Reyes, 1938).

Parasitological Diagnosis

A total of 213 EDTA blood samples were used for parasitological diagnosis of *Trypanosoma* spp. using the Woo test. The procedures were performed according to Jaimes-Dueñez *et al.*, (2020). The motility of trypanosomes was evaluated following the procedures described by Woo (1970).

DNA-Based Tests

T. evansi infection was evaluated using a convectional PCR based on the surface proteins (ESAG) of species using the primers ESAG/6ACATTCCAGCAGGAGTTGGAG, and ESAG/7CACGTGAATCCTCAATTTTGT (Holland *et al.*, 2001), which has an analytical sensitivity of 0.000001 ng *T. evansi* DNA, and a specificity of 100% for subgenus *Trypanozoon* (Fernández *et al.*, 2009). All PCR reactions were performed in a final volume of 25 μL containing 1 X reaction buffer (100 mm Tris-HCl, 50 mm KCl, pH 8.8), 1.5 mm of $MgCl_2$, 0.2 mm dNTP, 0.4 μm of each primer, 0.625 UI of Taq polymerase (Corpo Gen, Bogota, Colombia), and 5 μL (50 ng) of DNA sample. Thermal conditions were used as reported elsewhere (Jaimes-Dueñez *et al.*, 2017b). PCR products were separated on a 2% agarose gel for 45 min, stained with EZ-vision 10,000X (Amresco, Solon, United States), and visualized under UV light (Spectroline, NY, United States). Blood samples were considered positive for *T. evansi* when a PCR product of ~237 bp was observed in the gel (Holland *et al.*, 2001). PCR controls were obtained from canine blood samples previously diagnosed as positive and negative for *T. evansi* by (Jaimes-Dueñez *et al.*, 2017a). Finally, PCR inhibitors were evaluated as previously reported (Jaimes-Dueñez *et al.*, 2020). Samples in which this housekeeping gene could not be amplified were excluded from the analysis.

Clinical Information

Before blood sampling, animals were evaluated in a clinical examination, which considered body condition (scale 1 to 5), respiratory rates, heart rate, body temperature, and capillary refill time. Some hematological parameters were measured like Packed Cell Volume (PCV), total Red Blood Cells (RBCs), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), MCH Concentration (MCHC), Platelet Counts (PLT), White Blood Cells (WBCs), and leukocyte differential counts using an ABC VET® animal blood counter (Scil Animal Care Company, Gurnee, USA). Finally, Creatine Kinase Myocardial Band (CK-MB) and Aspartate Aminotransferase (ASAT) were estimated using the commercial kits (Spinreact, Girona, Spain), in a spectrophotometer GENESYS 10S (Thermo Scientific, Madrid, Spain).

Statistical Analysis

Molecular prevalence and 95% IC were calculated using SPSS v.18.0 statistical software (PASW Statistics for Windows, Version 18.0. Chicago: SPSS Inc; 2009). To identify, epidemiological variables associated with the *T. evansi* infection, a total of seven independent

variables (age, sex, breed, city, socioeconomic level, frequency of garbage collection, and access to rural areas) were analyzed with the result of the PCR (positive or negative) using binomial logistic regression. The level of statistical significance was set at P<0.05. Finally, quantitative parameters in the positive samples were shown as the median and Interquartile Range (IQR), whereas qualitative variables were described through frequency analysis.

Ethics Statement

All of the experimental procedures involving animals were conducted in accordance with the good animal practice as defined by the Colombian code of practice for the care and use of animals for scientific purposes, established by the law 84 of 1989. Informed consent about the risks and benefits of the study was completed by the owners. Ethical approval for analyzing animal specimens was obtained from the Animal Ethics Committee of the Cooperative University, Bucaramanga, Colombia (Act No. 043 of 2018).

Results

A total of 213 dogs were evaluated, of which 66.6% were females, 70.6% were adults, and 26.5% were puppies. According to the origin, 55.8, 20.1, 15, 9.1%, came from Bucaramanga, Floridablanca, Giron and Piedecuesta, respectively. Regarding the breed, 57.2% were Creole breed, and 12.5% were Labrador Retriever.

Animals were clinically healthy during the clinical examination. No positive animals to *Trypanosoma* spp. were detected in the Woo test.

Molecular Detection of T. Evansi Infection

Molecular analysis showed a total of 4 positive dogs to *T. evansi* (1.9, 95% CI = 0.04-3.68%). High frequencies were detected in Piedecuesta (10.5, 95% CI = -4.6-25.7%) and Bucaramanga (1.6, 95% CI = -0.65-3.96%), negative animals were observed in Giron and Floridablanca (Fig. 1).

Clinical and Epidemiological Variables Associated with T. Evansi Infection

The clinical analyses revealed that all parameters were within normal values in all animals except for the heart rate, Hb, HCT, MCV, MCH, WBCs, neutrophils, lymphocytes, and eosinophils (Table 1). All animals showed low levels of MCH and MCV. No animals showed the appearance of hemotropic agents in the blood smear (Table 1).

Binomial logistic regression did not show epidemiological variables significantly associated with the infection; however, the frequentist distribution of the cases revealed that of the four positive dogs to *T. evansi*, all corresponded to adults, three (3/4) to males, and two (2/4) to creole breed. Likewise, half (2/4) was from Bucaramanga and the other from Piedecuesta. Three animals (3/4) lived in areas of low socioeconomic status and two (2/4) in areas without weekly garbage collection. All animals received mixed feed and a half (2/4) have access to the rural area.

Table 1: Results of the clinical, hematological, and epidemiological analysis of the positive dogs to *T. evansi* in AMB.

Parameter	Reference	Case 1	Case 2	Case 3	Case 4	Median (IQR)
Clinical						
Body condition, 1 to 5	-	3	4	3	3	3 (0.7)
Temperature, °C	38–39.2	38	39.2	39.1	38.7	38.9 (1.0)
Capillary refill time, seconds	1–2	2	3	2	2	2 (0.7)
Heart rate, beats/min	60–180	98	74	64	56↓	69 (34)
Respiratory rate, breaths/min	10–30	24	30	30	30	30 (5)
Hematological						
Red blood cells, 1×10^6/ µL	4.8–9.3	6.75	7.50	9.15	8.90	8.2 (2.1)
Hemoglobin, g/dL	12.1–20.3	11↓	13	15	13	13 (3)
Hematocrit, %	36–60	33↓	39	45	40	39.5 (9.2)
Mean corpuscular volume, fL	58–79	49↓	52↓	49↓	45↓	49 (5.2)
Mean corpuscular hemoglobin, p.g.,	19–26	16.3↓	17.3↓	16.3↓	14.8↓	16.3 (1.9)
Platelets, 1×10^3/µL	170–400	320	256	254	233	255 (66)
White blood cells, 1×10^3/µL	4,000–15,500	13950	22450↑	11700	15500	14725 (8450)
Neutrophils, 1×10^3/µL	2,060–10,600	10044	11674↑	7722	7440	8883 (3756)
Lymphocytes, 1×10^3/µL	690–4,500	3208	8755↑	2457	5270↑	4239 (5239)
Monocytes, 1×10^3/µL	0–840	418	673	468	310	443 (284)
Eosinophils, 1×10^3/µL	0–1,200	279	1347↑	1053	2480↑	1200 (1725)
AST, U/L	15–66	21.3	27.9	41.3	22.6	25.2 (16.3)
CK-MB U/L	26.2–75.0	14.7	21.6	45.2	28.4	25 (24.6)
Hemotropic agents in the blood smear	Negative	Negative	Negative	Negative	Negative	-
Epidemiological						
Sex	-	Female	Male	Male	Male	-
Age	-	Adult	Adult	Adult	Adult	-
Breed	-	Creole	Creole	German shepherd	Cocker springer	-
Municipality	-	Bucaramanga	Piedecuesta	Piedecuesta	Bucaramanga	-
Socioeconomic level	-	1	1	1	3	-
Weekly garbage collection	-	Yes	No	No	Yes	-
Number of pets	-	2	4	3	2	-
Access to the rural area	-	no	yes	Yes	No	-
Feed type	-	Concentrate and food Waste	Concentrate and food Waste	Concentrate and food Waste	Concentrate and food Waste	-

Abbreviations: AST, Aspartate Aminotransferase; CK-MB, Creatine Kinase Myocardial Band. Values outside of reference value are shown in bold
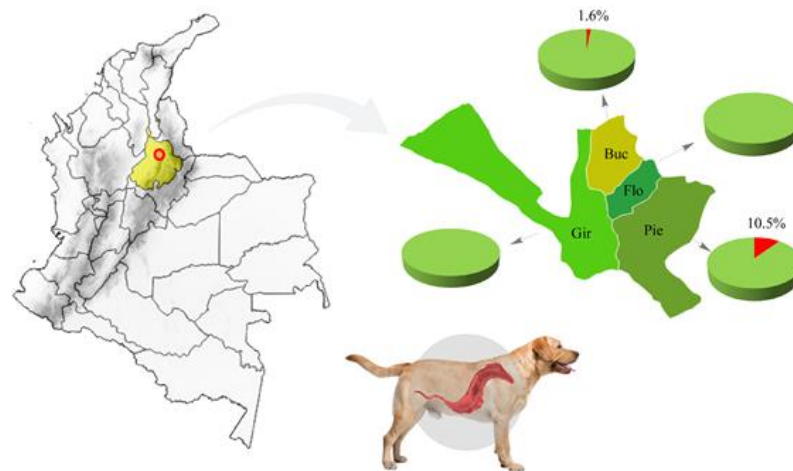
Fig. 1: Molecular prevalence of *Trypanosoma* evansi in dogs from the metropolitan area of bucaramanga, santander, Colombia. Buc, Flo, Gir and Pie, represent Bucaramanga, floridablanca, giron, and piedecuesta, respectively

Discussion

In Colombia, the prevalence of *T. evansi* infection has been reported in wild and domestic animals (Jaimes-Dueñez *et al.*, 2017a; Jaimes-Dueñez *et al.*, 2018; Jaimes-Dueñez *et al.*, 2019; Morales *et al.*, 1976); however, the epidemiology of this parasite in Colombian dogs is little known, with few case reports suggesting a high susceptibility of the dogs to development fatal cases (Correa-Salgado *et al.*, 2010; Jaimes-Dueñez *et al.*, 2017b). Herein, we addressed a *T. evansi* epidemiological surveillance in dogs from MAB, showing a considerable prevalence of infection and animals that can keep chronic infections without complicated clinical signs (Fig. 1). These results show the ability of the parasite to maintain a life cycle in urban areas and suggest a zoonotic risk for the human population of the AMB.

Molecular prevalence observed here is higher than that recently reported in dogs from the Brazilian Amazon (1.2%) (Filgueiras *et al.*, 2019), India, and Southeast Asia (1.5, 95% CI: 0.8-2.7%), using molecular methods (Nguyen *et al.*, 2021). The high prevalence of *T. evansi* infection in dogs from MAB could be associated with the high density of dogs in AMB (Florez and Solano, 2019) as well as the high infestation rates by hematophagous ectoparasites as *C. canis*, *R. sanguineus*, and *S. calcitrans*, which have been increased in recent years, probably associated to global warming, landscape modification, and resistance to acaricides and insecticides (Alkishe *et al.*, 2021; Barros *et al.*, 2019; Dantas-Torres, 2010). Nevertheless, *T. evansi* infection through oral mucosa penetration of the parasite during the ingestion of contaminated food or consumption of infected rodents also be considered (Raina *et al.*, 1985; van Bree *et al.*, 2018). Although the statistical analysis did not show

epidemiological variables associated with the infection, probably due to the few numbers of positive cases in the population, the infected dogs observed here received constant food waste, with most living in low-socioeconomic areas with a lack of weekly garbage collection. As shown in previous studies, these conditions favor the abundance of rodents and other synanthropic animals that are important reservoirs of *T. evansi* infection (Aregawi *et al.*, 2019; Desquesnes *et al.*, 2013; Echeverria *et al.*, 2019), and that could transmit the infection to dogs through food contamination or direct consumption from these reservoirs. Further studies evaluating synanthropic reservoirs of *T. evansi* in the AMB are necessary to describe a complete trasmisión cycle in these urban zones.

In Colombia, *T. evansi* infection in dogs has been reported in three animals with fatal outcomes after experiencing clinical signs such as cachexia, anemia, thrombocytopenia, and hepatosplenomegaly (Correa-Salgado *et al.*, 2010; Jaimes-Dueñez *et al.*, 2017a). Interestingly, epidemiological surveillance performed here showed that positive animals did no evident clinical signs, however, hematological alterations were observed in this group. In this case, infected dogs showed a significant reduction of the MCH with a low MCV that suggests hypochromic microcytic anemia, the same as those observed during *T. evansi* infection in dogs from Brazil (Silva *et al.*, 1995). Anemia is one of the most consistent findings in trypanosomiasis. Three phases of anemia have been reported in this pathology, phase I (acute crises), phase II (chronic), and phase III (recovery) (Mbaya *et al.*, 2012). During the first one (acute crises), the parasitemia is usually high, fluctuating, and evident on most days. In this phase, the anemia is morphologically classified as macrocytic and normochromic, and the death commonly occurs due to

severe pancytopenia and other pathologies. The second is characterized by low levels of parasitemia with fluctuant erythrocyte values, in this point, the anemia could vary from normochromic and normocytic to hypochromic and microcytic. In the last phase, hematological parameters return to pre-infection values (Anosa, 1988). These postulates, plus the lack of positive animals in the Woo test, suggest that the positive dogs observed here correspond to chronic infections in which the parasitic load can only be detected by molecular methods. These findings show that although the mortality rate of *T. evansi* in dogs is high (>90%) (Echeverria *et al*., 2019; Eloy and Lucheis, 2009), someone's could be chronically infected and maintain low parasitic loads, which suggests they are not an effective source of infection to spread the disease. Further studies involving parasitological and immunological analyses are necessary to confirm these hypotheses.

Conclusion

An epidemiological survey of *T. evansi* in dogs from urban areas of Colombia allowed detected a considerable prevalence of animals chronically infected, with more frequency in the neighborhoods of a low socioeconomic level where animals have more risk of oral transmission; in addition, chronic infections were associated with signs of anemia, which considerably impairs the health and quality of life of these pets. This study provided relevant information about clinical and socioeconomic features associated with *T. evansi* infection in dogs from AMB, which must be taken into consideration when Surra control programs are implemented in these municipalities.

Declaration of Interest

All the authors have participated in the study and no conflicts of interest have been disclosed.

Acknowledgment

Author's Contributions

Jeiczon Elim Jaimes-Dueñez: Designed the study, conducted the analysis, and drafted the manuscript.

Ángela Patricia Jiménez-Leaño: Designed the study and conducted the analysis.

Daniela Montenegro-Ayala: Made experiments and provided critical revision of the manuscript.

Maria Esteban-Mendoza: Designed the study, made experiments, and provided critical revision of the manuscript.

All authors contributed to and commented on subsequent revisions of the manuscript. All authors approved the final manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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