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Trypanosoma cruzi co-infections with other vector borne diseases are frequent in dogs from the pacific coast of Ecuador

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ABSTRACT

Dogs are a reservoir for Chagas disease, caused by *Trypanosoma cruzi* (*T. cruzi*), and other companion vector-borne diseases, including ehrlichiosis (*Ehrlichia canis* and *Ehrlichia ewingii*), anaplasmosis (*Anaplasma phagocytophilum* and *Anaplasma platys*), dirofilariasis (*Dirofilaria immitis*) and Lyme disease (*Borrelia burgdorferi*). This study has two key objectives: 1) to determine seroreactivity against *T. cruzi* in dogs from the town of Colón, in Portoviejo city, in the central coast of Ecuador; and 2) to establish the coinfection frequency of other companion vector-borne diseases in dogs positive for *T. cruzi*. Antibodies against *T. cruzi* were detected using two enzyme-linked immunosorbent assays. Diagnostic consensus between ELISA tests was established using the Cohen's Kappa coefficient. Other haemoparasitic diseases were detected using the IDEXX SNAP® 4Dx® kit in dogs previously diagnosed as *T. cruzi*-seropositive. From 84 dogs sampled, 57.14% (48/84) tested positive for *T. cruzi*.

Co-infection analysis of 25 dogs positive for *T. cruzi* revealed antibodies also against *Ehrlichia* spp. (48%), *Anaplasma* spp. (28%), and *Dirofilaria immitis* (12%). These results provide a novel perspective regarding the status of these pathogens which co-infect dogs in Colón. Since all these pathogens are zoonotic, our findings should warn regional health authorities to implement sanitary programs, to better prevent and control vectors associated to these pathogens. On the other hand, human and veterinarian doctors, should consider that patients with a cardiac infection condition could be suffering co-infections with two or more vector transmitted pathogens.

1. Introduction

Companion vector-borne diseases (CVBD) encompass a group of afflictions transmitted by a variety of arthropods, including ticks, fleas, triatomines, mosquitos, and sand flies [1]. These diseases present a major problem of different zoonoses, especially in regions where the climate meets the ideal conditions for vector reproduction and transmission of etiological agents [2]. Domestic dogs are hosts of these diseases, and frequently may present clinical signs with non-specific hematological or biochemical alterations [3]. Therefore, these diseases are not easily recognized by veterinarians, and consequently are frequently fatal. These pathogens are also a source of risk of disease for

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humans and other susceptible animal species [1].

T. cruzi is the causative agent of Chagas disease. It naturally is able to infect many mammalian species, including humans and domestic dogs. In both cases, humans and dogs, characteristic clinical symptoms of acute Chagas disease include lymphadenopathy, splenomegaly and myocarditis, while chronic Chagas disease presents cardiomegaly with inflammation and fibrosis. Cardiac damage in the chronic form of *T. cruzi* infection results from a strong Th1 immune response promoted since the acute phase of infection [4]. While serological tests and xenodiagnostic methods are often used to diagnose Chagas disease, Polymerase Chain Reaction (PCR) can also be used for experimental diagnosis.

Triatomines are responsible for vectorial transmission of *T. cruzi* in most sylvatic, peridomiciliary and domestic cycles of the disease [5]. In the sylvatic cycle, many mammalian species act as reservoirs [6]. However, in the domestic cycle, domestic dogs are the primary reservoir [7–9]. Although the presence of the triatomines *Rhodnius ecuadoriensis* (*R. ecuadoriensis*), *Triatoma dimidiata* (*T. dimidiata*), *Panstrongylus howardi* (*P. howardi*), *Panstrongylus chinai* (*P. chinai*), *Panstrongylus rufotuberculatus* (*P. rufotuberculatus*) has been previously studied in Ecuador [10–12], and Chagas disease has also been reported for humans in the region of Portoviejo city in Ecuador [13,14], no information is available for the prevalence of this disease in dogs. This animal is considered a sentinel species par excellence [9,15]. There are several factors that may affect *T. cruzi* transmission in domestic dogs and in humans in endemic areas. These factors mainly include the type of housing materials (e.g., hatched or tiled roofing, dirt floor, wooden or adobe walls) that can provide shelter to triatomines. Peridomicile conditions such as the presence or absence of palm trees, bushes, rock fence walls, animal housings, etc., may also contribute to spread of triatomines at a close distance to the house. These housing conditions are frequently present in the suburbs and rural areas under study. Further, humans and dogs living in the rural areas frequently invade nearby woods or jungle, which are endemic for triatomines, increasing the probability of these insects to switch their food source from wild to domestic animals [6,16].

Environmental conditions of the area of study also contribute to the presence of *Rhipicephalus sanguineus* (*R. sanguineus*), which is a tick that infests dogs and can transmit pathogens such as *Ehrlichia* spp. [17,18], *Anaplasma* spp. [19], and *Borrelia burgdorferi* (*B. burgdorferi*) [20], the causative agents of ehrlichiosis, anaplasmosis, and Lyme disease, respectively. These agents can affect domestic and wild animals and are potentially zoonotic [21,22]. Similarly, mosquito species such as *Culex* spp., *Aedes* spp., and *Anopheles* spp., can transmit *Dirofilaria immitis* (*D. immitis*), the causative agent of dog heartworm in the Americas, which primarily affects dogs and cats [23], and is also considered a zoonotic disease [24].

The occurrence of CVBD in dogs has been reported in different regions of Latin America [22]. However, as far as we are aware of, there is only one scientific report [25] on CVBD prevalence in the area of study (Colón, Portoviejo, Ecuador). In that report, a prevalence of 56.75% of *Ehrlichia canis* (*E. canis*) and 0.67% *D. immitis* was described. Since this study was conducted more than a decade ago and did not include all of the main CVBD diseases, it is important to update this information and to widen the spectrum of CVBD pathogens under study. It should be noted that the transmission of these diseases in Portoviejo is also related to human behavior, regarding the culture of pet ownership, since dogs are frequently allowed to freely roam streets, agricultural and wild areas, where they are exposed to a plethora of arthropods responsible for transmission of CVBD. Additional factors contributing to CVBD transmission risks by dogs include poor sanitary and medical management of dog populations in agricultural sectors.

The results provided in this study could serve as a reference in the diagnosis, treatment, control, and prevention of the aforementioned infections in dogs, for veterinary doctors who are working on small animal clinics. It also provides relevant information for health authorities to carry out preventive measures for these potentially zoonotic diseases. The main objective of the present study was to determine *T. cruzi* seroprevalence in dogs living in the rural community of Colón, and also to determine seroreactive frequency of CVBD, including *E. canis, Ehrlichia ewingii* (*E. ewingii*), *Anaplasma phagocytophilum* (*A. phagocytophilum*), *Anaplasma platys* (*A. platys*), *D. immitis,* and *B. burgdorferi* in *T. cruzi* seroreactive dogs.

2. Materials and methods

2.1. Study area

The present study was conducted in the rural community of Colón in Portoviejo city, which is located in the center of the province of Manabí, on the pacific coast of Ecuador (Fig. 1). Colón is home to 16,206 inhabitants and the primary economic activity is agriculture (corn and cacao crops). The climate is warm and varies between tropical, semi-humid and humid. The average temperature is 24 °C, average relative humidity is 76.2%, and annual precipitation varies between 500 and 823 mm [26]. No census of the canine population in Colón exists, but in Portoviejo there are approximately 41,637 dogs (personal communication: Martha Vanessa Pita Macías, Directora Distrital de Salud 13D01 – Portoviejo, Ministerio de Salud Pública del Ecuador). The province of Manabí is an endemic zone for Chagas disease in humans [27], and the Ecuadorian Ministry of Public Health continually conducts prevention campaigns, with periodic fumigation for vector control [13]. This study was conducted in the above-mentioned region to gain information about the prevalence of *T. cruzi* infection in dog populations. The data gathered here might be useful as indicator to implement triatomines control/eradication programs in order to reduce the incidence of human Chagas disease in the region.

2.2. Study population

Data collection was conducted in July 2018. Since data were unavailable for the canine population in Colón, the human population was used for calculations. In 2017, the Ecuadorian National Secretary of Planning and Development reported the human population in Colón as 16,206 [28]. Considering a dog: human ratio of 1:6 [29], the estimated canine population would be approximately 2701. Using Epi Info v.7.2 statistical software, a minimum sample group of 66 dogs was calculated, with 50% expected *T. cruzi* seroreactivity frequency and a 10% margin of error. Initially, in this study, a total of 84 samples were collected to detect seroreactivity to *T. cruzi*. Dogs were chosen opportunistically (convenience sampling) since in the area of study there are not enough private veterinarian services and dogs normally do not receive professional medical assistance, there are not registers about animals health. Therefore, the sample collection was conducted with the assistance of residents who informed which houses to visit. Once informed consent was obtained from the dogs' owners, sample was taken from all animals regardless of clinical condition, sex, age, or breed. The only inclusion criterion was that the dogs had owners to provide informed consent for blood sampling.

Analyzing samples for several vector borne diseases was not considered in first place in this study, however in our first visit we suspected the presence of some other vector borne diseases in the dog population studied. We concluded the study for Chagas but we wanted to know if dogs were concomitantly infected with other vector borne pathogens. Therefore, three months later, we returned to Colón to collect serum samples from dogs that previously had resulted seroreactive to *T. cruzi*. From the original 48 *T. cruzi* seroreactive dogs, only 25 were located in our second visit to the community. This low number of animals found could be explained because, on one hand, some animals had already died and, on the other, because in these suburbs dogs are allowed to roam the streets, agricultural or sylvatic areas, and they are not easy to find at any specific time point. Consequently, we were

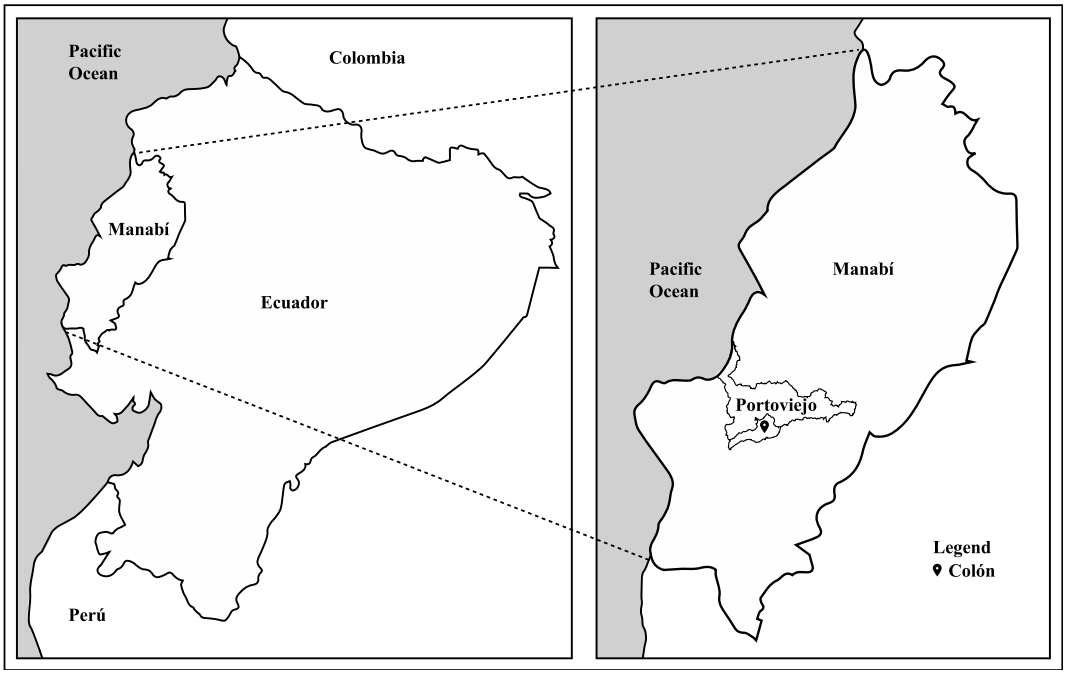

Fig. 1. Study area: rural community of Colón and Portoviejo city in Manabí Province, Ecuador.

able to collect blood only from the 25 dogs that were available at the time of visit. Samples were taken to the lab to determine presence of antibodies against *E. canis*, *E. ewingii*, *A. phagocytophilum*, *A. platys*, *B. burgdorferi* and antigens against *D. immitis*.

2.3. Blood samples collection

Animals were restrained with the help of the owner and an expert veterinarian. Blood was collected through jugular vein puncture using a vacutainer system. The sampling area was shaved and disinfected beforehand. Four mL of blood was collected in 5 mL vacutainer tubes without anticoagulant. Serum was obtained through centrifugation (1500×*g* for 10 min) and frozen at −20 °C until processing.

2.4. Anti-T. cruzi antibody detection

Two enzyme-linked immunosorbent assay (ELISA) kits 'Chagas recombinant microELISA' (Recombinant protein) and 'Chagas microELISA test system' (Trypomastigote lysates) were used to detect anti-*T. cruzi* antibodies. ELISA was performed following the instructions provided by the manufacturer (Accutrack, Laboratorios Lemos S.R.L., Buenos Aires, Argentina), with modifications according to Aparicio-Burgos [30]. Briefly, samples were diluted to 1/100 in PBS solution containing 0.05% Tween-20 and 3% nonfat milk (dilution buffer). Each diluted sample (200 μL) was transferred in duplicate into 96-well ELISA plates. Samples were incubated at 37 °C for 30 min and then at room temperature for 20 min. The wells were then washed six times with 300 μL of washing buffer provided by the manufacturer. Then 100 μL of horse-radish peroxidase-conjugated sheep anti-dog (IgG) secondary antibody (Bethyl Laboratories Inc., TX, USA) diluted to 1:5000 in dilution buffer was added. The plates were then incubated at 37 °C for 30 min and then at room temperature for 30 min. Reactions were developed by adding 100 μL/well of TMB (Kirkegaard & Perry Labs) and hydrogen peroxide solution premixed at a 1:1 ratio, following the manufacturer's instructions. Samples were then incubated in dark for 20 min at room temperature. The reaction was stopped with 100 μL sulfuric acid (1 M) and change in color was recorded 450 nm using an Epoch microplate reader equipped with Gene 5 v.2.0 software (Biotek, VT, USA). The cutoff value was set as the average reading of negative samples plus 0.1 optical density. The same methodology was used for both ELISA tests [30].

2.5. Seroreactivity against CVBD agents in dogs positive for T. cruzi

Sera from the 25 dogs seroreactive for *T. cruzi* were analyzed using a commercial kit (IDEXX SNAP® 4Dx® Plus) for the detection of antibodies against *E. canis*, *E. ewingii*, *A. phagocytophilum*, *A. platys*, and *B. burgdorferi*, as well as *D. immitis* antigens. Samples were processed according to manufacturer's protocols [31].

2.6. Statistical analysis

Diagnostic consensus (accuracy and inter-reliability) between the two ELISA tests used to detect anti-*T. cruzi* antibodies was established by Cohen's Kappa coefficient, using the statistical language R Core Team (2013).

3. Results

3.1. Seroreactivity against T. cruzi

Comparison of the results of the ELISA tests (recombinant protein or parasite lysates) resulted in the following findings: Reactive agreement was 57.1% (48/84), non-reactive agreement was 34.52% (29/84) and tests agreement was 0.917. Of the 84 samples tested, 48 samples were positive by both tests, 4 were identified as seropositive by parasite lysate based ELISA only, and 3 were identified as seropositive by recombinant

Table 1
Seroreactivity and diagnostic agreement between the ELISA tests, based on recombinant protein or parasite lysates, used to detect antibodies against *T. cruzi*.

		Recombinant proteins based ELISA		
	Sero-reactivity	Positive	Negative	Total
Parasite lysate-based ELISA	Positive	48	4	52
	Negative	3	29	32
	Total	51	33	84

Observed agreement = 0.917; Agreement of chance = 0.526; Cohen's kappa value = 0.824.

Protein based ELISA only. Calculated Cohen's kappa statistic value (0.824) indicates strong agreement between tests (Table 1).

3.2. Seroreactivity against CVBD agents in dogs seroreactive for T. cruzi

Out of the 48 dogs initially found seroreactive to *T. cruzi*, 23 were lost for the follow-up study conducted three months later for reasons such as owners not allowing a new sampling of animals, or because animals were not at home due to death or other reasons. Therefore, sera from only 25 dogs seroreactive for *T. cruzi* were further diagnosed for other CVBD. Sixty four percent of dogs (16/25) resulted seropositive for one or more infectious agents detected by the IDEXX SNAP ® 4Dx® Plus Kit. The frequency of infections detected in chagasic animals was as follows: 48% (12/25) *E. canis/E. ewingii*, 28% (7/25) *A. phagocytophilum/A. platys*, 12% (3/25) *D. immitis,* and 0% (0/25) *B. burgdorferi* (Fig. 2).

Columns show frequency of infections of *Ehrlichia* spp*, Anaplasma* spp*, Dirofilaria immitis* and *Borrelia burgdorferi* in 25 *Trypanosoma cruzi* infected dogs.

However, animals could have co-infections with *T. cruzi* either with one, two or three CVBD additional pathogens. When classifying animals according to the number of pathogens that they were diagnosed with, we observed that 44% (11/25) had at least one additional pathogen: from which 28% (7/25) were co-infected with *Ehrlichia* spp., 8% (2/25) with *Anaplasma* spp. and 8% (2/25) with *D. immitis*. Triple infection was found in 16% (4/25) of the animals, all of them infected with *Ehrlichia* spp. and *Anaplasma* spp., in addition to *T. cruzi*. Only one animal (4%; 1/25) was infected with four pathogens, i.e., *T. cruzi, Ehrlichia* spp., *Anaplasma* spp. and *D. immitis* (Fig. 3).

Infection with each one of the pathogens is represented with a different colored circle, overlapping between and among circles indicates co-infection with 2 or more pathogens.

4. Discussion

To detect anti-*T. cruzi* antibodies, two diagnostic tests were used, as is recommended by the World Health Organization [32]. In this study, we found much higher prevalence of *T. cruzi* sero-reactivity in dogs, which was 57.14%, in Colón, Portoviejo city in Manabí Province of Ecuador. Such high rate of prevalence of *T. cruzi*-specific antibodies in dogs was reported in Argentina in 1985 and 1989 [33,34], before the South Cone Initiative to control/eradicate Chagas disease (INCOSUR-Chagas) was implemented in 1991 [35]. The prevalence of *T. cruzi* reactive antibodies in dogs is reported at 10%–30% in the Americas, including Mexico, Argentina, Chile, the Amazon basin, USA and Colombia, [9,15,36,37]. The prevalence of *T. cruzi* sero-reactivity in the province of Guayas, Ecuador was reported at 11.3% in the year 2012 [38]. The regional triatomine vectors (*R. ecuadoriensis, P. howardi, P. rufotuberculatus*) in Ecuador are found in abundance and up to 42% of these are reported to be infected with *T. cruzi* [11,39,40]. The observed high levels of seroprevalence for *T. cruzi* specific antibodies in dogs in this study could be an outcome of increased activity of the regional triatomines in the sylvatic environment. This is an important observation as dogs serve as an important reservoir of T. cruzi in the domestic cycle carrying a risk for human population [41] and provide a sentinel species for evaluating the epidemiologic disease control programs [9,42,43]. The presence of dogs in the domicile and peridomiciliary areas, increases the risk of infection for humans, both as a primary reservoir and food source for triatomines and as the most common house pet, living in close contact with people [43]. Our findings indicate the need for improve of vector control programs and consistent monitoring of seropositivity in dogs to assess the risk of parasite transmission to humans.

Regarding other CVBD, various studies have reported the status of pathologies associated with ehrlichiosis, anaplasmosis, dirofilariasis and Lyme disease in the Latin and North America [22,36,44]. In Ecuador, in recent years, prevalence of *E. canis* and/or *E. ewingii* in dogs ranged from 37.84% to 56.75% in Santa Cruz island and coastal Manta, Guayaquil, and Portoviejo cities [25,45,46]. The prevalence of *A. platys/A. phagocytophylym* has also been observed in the range of 12.1%–22% in Santa Cruz and Guayaquil regions of Ecuador [45–47]. A 26% co-infection prevalence was reported for *E. canis* and *A. phagocytophylum* in Manta and Guayaquil cities [46], while a very low co-infection prevalence of 1% was reported in Isabela Island, Ecuador [48]. None of the above studies have assessed *T. cruzi* co-infection with *Ehrlichia* spp. and/or *Anaplasma* spp. infection rate in general dog population. We found a prevalence of 48% for *E. canis/E. ewingii* and a 28% prevalence for *A. platys/A. phagocytophilum* in *T. cruzi* sero-reactive dogs in Colón and Portoviejo cities in Manabí Province of Ecuador. Our finding about *Ehrlichia* spp. and *Anaplasma* spp. prevalence were as high as those reported by others in the cities of Manta, Guayaquil [46], and Santa Cruz

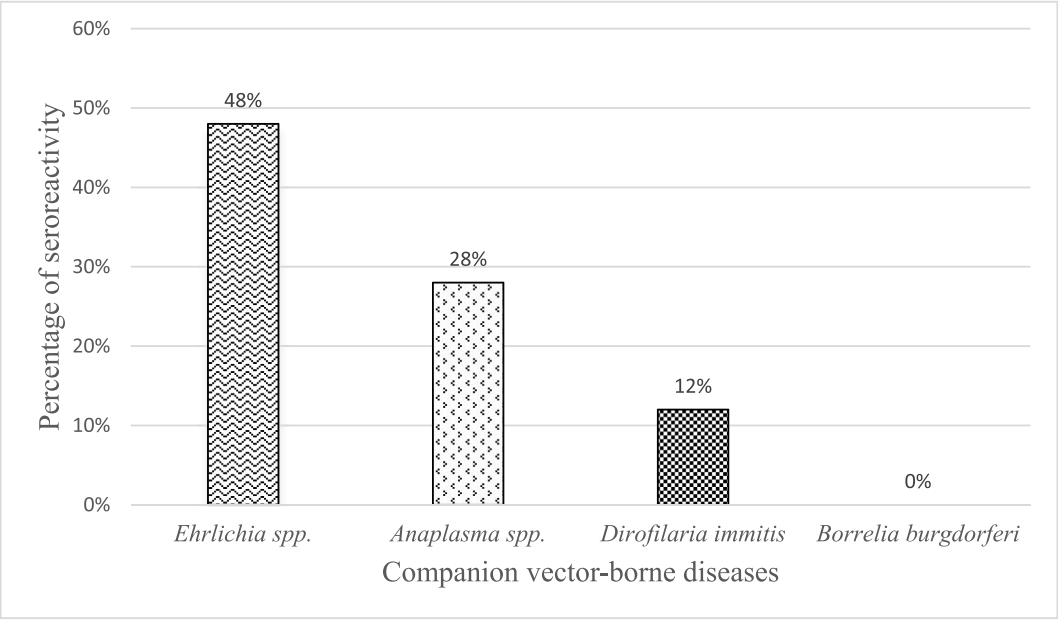

Fig. 2. CVBD found within a *T. cruzi* seroreactive dog population.

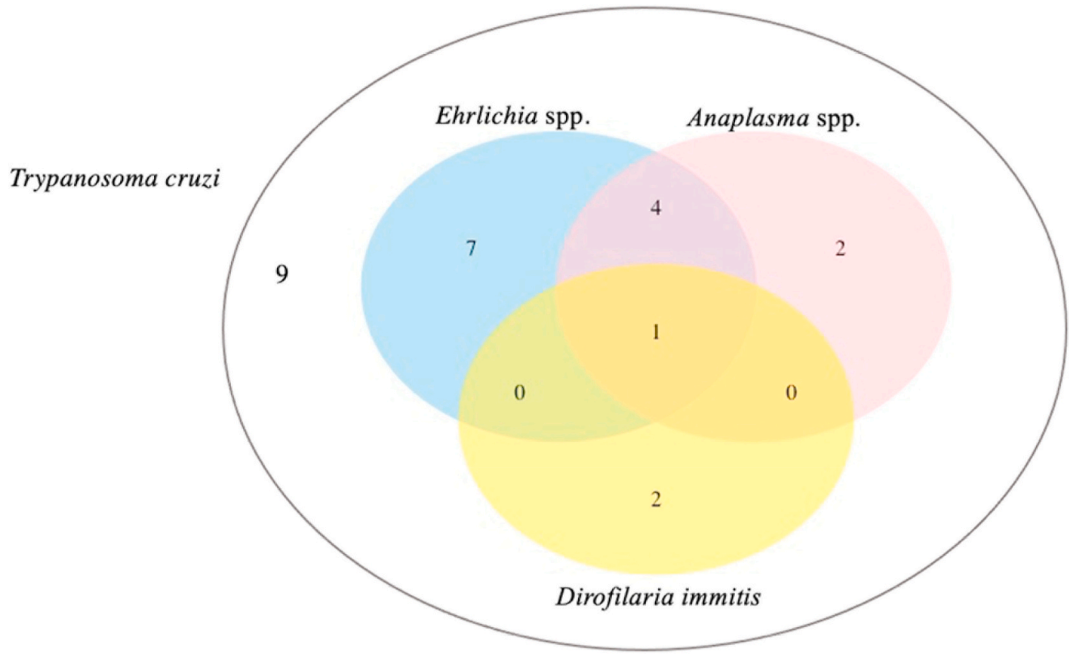

Fig. 3. Venn diagram shows companion vector-borne disease agents seroreactivity in dogs previously diagnosed as seropositive for *T. cruzi*.

Island [47]. Prevalence differences of continental Ecuador with Santa Cruz [45] and Isabela islands [48], both part of the Galápagos Archipelago, could be due to the isolation of the islands from the continent, tick geographical distribution [49] and the strict migration control of domestic animals by Ecuadorian health authorities.

The high rates of seroreactivity to *E. canis/E. ewingii*, and A. p*hagocytophilum/A. platys,* is most likely related to the presence of ticks that infest dogs and the lack of preventive measures by owners to control and reduce such infestation [22,50].

Canine dirofilariasis (*D. immitis*) has been reported in many regions of the world with prevalence varying from 0% to more than 30%. For example, *D. immitis* infection prevalence in dogs was reported at 16% in several regions of Texas, USA [36]; 13.18% in Henan province of central China [51]; 0.2% in Canada [52]; 13% in Iranshahr city in southeast of Iran [53]; and 36.3% and 0% in two regions of Brazil (São Joaquim de Bicas, Pernambuco and Goiana, Minas Gerais, respectively) [54]. Prevalence variability of canine dirofilariasis has also been observed in different areas of Ecuador. For example, in the Galápagos Islands, *D. immitis* prevalence in dogs was reported at 0%–6.9% in Santa Cruz island [45,47] and at 34% in Isabela island [48]. Similarly, we report a 12% prevalence of *D. immitis* in dogs from Colón, a rural town, while Morán [25] reported 0.67% prevalence in Portoviejo located very close to Colón. The main difference between these two communities in epidemiological terms could be the level of urbanization, which may have an impact on the exposure of dogs to mosquito populations and probably also due to prophylactic medicine that is provided to pets in urban areas that are not available in rural communities.

Prevalence fluctuations in canine dirofilariasis are influenced by mosquitoes (*Culex* spp. and *Aedes* spp., among others) populations, which are determined by geo-ecological factors such as humidity, temperature, altitude and type and amount of existing vegetation [55]. Additionally, some human activities may have an impact on mosquito populations. Health authorities regularly promote proper cleaning awareness for homes, including peridomiciliary areas such as patios, gardens and sidewalks (avoiding puddles, objects and places where stagnant water persists) and to restrict plant overgrowth in order to limit the propagation of mosquitoes. Other human activities that may have an impact on the control of canine dirofilariasis prevalence could be customs and habits of pet owners, including keeping dogs indoors in houses protected with mosquito nets on windows and doors and the use of insecticides to keep mosquito populations under control. Since Portoviejo and Colón are in the same geographic region and share geo-ecological characteristics, most likely cultural features would explain why prevalence is higher in Colón than in Portoviejo. Additional circumstances that may explain differences between these regions, could be the study's design and methodology. It is important to point out that the present study is limited from a statistical viewpoint, as sample size is small ($n = 25$) for epidemiologic study to detect seroreactivity against CVBD agents in dogs seroreactive for *T. cruzi.* However, the identification of 12% prevalence suggests that *D. immitis* is more important in Portoviejo region than previously reported by Morán [25].

Lyme disease is normally present in Nearctic and Palearctic regions. In Americas, it has mainly been reported in the USA, Canada, Mexico, and Costa Rica, and the prevalence ranged from <1% up to 30% [36, 56, 57, 58, 59, 60]. South America is not an endemic area for Lyme disease, although sporadic cases have been reported in dogs from Peru [61] and in humans from Ecuador [62]. There is no epidemiological study that explains how these people were infected with *B. burgdorferi*, but most likely they were imported from other latitudes. In the case of Ecuador, studies conducted in Portoviejo [25], Manta, Guayaquil cities and the Galápagos archipelago [45, 46, 47] found no serological evidence of the disease in dogs. A study performed in the neighboring country of Colombia found no serological evidence of Lyme disease either [63]. Our findings support those previous reports that indicate that Lyme disease is not an endemic disease in the area of study.

The sample size of the present study did not allow the inference of risk factors associated with the presentation of different CVBD in Colón and it would be necessary to conduct further studies with larger numbers of animals to find out which risk factors are associated with the different CVBD present in this socioeconomic and geographic region.

The environmental conditions of the study area contribute to the propagation of vectors (e.g., triatomines, ticks, and mosquitoes) that are the etiological agents of CVBD. Finally, it should be noted that dogs carry concomitant *T. cruzi, E. canis, A. phagocytophilum,* and *D. immitis* infections and this could represent an added risk for human infection with CVBD [22,64]. Therefore, health authorities should consider taking adequate measures to control vectors, preventing dogs from becoming a reservoir in an ecological niche where various vectors, reservoirs and

pathogens coexist.

It is important to note that serologic studies detect antibodies against pathogens and may or may not be an indication of active infection. However, it should also be considered that in suburban and rural areas in Ecuador, people do not have a strong pet care culture. It is therefore likely that most of the animals with non-symptomatic infection, which is the case for the cluster of CVBD, may live their lives with the infection and continue to serve as reservoir host.

The present report did not include clinical studies involving coinfections of the studied pathogens. However, it would be interesting to study such interactions and the additional health risks involved in animals presenting coinfections. Furthermore, the knowledge that coinfections are apparently common in dogs suggests that they could be present in humans with some frequency, and therefore, this report represents a warning to be considered by public health authorities, to implement and reinforce disease prevention and treatment programs.

5. Conclusions

This study detected a high seroprevalence of *T. cruzi* in dogs, and additionally, coinfections with ehrlichiosis, anaplasmosis, and dirofilariasis. The conducted research is important from both veterinary and human health perspectives, due to the zoonotic nature of these diseases. Our results could be useful in the development of programs that prevent the transmission of these diseases to people and animals.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations

CVBD	Companion vector-borne diseases
ELISA	Enzyme-linked immunosorbent assays
INCOSUR-Chagas	South Cone Initiative to control/eradicate Chagas disease
PCR	Polymerase Chain Reaction

Ethics approval and consent to participate

Samples were collected only after obtaining the consent of each dog owner. Blood sera were collected for diagnostic purposes, and no ethical considerations were necessary.

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CRediT authorship contribution statement

Pilar Rivadeneira Barreiro: Conceptualization, Methodology, Investigation, Writing – Original Draft. **Roberto Montes de Oca-Jiménez:** Funding Acquisition, Project administration, Supervision. **Juan Carlos Vázquez Chagoyán:** Investigation, Visualization, Supervision. **Silvia Martínez Subiela:** Writing – Original Draft. **Laucel Ochoa García:** Data Curation. **Pablo Zambrano Rodríguez:** Methodology, Investigation, Formal analysis. **Adolfo Morán Loor:** Conceptualization, Methodology, Investigation, Resources. **Jorge Varela Guerrero:** Validation. **Nisha Jain Garg:** Writing – Review and Editing.

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