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Original

# Knowledge and seroprevalence of *Trypanosoma cruzi* in dogs in Valledupar, Colombia

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## ABSTRACT

**Objective.** Determine the seroprevalence of IgG antibodies against *Trypanosoma cruzi* in dogs in the city of Valledupar, department of Cesar in Colombia, as a possible Chagas disease sentinel species. **Materials and methods.** An awareness talk was given and a survey applied to participants regarding knowledge of Chagas disease, previously standardized by the Instituto National de Salud (INS – Colombia). A total of 80 dog samples were tested using an ELISA recombinant Chagatest v3.0. Sample reading was done using an ELISA strip reader Stat Fax 303 Plus and statistical analyses were done with SPSS v.22.0. **Results.** Data captured by 66 surveys indicates 37.88% of people relate the insect vector and the symptoms with the pathology; most of the surveyed performed vector control at home (cleaning, fumigation). Of the 80 samples evaluated, 95% were negative and 5% positive. No significant association was found between the variables breed, sex and age, and seropositivity to *T. cruzi*. **Conclusions.** Identification of seropositive samples in dogs represents an important epidemiological indicator for the Caribbean region and the department of Cesar. Implementation of strategies to strengthen interinstitutional alliances for disease monitoring are recommended, including the use of dogs as sentinel species. This is the first report of seroprevalence of *T. cruzi* in dogs in Valledupar, Colombia and is expected to increase the knowledge on the behavior of the infection in domestic reservoirs of the region.

**Keywords:** Chagas disease; seroepidemiologic studies; seroprevalence; Immunoglobulin G; *Canis familiaris*; canine (*Source: DeCS, CAB*).

## RESUMEN

**Objetivo.** Determinar la seroprevalencia de anticuerpos IgG contra *Trypanosoma cruzi* en caninos de la ciudad de Valledupar, en Cesar - Colombia como posible especie centinela de la enfermedad de Chagas. **Materiales y métodos.** Se dictó una charla de sensibilización y se aplicó una encuesta

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con preguntas sobre el conocimiento, actitudes, prácticas y factores de riesgo de la enfermedad de Chagas, previamente estandarizada por el Instituto Nacional de Salud. Se analizaron 80 sueros caninos mediante la técnica Chagatest ELISA recombinante V3.0. La lectura de las muestras se efectuó con el lector de ELISA en tiras Stat Fax 303 Plus y el análisis estadístico se realizó usando SPSS v.22.0. **Resultados.** Los datos captados por 66 encuestas indican que el 37.88% de las personas relacionan el insecto vector y los síntomas con la patología; la mayoría de encuestados realizan control de vectores en sus viviendas (limpieza, fumigación). De las 80 muestras evaluadas 95% fueron negativas y 5% positivas. No se encontró relación significativa entre las variables raza, sexo y edad con la seropositividad a *T. cruzi*. **Conclusiones.** La identificación de muestras seropositivas en perros representa un importante indicador epidemiológico para la Región Caribe y el departamento del Cesar. Se recomienda implementar estrategias para fortalecer alianzas interinstitucionales en el monitoreo de la enfermedad, incluyendo el uso de perros como centinelas. Este es el primer reporte de serologías caninas positivas para *T. cruzi* en Valledupar, Colombia por lo que se espera incremente el conocimiento sobre el comportamiento de la infección en reservorios domésticos en la región.

**Palabras clave:** Enfermedad de Chagas; estudios seroepidemiológicos; seroprevalencia; Inmunoglobulina G; *Canis familiaris*; perros (*Fuente: DeCS, CAB*).

## INTRODUCTION

Chagas disease is a zoonotic public health problem affecting 6 to 7 million people worldwide, mainly in Latin America with 28000 cases and around 12000 deaths per year on average (1). It is estimated 100 million people are at risk of contracting the disease in endemic areas (2). In Colombia, estimated prevalence is between 700 thousand and 1.2 million infected inhabitants, and 8 million are considered at risk of acquiring the infection, and the annual cost of medical care for the disease was estimated at 267 million dollars (USD) in 2008 (1).

Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi* which disseminates making use of Triatominae species as vectors in its life cycle (3) and uses wild and domestic mammals as reservoirs (4,5). Among its reservoirs, dogs have been studied in different countries of America including Colombia (6,7), Venezuela (8), Argentina (9,10), Brazil (11), Panama (12), Costa Rica (13), Mexico (14,15) and the United States (16). Interest in dogs rises from the preference of triatominae insects for feeding on canines and given their proximity to humans compared to other reservoir species, which makes them a suitable sentinel species of the disease (17).

Significant advances have been made in America in the study of canine Chagas disease. In Colombia, research is being developed to understand the dynamics of *T. cruzi* transmisión in dogs (18). However, in 2014 Cesar department of Colombia had one of the highest number of cases reported and high rates of triatomines infestation were demonstrated in indigenous communities of Valledupar and the Sierra Nevada of Santa Marta (19). Active transmisión of *T. cruzi* with overlap between domestic and wild transmisión cycles was determined in four different indigenous communities, as a result of the social, environmental and cultural conditions of the populations (20). The previous reports show a predisposition for increased prevalence and incidence of Chagas disease in humans and susceptible animals in the city of Valledupar. The objective of the current study was to detect levels of seropositivity to *T. cruzi* in a sample of dogs from the city of Valledupar, in order to demonstrate a posible zoonotic risk and validate the feasibility to use canines as sentinel species for Chagas disease in humans.

## MATERIALS AND METHODS

**Study type.** A cross-sectional descriptive study was performed with 80 canines sampled and 66 people attending an awareness talk and performing a standardized survey.

**Study area and geoclimatic conditions.** The study was performed in the city of Valledupar, department of Cesar in Colombia. Valledupar has a dry tropical weather with 970 mm average annual rainfall. With two rainy and two dry seasons, the months of December through April and June through July are usually dry season, while rainy seasons take place in May and August through November. Average temperature is 28.4°C, with maximum

temperatures at midday reaching between 33 and 36°C, and minimum temperatures before sunrise of 23 to 24°C (21).

**Ethical Aspects.** All dogs included in the study were taken by their owners to five different veterinary clinics from the city associated to the study and their owners granted permission to participate in the study by written informed consent. All samples were taken by licensed veterinarians in registered veterinary centres from Valledupar associated to this study. Animals were treated with proper care and ethical management in accordance with the stipulations in the national regulations the protection of animals: Law 1774 of 2016, Law 044 of 2009, and its predecessor Law 84 of 1989.

**Surveys.** An awareness talk followed by a survey was given to 66 dog owners, some of them owning more than one animal, to inform them about the risks that Chagas disease implies in the health of their pets. The survey, standardized by the Instituto Nacional de Salud, assessed their knowledge, attitudes, practices and risk factors for Chagas disease.

**Sampling.** For each dog included in the study, a full health check-up was performed and blood samples were drawn by puncturing the cephalic vein. Dogs residing in Valledupar that attended the veterinary centres associated, whose owners granted permission to participate in the study by written informed consent, were included in the study until the sample was completed. Canines that were not residing in Valledupar, those who presented clinical signs of anaemia and those whose owners did not grant permission to participate were excluded from the study.

**Sample analysis.** Once the blood samples were collected, serum was separated following (9,10) centrifuged at 2500 rpm for 15 minutes. The serum obtained was placed in a 1.5mL Eppendorf tube, labelled with the date, name, age, breed and sex of the canine and sealed for cold storage at -20°C until analysis. The serums were analysed using the Chagatest ELISA Recombinant V.3.0® (100% sensitive and 99,7% especific, Wiener Laboratorios SAIC-Rosario, Argentina) following the manufacturer's specifications. Samples were read using an ELISA reader equipped with Stat Fax 303 Plus strips.

**Statistical Analysis.** Results were analysed using SPSS® version 22.0. The results referring to the level of knowledge were expressed in the form of frequencies and percentages. Quantitative variables such as age and sex were expressed as frequencies and percentages. The association between variables was presented by contingency tables using the chi square test. A value of $p<0.05$ was considered significant.

## RESULTS

The sample included 40 males and 40 females for a total of 80 canines with an age range between 3 and 180 months. A total of 16 breeds were included in the study: 30% Cimarron Creole (24), 17.5% Pitbull (14), 11.25% Schnauzer (9), 10% Poodle (8), 7.5% Doberman Pinscher (6), 3.75% Labrador (3), 2.5% (2 individuals each) of the Beagle, Cocker Spaniel, Pug, Border Collie, and Siberian Husky breeds, and 1.25% (1 individual each) of the Boston Terrier, German Shepherd, Jack Russell Terrier, Weimaraner and Bulldog breeds.

Of the 80 serological samples collected 4 individuals were positive for *T. cruzi* antibodies, representing a 5% general prevalence. Three of the samples were from male dogs (75%) and one came from a female (25%). No significant association was found between seropositivity to *T.cruzi* and sex or age ($p>0.05$) (Table 1).

**Table 1.** Distribution of seroprevalence of *Trypanosoma cruzi* antibodies in canines by age and sex in the city of Valledupar – Colombia.

| Variable | N | + | F | - | F % | P |
|---|---|---|---|---|---|---|
| **Sex** | | | | | | |
| Female | 40 | 1 | 1.25 | 39 | 48.75 | >0.05 |
| Male | 40 | 3 | 3.75 | 37 | 46.25 | |
| **Age** | | | | | | |
| 0–3 years | 31 | 2 | 2.5 | 29 | 36.25 | >0.05 |
| 4–7 years | 49 | 2 | 2.5 | 47 | 58.75 | |

+: Positive; F: Frequency %; -: Negative.

Participants of the awareness talk expressed great interest, with women showing a higher participation. Survey outcomes clearly showed a lack of knowledge about the disease and the risks it entails. Only 37.9% (26 respondents) associated the vector insect and the symptoms with the pathology. (Table 2)

**Table 2.** Chagas disease epidemiological survey conducted on canine owners of Valledupar.

| Question | Answer | | | |
|---|---|---|---|---|
| | Yes | | No | |
| | n | % | n | % |
| Do you know the vector? | 25 | 37.88 | 41 | 62.13 |
| What is the name of the insect? | | | | |
| Triatominae | 4 | 6.06 | 62 | 93.94 |
| Kissing bug | 21 | 31.82 | 45 | 68.18 |
| Other | 41 | 62.12 | 25 | 37.87 |
| Does the insect vector transmit the disease? | 18 | 27.27 | 48 | 72.73 |
| You know a disease that is transmitted by an insect, whose symptoms in humans are fever, dizziness, fainting, palpitations, difficulty breathing, fatigue and heart problems? | 25 | 37.89 | 41 | 62.12 |
| What is the name of the disease? | | | | |
| Chagas | 13 | 19.70 | 53 | 80.30 |
| Leishmaniasis | 5 | 7.58 | 61 | 92.42 |
| Other | 48 | 73.72 | 18 | 27.27 |
| What type of walls does your home have? | | | | |
| Block or brick | 66 | 100 | 0 | 0 |
| Type of plaster on the walls | | | | |
| Completely plastered | 47 | 71.21 | 19 | 28.79 |
| Partially plastered | 18 | 27.27 | 48 | 72.72 |
| Not plastered | 1 | 1.52 | 65 | 98.48 |
| What type of roof does your home have? | | | | |
| Zinc | 5 | 7.57 | 61 | 92.42 |
| Eternit roof | 59 | 89.4 | 7 | 10.60 |
| Other | 2 | 3.04 | 64 | 96.96 |
| What type of flooring does your home have? | | | | |
| Tiling | 49 | 74.25 | 17 | 25.75 |
| Concrete | 16 | 24.25 | 50 | 75.75 |
| Wood | 1 | 1.52 | 65 | 98.48 |
| Has the Secretary of Health inspected your home? | 5 | 14.29 | 61 | 85.71 |
| What type of check did they do in your home? | | | | |
| Home check | 25 | 37.88 | 41 | 61.12 |
| Home fumigation | 10 | 15.15 | 56 | 84.84 |
| Other | 31 | 46.97 | 35 | 53.03 |

## DISCUSSION

The general prevalence found in dogs (5% or 4/80) is similar to the reports from Costa Rica using the same technique (13). Other studies conducted in Venezuela (8), Argentina (10), Brazil (11) and Mexico (14,15) also obtained seropositive results for trypanosomiasis in canines with ELISA techniques, which supports that the method is a valid tool for the diagnosis of the disease. However, it is recommended to use molecular techniques as additional confirmation for the serology tools in order to detect active infections in dogs and cats, and even to replace xenodiagnosis and blood culture techniques (9). Seropositive dogs belonged to the Cimarron Creole, Cocker Spaniel and German Shepherd breeds. Age was also variable (6, 36, 60 and 84 months) and both sexes were affected in the same way, indicating these variables did not show a significant relationship with seropositivity. According to the outcomes of the survey, seropositive canines coexist with several people) and other pets, which could expose them to a greater probability of completing the pathogen transmission cycles and spreading the disease (9). Further on, the location and type of housing of the pet owners does not support the hypothesis that canines that live in rural areas may be more likely to be positive than those in urban areas (22).

In studies previously conducted in urban regions of Brazil (Sao Paulo) and Colombia (Bogotá), no antibodies against *T. cruzi* were detected in any of the groups of dogs analysed, suggesting that in these urban centers canines have a limited role in the transmission of *T. cruzi* to humans (22). However, in a different region of Colombia located in the Caribbean (Valledupar), there is scientific evidence of the presence of triatomines (19), and the active transmission of *T. cruzi* in indigenous communities of the Sierra Nevada de Santa Marta (20), exhibiting different behaviours in different geoclimatic zones.

Most of the survey respondents conducted checks on their homes (cleaning, fumigation) even without knowing the existence of Chagas disease, reducing the probability of spreading the disease vector and therefore protecting the people who live in the home. It is suggested that the risk of vector transmission is relatively different for the two species (human/canine) because dogs are more predisposed to contact with the parasite (both via vector and via oral contact) each time they are taken for walks in fields, parks, or farms, when they go hunting and from their neighbourhoods, which indirectly increases the risk of infection in humans, regardless of other significant risk factors (23).

The present study has limitations related to the techniques used and the group of canines sampled, in which those that are not taken to veterinary clinics were not included, suggesting the conduct of new investigations that include complementary diagnostic techniques and the inclusion of the group of canines that are not taken to veterinary clinics in the city of Valledupar, Cesar - Colombia. The evidence

obtained in this study is an important epidemiological indicator for the Caribbean Region and the Department of Cesar, given that the incidence and prevalence of Chagas disease in humans is high for this region. Is important to further investigate the role dogs play in the transmission of this parasite to humans, the maintenance of the different cycles of infection, their role as a reservoirs and potential use as sentinel species of the disease. Identification of seropositive samples in dogs represents an important epidemiological indicator for the Caribbean region and the department of Cesar. Implementation of strategies to strengthen interinstitutional alliances for disease monitoring are recommended, including the use of dogs as sentinel species. This is the first report of seroprevalence of *T. cruzi* in dogs in Valledupar, Colombia and is expected to increase the knowledge on the behavior of the infection in domestic reservoirs of the region. This study represents a valuable contribution to the scientific community and highlights the importance of implementing control and surveillance strategies to reduce the transmission of *T.cruzi* in the region.

## Conflicts of interest

Authors declare no conflicts of interest.

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