

Chagas disease in northern Chile: Detection of *Trypanosoma cruzi* in children, dogs and triatomine bugs

Carolina Reyes^{a,*}, Christian R. González^b, Sergio Alvarado^{c,d}, Leticia Flores^e, Catherine Martin^e, Alan Oyarce^f, María Paz Aylwin^g, Mauricio Canals^h, Alonso Parraⁱ, Lara Valderrama^a

^a Laboratorio de Entomología, Subdepartamento de Genética Molecular, Instituto de Salud Pública de Chile, Santiago, Chile

^b Facultad de Ciencias Básicas, Instituto de Entomología, Universidad Metropolitana de Ciencias de la Educación, Santiago, Chile

^c Facultad de Medicina, Programa de Bioestadística, Escuela de Salud Pública, Universidad de Chile, Santiago, Chile

^d Facultad de Ciencias de la Salud, Universidad de Tarapacá, Arica, Chile

^e Oficina Provincial El Loa, Secretaría Regional Ministerial de Salud Región de Antofagasta, Calama, Chile

^f Sección Parasitología, Subdepartamento de Enfermedades Infecciosas, Instituto de Salud Pública de Chile, Santiago, Chile

^g Subdepartamento de Genética Molecular, Instituto de Salud Pública de Chile, Santiago, Chile

^h Departamento de Medicina y Programa de Salud Ambiental, Facultad de Medicina, Escuela de Salud Pública, Universidad de Chile, Santiago, Chile

ⁱ División de Políticas Públicas Saludables y Promoción, Oficina de Zoonosis y Vectores, Subsecretaría de Salud Pública, Ministerio de Salud, Chile

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ABSTRACT

Chagas disease is an anthroponozoonotic disease caused by the protozoan *Trypanosoma cruzi*, transmitted by triatomine vectors. In Chile, there are four species of triatomine bugs that are potential vectors of *T. cruzi*, being *Triatoma infestans* the main vector in endemic areas of the country. The “Programa Nacional de Control Vectorial de la Enfermedad de Chagas de Chile” has significantly reduced the rates of home infestation to less than 1% and has interrupted vectorial transmission since 1999. The objective of this study was to evaluate the impact of vectorial control and the continuity of the interruption of vectorial transmission in northern Chile (provincia de El Loa, región de Antofagasta). The study comprised fingerstick blood samples of 2104 children, attending local school, venous blood samples of 65 dogs, associated to houses with *T. infestans* unique findings and vector infestation, and intestine samples of 284 *T. infestans* specimens, from the provincia de El Loa, during 2014–2016 period. The samples were analyzed by enzyme-linked immunosorbent assay (ELISA), indirect immunofluorescence and/or polymerase chain reaction (PCR) techniques. A total of 5 children (0.24%), 7 dogs (10.8%), and 6 specimens of *T. infestans* (2.1%) resulted positive to *T. cruzi* infection. This study showed that the risk of transmission of Chagas disease is low in the north of Chile (provincia de El Loa), detected a low positive rate of chagasic children and of infected triatomine bugs, and showed the existence of *T. cruzi* transmission in dogs, which are used as natural sentinels for the detection of *T. cruzi* infection, being especially useful during surveillance program in human population characterized by low seroprevalence.

1. Introduction

Chagas disease or American trypanosomiasis is described by the World Health Organization (WHO) as a worldwide existing major neglected disease. Up to 7 million people worldwide are estimated to be infected (WHO, 2017). This sickness is a zoonotic parasitic vector borne disease caused by *Trypanosoma cruzi*, which is transmitted when infected feces of triatomine vectors are introduced through a bite site or through an intact mucous membrane of a mammalian host (Schofield, 2000).

T. cruzi infects a broad range of mammalian hosts, both wild and domestic ones. Furthermore, opossum (*Didelphis sp.*) is described as a major reservoir in sylvatic areas (Gürtler and Cardinal, 2015). Probably the parasite would have evolved from this reservoir to infect triatomines (Jansen, 1998). Four species of Triatominae bugs have been described for Chile: *Triatoma infestans* Klug, *Mepraia spinolai* Porter, *Mepraia gajardoi* Frías, Henry & González and *M. parapatrica* Frías. *T. infestans* is the main vector of this pathology, both in southern cone of South America and in Chile (Canals et al., 1999; Ceballos et al., 2011), because

* Corresponding author.

E-mail address: creyes@ispch.cl (C. Reyes).

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of its feeding-defecation patterns, *T. cruzi* infection rate and domiciliary habits (Canals et al., 1999, ISP, 2018).

There is a large number of vertebrate reservoirs involved in the cycle of transmission and maintenance of this disease. Domestic animals, especially dogs (*Canis lupus familiaris*), have an important epidemiological role, because they serve as a link between domestic, peridomestic and wild cycles of *T. cruzi* (Rojas et al., 2008; Pineda et al., 2011; Enríquez et al., 2013; Ramírez et al., 2013; Arce-Fonseca et al., 2017; Freitas et al., 2018). Therefore, it is possible to use this species as natural sentinels for detection of *T. cruzi* in the peridomestic and domestic cycle, being especially useful during surveillance program in human population characterized by low seroprevalence (Gürtler et al., 1988; Rojas et al., 2008; Castillo-Neyra et al., 2015). There is only one study carried out previously regarding *T. cruzi* prevalence in dogs from the north coast of Chile, which detected 35 positive dogs (Ortiz et al., 2016).

The “Programa Nacional de Control Vectorial de la Enfermedad de Chagas de Chile”, applied in historically endemic areas of *T. infestans* vector distribution (18°30'–34°36'S), has obtained good results, such as the reduction of *T. infestans* home infestation indices to less than 1% and the certification of interruption of vectorial transmission of Chagas disease in the national territory by Pan American Health Organization (PAHO) since 1999. Nevertheless, according to the analyzes carried out in *T. infestans* by the Instituto de Salud Pública de Chile (ISP) between 2005 and 2016, trypano-triatomine indices remained high (prevalence $P= 49,2\%$ for the period 2005–2013, $P= 36,5\%$ for the period 2014–2017) (ISP, 2014; ISP, 2018; Canals et al., 2018).

In order to evaluate the impact of vector control and certify the continuity of vector transmission interruption, seroepidemiological studies are carried out in children under the age of 10, residents of

historically endemic areas of the main vector distribution of Chile (ISP, 2014; MINSAL, 2016). *T. cruzi* prevalence in these children had reduced from 5,4% in the early 1980s to 0,4% in 1999 (Lorca et al., 2001).

The aim of this study was to evaluate the impact of vector control program and the continuity of vector transmission interruption in the provincia de El Loa, región de Antofagasta.

2. Material and methods

2.1. Study area

The study was conducted in the comuna de Calama (-22°28'S; -68°55'W) and San Pedro de Atacama (-22°55'S; -68°13'W) (Fig. 1), including the localidades de Chiu Chiu, Lasana, Ayquina, Caspana and the city of Calama, and the localidades de Toconao, Peine, Socaire, Talabre, Camar, Río Grande, Aylluso, Ollagüe and San Pedro de Atacama respectively, during the period since 2014 to 2016.

According to the data provided by the Secretaría Regional Ministerial de Salud de la región de Antofagasta (SEREMI de Salud), 27 colonized houses and 31 houses with unique vector findings have been identified during the 2014–2016 period, which were nominated as ‘index houses’ for this study. Both serological samples of children and dogs were obtained taking into account a radius of 200 meters around these houses. Triatomine samples corresponded to specimens of *T. infestans* from the houses included in this perimeter from ‘index houses’ and were studied at the Laboratorio de Entomología ISP for their taxonomic identification and molecular analysis.

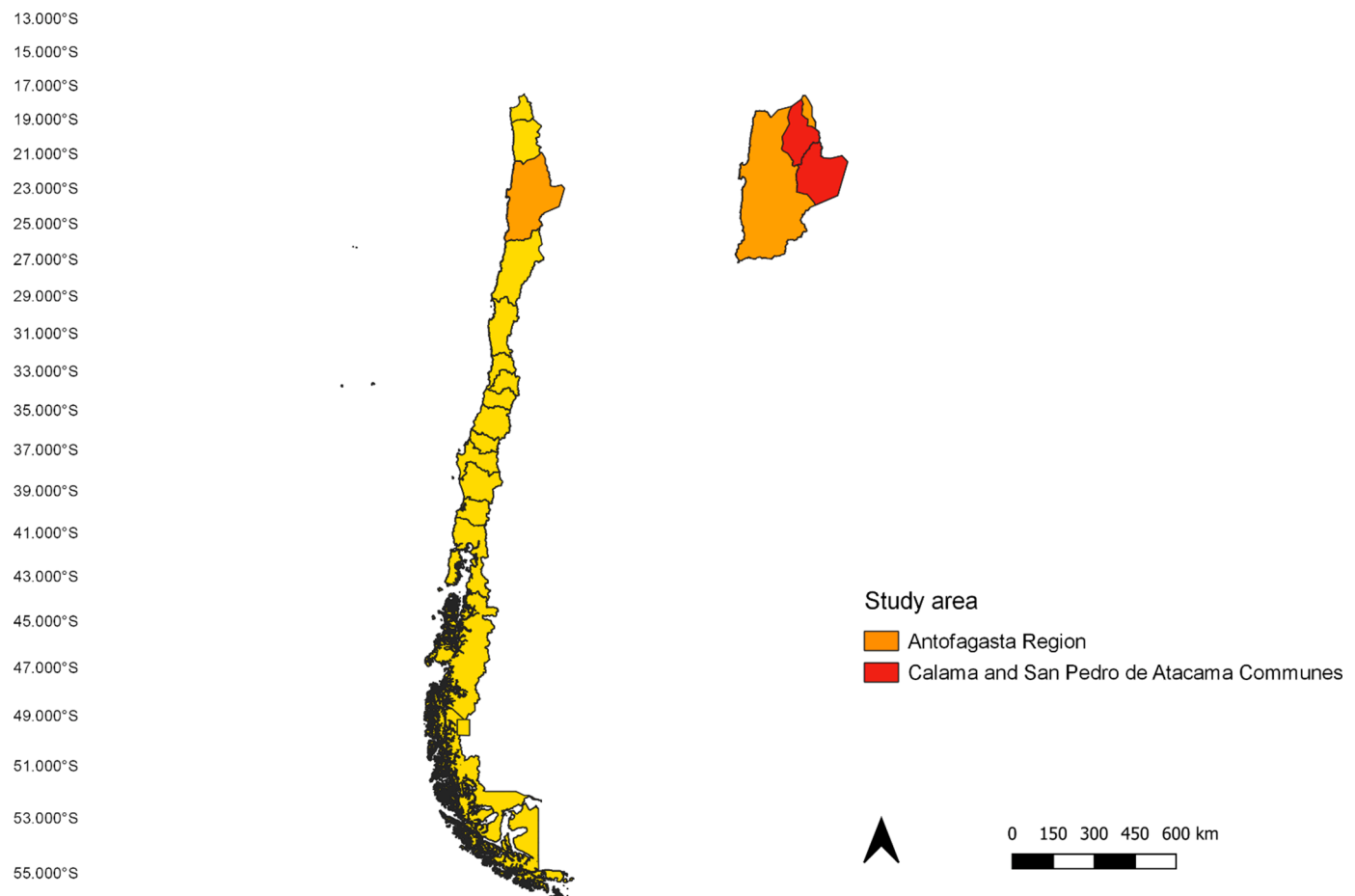


Fig. 1. Study area: Comunas de Calama and de San Pedro, región de Antofagasta, Chile. Source: Laboratorio de Entomología ISP.

2.2. Children sampling

Prior to obtaining the samples from children, SEREMI de Salud held awareness talks with the community and requested the signing of an informed consent by the legal guardian of each child, previously approved by the ISP Ethics Committee. Subsequently, a capillary blood sample by finger puncture with a sterile lancet collected on completely soaking 2cm² of filter paper grade 4 (Cat. No. 1004-917) was taken from each child in his corresponding educational or health establishments, which were later transferred at room temperature to the Laboratorio de Parasitología ISP for analysis (Contreras et al., 1984; Lorca et al., 1996). A total of 2,106 children under the age of 10 were sampled for this study, 1,362 belonging to the comuna de Calama and 744 to the comuna de San Pedro de Atacama (Table 1).

2.3. Dogs sampling

Only specimens older than 4 months were included in this study to avoid false positive results for maternal antibodies found in younger individuals. Selection of canids was based on domiciliary units that presented findings of *T. infestans* between the period 2012 and 2015, including the dwellings located up to 200 m around the 'index houses'. A total of 65 dogs (46 from comuna de Calama and 19 from comuna de San Pedro de Atacama) (Table 2) were sampled on site by trained personnel, after obtaining a signed consent form from the dog's owners. Dogs tested were taken by their owners to places prepared for sampling, and approximately 3 mL of venous blood was extracted from cephalic vein. Each sample was then transferred immediately to a Falcon tube and was maintained at room temperature for 24 h to remove the serum, then it was transferred to 2 mL tubes that were frozen and stored until analysis at the Laboratorio de Parasitología ISP. The rest of the sample was eliminated.

2.4. Triatomines sampling

Data of *T. infestans* was extracted from databases of the Laboratorio de Entomología ISP, corresponding to samples of this species from the provincia de El Loa, región de Antofagasta, received for their taxonomic identification and analysis by PCR for detection of *T. cruzi*, between 2014 and 2016. A total of 545 specimens were analyzed, 279 from the comuna de Calama and 266 from the comuna de San Pedro de Atacama.

Table 1

Sampled children distribution according to place of residence. Comunas de Calama and de San Pedro. Región de Antofagasta, Chile. 2014–2016.

Residence place	Number of sampled children
Comuna de Calama	1492
Ayquina	4
Calama	1362
Caspana	10
ChiuChiu	107
Lasana	9
Comuna de San Pedro	612
Camar	12
Ollague	17
Peine	20
Río Grande	5
San Pedro de Atacama	401
Socaire	37
Solor	23
Talabre	6
Toconao	91
Total	2104

Source: Laboratorio de Parasitología ISP

Table 2

Trypanosoma cruzi positive children distribution according to the comuna and the localidad of residence. Región de Antofagasta. Chile, 2014–2016.

Comuna and localidad	Positive children number / Total (%)
Calama	4/1492 (0,27)
Ayquina	0/4
Calama	3/1362 (0,22)
Caspana	0/10
Chiu Chiu	1/107(0,93)
Lasana	0/9
San Pedro de Atacama	1/611 (0,16)
Camar	0/12
Ollague	0/17
Peine	0/20
Río Grande	0/5
San Pedro	1/401(0,25)
Socaire	0/37
Solor	0/23
Talabre	0/6
Toconao	0/91
Total	5/2104 (0,24)

Source: Laboratorio de Parasitología ISP

2.5. Parasitological diagnosis

2.5.1. Children

Collected samples were stored at 4°C until processing. Antibodies were then eluted from 1cm² filter paper during 24 h at 4°C in 500 µL phosphate-buffered saline (PBS) solution at pH 7,2 and subsequently centrifuged for 10 min at 5000 rpm. Screening of the samples was carried out by the enzyme-linked immunosorbent assay (ELISA) technique, using an in-house methodology, developed in the Laboratorio de Parasitología ISP, to detect the presence of IgG antibodies produced against the parasite to perform diagnosis of Chagas disease in humans. ELISA was performed as follows: microplates were coated with whole *T. cruzi* (Tulahuen strain) isolated extract (5 µg/mL) cultivated at 18–28°C in LIT (liver-infusion tryptose medium). Plates were blocked with buffer PBS-Tween 0.1%–milk 3% for at least 60 min at 37±1°C. Eluted serum samples were diluted in buffer PBS-Tween 0.1%–milk 3% at a dilution of 1:10 and incubated (30 min/37±1°C). Subsequently, the plates were washed 3 times in buffer PBS-Tween 0.1% and incubated (30 min/37±1°C) with Alkaline Phosphatase-conjugated Human IgG (Sigma-Aldrich Cat. No. A3150) diluted 1:1000 in buffer PBS-Tween 0.1%–milk 3%. Finally, the plates were washed 3 times in buffer PBS-Tween 0.1% and P-Nitrophenyl-phosphate (Sigma-Aldrich Cat. No.2770) was used as substrate for 30 min at 25°C and the results were read at 405 nanometers (Multiskan Ex, Thermo Scientific®).

In accordance with World Health Organization recommendations to use at least two assays based on distinct techniques to confirm specific anti-*T. cruzi* antibodies in Chronic Chagas disease (WHO, 2002), a new whole blood sample was collected for all positive results by ELISA on filter paper and were analyzed by ELISA IgG and indirect immunofluorescence IgG standardized in the reference laboratory of the ISP (Lorca et al. 1990; Astorga et al. 1988).

2.5.2. Dogs

Circulating antibodies directed against *T. cruzi* antigens were screened in sera obtained from dogs through ELISA for Chagas disease as described for humans and using conjugated antibody IgG anti-dog (Sigma-Aldrich Cat No. A6792). This technique was used in dogs only due to the characteristics of chronicity of this disease, where it is more likely to find antibodies than parasites in blood as in acute patients.

2.5.3. Triatomines

Taxonomic identification of the specimens collected was conducted based on Lane and Wygodzinsky (1979) and González and Reyes (2015). Detection of *T. cruzi* DNA was conducted with PCR technique, using homogenate of intestine distal portion of triatomine samples.

DNA extraction from intestinal tract of triatomines was performed in a Maxwell 16MDx instrument (Promega, Madison, WI, U.S.A.), using a Maxwell 16 Tissue DNA Purification Kit (Promega). Conventional PCR was performed using an Applied Biosystems® 2720 Thermal Cycler (Applied Biosystems, Carlsbad, CA, U.S.A). Master mix was composed by 1 × PCR reaction Buffer; 0.25mm deoxynucleotide triphosphate solution (dNTPs); 3mm MgCl₂ solution; 0.6U Taq Platinum (Invitrogen, Carlsbad, CA, U.S.A). 2.5 µm kDNA specific primers 121 (5'-AAATAATGTACGGGGGAGATGCATGA-3') and 122 (5'-GGTTCGATTGGGGTTGGTGTAAATATA-3'); 5 µL of template DNA and a quantity of water sufficient for a final volume of 30 µL (Ortiz et al., 2016). Cycling parameters were one step of 5-min denaturation at 94°C; 33 cycles of 1 min at 94°C, 1 min at 65°C and 1 min at 72°C; and one final extension step of 10 min at 72°C. PCR products of 330 pb were analyzed in 1% agarose gels stained with GelRed Nucleic Acid Gel Stain 1 × (Biotium, Hayward, CA, U.S.A) (Schijman et al., 2011).

2.6. Statistical analysis

For the three types of samples (children, dogs and triatomines) descriptive statistics were performed. For *T. cruzi* seropositivity rates in children, dogs and triatomines, 95% confidence intervals were constructed.

3. Results

A total of 2,103 samples were obtained from 2,106 children selected for the study, of which 51.52% were males and 48.48% females. The age of the 2,079 children analyzed were recorded, obtaining an average of 5 years and 9 months (Standard deviation= 2 years 5 months). Of the children sampled, 70.91% were residents of the comuna de Calama and 29.09% of the comuna de San Pedro de Atacama. Of the total of samples, 99.76% gave a negative result to ELISA technique; while 5 children (prevalence $P=0.24\%$) of the total studied were positive to the technique [$IC_{95\%}(P) = (0.07\%; 0.55\%)$], 4 belonged to the comuna de Calama and 1 to the comuna de San Pedro de Atacama (Table 2). Of the 5 positive children, 2 had Chilean nationality, 2 had Bolivian nationality and 1 had Argentine nationality.

Of the 65 dogs analyzed for the detection of antibodies circulation against *T. cruzi* antigen by ELISA, 71.64% belonged to the comuna de Calama and 28.36% to the comuna de San Pedro de Atacama. The average age of the dogs included in this study was 4 years and 4 months ($DE= 3$ years and 10 months). Of the dog blood samples, 81.54% (53/65) were negative, 10.77% (7/65) were positive [$IC_{95\%}(P) = (4.44\%; 20.94\%)$] and 7.69% (5/65) were undetermined. Of the positive dogs to *T. cruzi*, 4 belonged to the comuna de Calama and 3 to the comuna de San Pedro de Atacama. Nevertheless 1 of the dogs from the comuna de San Pedro de Atacama had been born in Calama (Table 3).

During the period covered by the study, 545 *T. infestans* specimens (285 adults and 260 nymphs, Table 4) were received at the Laboratorio de Entomología ISP from the region de Antofagasta, 51.2% of the

Table 3

Trypanoma cruzi positive dogs and triatomines bugs according to the comuna of origin. Región de Antofagasta, Chile. 2014–2016.

Comuna	Positive dogs number / total (%)	Positive triatomine bugs number / total (%)
Calama	4/46 (8,7)	4/95 (4,21)
Calama	3/41(7,3)	4/94 (4,25)
Chiu Chiu	0/5	0/1
San Pedro de Atacama	3/19 (15,8)	2/188 (1,26)
Peine	1/6 (0,17)	2/167(1,2)
Toconao	2/13 (0,15)	0/21
Total	7/65 (10,8)	6/283 (2,12)

Source: Laboratorios de Parasitología y Entomología ISP

Table 4

Distribution of *Triatoma infestans*, identified in the Laboratorio de Entomología ISP, according to the bug development stage. Región de Antofagasta, Chile. 2014–2016.

Stage of development	Triatomine bugs number (%)
Adult	285 (52,3)
Nymph	260 (47,71)
Stage 1	18 (3,3)
Stage 2	34 (6,3)
Stage 3	26 (4,8)
Stage 4	10 (1,8)
Stage 5	42 (7,7)
Stage not identified*	130 (23,8)
Total	545

* The stage of development of these nymphs could not be identified because the samples were not well preserved. Source: Laboratorio de Entomología ISP

triatomine bugs belonged to comuna de Calama and 48.8% to comuna de San Pedro de Atacama. All the specimens received were identified based on morphological characteristics and *T. cruzi* detection using PCR technique was carried out only in 284 of them. Triatomine bugs belonging to colonize houses were divided into pools to perform PCR analysis. According to the protocol of the Laboratorio de Entomología ISP, specimens from a colonized house were analyzed by pools until a positive result by PCR was obtained, consequently all specimens from that colonized house were reported as positive for the parasite, even if they weren't analyzed. Of the 284 triatomines analyzed, 6 were positive for *T. cruzi*, with a prevalence of $P= 2.1\%$ and $[95\% CI (P) = (0.78\%; 4.54\%)]$. Of the 6 positive bugs, 4 belonged to comuna de Calama (3 adults and 1 nymph) and 2 to the comuna de San Pedro de Atacama (2 nymphs) (Table 5).

4. Discussion

The seroprevalence value obtained from children's samples was 0.24% with a 95% CI [0.07–0.5%]. This value was within the expected (<2%), considering an interruption on the vectorial transmission chain (Salvatella et al. 2014). Furthermore, this seroprevalence value supports the results obtained from other studies based on children under the age of 5 years old seroprevalence made in Chile. These studies have reported that *T. cruzi* prevalence in children has decreased from 5.4% in 1982 (Lorca et al. 2001) to 0.47% between 2005 and 2015 (MINSAL, 2016). This is a strong argument to determine that vector transmission chain by *T. infestans* is interrupted in this region. Observed prevalence decrease is similar to the one seen today in the total population of Chile, with a 1.2% seroprevalence reported in the endemic area (north of Chile, from 18° 30'S to 34°36'S) (Canals et al., 2020). Nevertheless, prevalence continues to be higher in rural areas (2.3%) compared to urban areas (1.1%). Chagas disease seroprevalence is 1% in región de Antofagasta (23°39'S; 70°25'W) (MINSAL, 2017), whereas prevalence reaches 2.8%

Table 5

Distribution of *Triatoma infestans* positives to *Trypanosoma cruzi* according to the comuna and the localidad of capture and bug developmental stage. Región de Antofagasta, Chile. 2014–2016.

Comuna and localidad	Positive adult bugs number/ total (%)	Positive nymph bugs number / total (%)	Positive bugs number / total (%)
Calama	3/62 (4,84)	1/33 (3,03)	4/95 (4,21)
Calama	3/61(4,92)	1/33(3,03)	4/94(4,25)
Chiu-Chiu	0/1	0/0	0/1
San Pedro de Atacama	0/79 (0)	2/109 (1,9)	2/188 (1,06)
Peine	0/68	2/99(2,02)	2/167(1,2)
Toconao	0/11	0/10	0/21
Total	3/141 (2,13)	3/142 (2,1)	6/283 (2,11)

Source: Laboratorio de Entomología ISP

in región de Coquimbo (30°45'S; 71°00'W), the most endemic area of Chagas disease in Chile (Salas, 2020). Between 1989 and 1999, before vectorial transmission interruption declaration in Chile, children under the age of 5 years old represented 1.24% and people between the age of 5 years old and the age of 20 years old represented 5.27% of the total number of Chagas reported patients. Between 2011 and 2017, these values have decreased to 0.77% and 2.56%, respectively (Lorca et al., 2001). However, it should be noted that this seroprevalence decrease could be explained by Chilean population aging, based on an increase in life expectancy, with a lower proportion of people belonged to lower age bracket (MINSAL, 2017; Salas 2020; Canals et al., 2020), although, 6% of Chagas cases still corresponded to patients under the age of 20 years old in región de Coquimbo (Salas, 2020). Moreover, in this study, only two of the positive children were Chilean, while two were Bolivian and one Argentinian, and it is probable that these three last cases have been infected in their countries of origin, supporting the continuity of the vectorial transmission interruption in Chile. The two Chilean cases could have a congenital origin, since their respective mothers were positive to the ELISA test for Chagas disease, although a vector origin cannot be completely ruled out.

Several studies have been carried out in South America where the presence of specific anti-*T. cruzi* antibodies in dogs demonstrated the importance of this specie as reservoir of *T. cruzi*, maintaining the parasite's urban cycle (Ramírez et al., 2013; Ortiz et al., 2016; Araújo-Neto et al., 2019). These studies demonstrated that dogs have an important epidemiological role, because they serve as a link between domestic and wild cycles of *T. cruzi* (Rojas et al., 2008; Pineda et al., 2011; Enríquez et al., 2013; Ramírez et al., 2013; Arce-Fonseca et al. 2017; Freitas et al. 2018). Dogs play a very important role in the prevalence of this disease in human population because they are the most common mammal found in homes and they usually come into close contact with their owners, increasing the risk of transmission to humans (Gürtler et al., 1991). Thus, it is possible to use this species as natural sentinels for detection of *T. cruzi* infection in domestic cycle, being especially useful during surveillance program in human population characterized by low seroprevalence (Gürtler et al., 1988; Rojas et al., 2008; Castillo-Neyra et al., 2015). In our study we detected an alarming 10.8% positive rate in domestic dogs. On one hand this means that dogs are an important reservoir for the maintenance of the domestic cycle of Chagas disease in the provincia de El Loa, región de Antofagasta, and on the other hand, it means that *T. infestans* is the species responsible for the persistence of vector transmission in peridomicile, since this is the most commonly found species that inhabits the interior of houses in región de Antofagasta (Torres-Pérez et al., 2011). It would be recommendable to incorporate *T. cruzi* blood screening of dogs to the Chagas disease program of Chile to use them as sentinels, like it is done in other countries.

Trypano-triatomine index of *T. infestans* detected in this study was 2.1%, lower than the 6.7% regional index obtained by the ISP, from región de Antofagasta between 2012 and 2013 (ISP, 2014). The index obtained in this study is also much lower than the national average index (36.5%) obtained from the analysis of 2,085 *T. infestans* specimens from historically endemic regions of the vector distribution, between 2014 and 2017, studied at the ISP (with CI0.95 [29.14-44.12]) (ISP, 2018). This result is a good indicator that the infection of *T. infestans* has decreased. That could be explained by the lower access of the vector to homes and, therefore, to biting human reservoirs, due the activities applied by the Programa Nacional de Control Vectorial de la Enfermedad de Chagas de Chile. Nevertheless, the presence of sylvatic populations of *T. infestans*, which diet is based on wild and peridomestic mammals, maintains the high trypano-triatomine index (Canals et al., 2018).

Since 1999 Chile has been declared free from Chagas disease vectorial transmission by PAHO and it was recertificated by this same entity in 2016. Nevertheless, a high proportion of *T. infestans* specimens, detected in the vector's distribution range, is maintained with high rates of infection by *T. cruzi* in Chile (Canals et al., 2017).

This research constitutes the second study in northern Chile to include human, canine and vector components seeking to evaluate vector transmission of Chagas disease (González et al., 2015). Our results are strong evidence of a low trypano-triatomine index and a very low seroprevalence in children, which supports the continuity of the vector transmission interruption in humans in the provincia de El Loa, región de Antofagasta. However, they are also a warning voice, showing the existence of domiciliary and peri-domiciliary *T. cruzi* transmission in dogs that, on one hand, establishes a bridge between sylvatic and domiciliary cycle and, on the other hand, constitutes the basis for a resurgence of *T. cruzi* infection in humans and an increase in infected populations of *T. infestans* in the provincia de El Loa, región de Antofagasta.

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.

Data availability

Data will be made available on request.

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