Trypanosoma cruzi over the ocean: Insular zones of Chile with presence of infected vector Mepraia species

Ninette Rives-Blanchard[a], Fernando Torres-Pérez[b], Silvia Ortiz[c], Aldo Solari[c], Ricardo Campos-Soto[b],[*]

[a] Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile
[b] Instituto de Biología, Facultad de Ciencias, Pontificia Universidad Católica de Valparaíso, Valparaíso 2373223, Chile
[c] Programa de Biología Celular y Molecular, ICBM, Facultad de Medicina, Universidad de Chile, Santiago 8380453, Chile

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ABSTRACT

Chagas disease is one of the main zoonosis mediated by vectors in America. The etiologic agent Trypanosoma cruzi infects different mammals and is transmitted principally by the subfamily Triatominae. Mepraia is a genus endemic to Chile, responsible for transmitting T. cruzi in the sylvatic cycle. Mepraia includes three species: M. gajardoi and M. parapatrica inhabit coastal areas, while M. spinolai inhabits coastal and interior valleys. Previous studies reported the occurrence of Mepraia in Pan de Azucar Island, currently classified as M. parapatrica, but T. cruzi has not been reported in these insects. It is suggested that this could be due to infrequent insect feeding on mammalian hosts. In order to detect T. cruzi in insects from coastal islands, specimens from Pan de Azucar and Santa Maria Islands were examined. A region of kDNA of T. cruzi was amplified by PCR and hybridization assays were performed for T. cruzi genotyping of insect feces. The presence of infected insect and mixed T. cruzi infections was demonstrated. This is the first report of infected Triatominae in coastal islands in Chile. We discuss T. cruzi detection in insular zones, and the presumptive reservoirs that may participate in maintaining its transmission cycle in this habitat. Mixed and unidentified infections suggest that there are complex and unknown reservoir interactions in these habitats.

1. Introduction

Chagas disease is one of the main zoonosis diseases mediated by vectors in America. The etiologic agent is the protozoan *Trypanosoma cruzi*, which infects different mammals and is transmitted principally by hematophagous hemipterans of the subfamily Triatominae. Species of the genus *Mepraia* (Mazza et al., 1940) are vectors that play an important role in the transmission of *T. cruzi* in the wild cycle of Chile (Botto-Mahan et al., 2008; Campos-Soto et al., 2016). *Mepraia* is endemic to semiarid and arid regions, and is distributed in coastal and interior valleys of northern and central Chile (Campos et al., 2013; Frías et al., 1998). Three species are currently included in the genus: *M. spinolai* Porter 1943, *M. gajardoi* and *M. parapatrica* (Frías, 2010; Frías et al., 1998). *M. parapatrica* is distributed in the coastal desert of the Antofagasta and Atacama Regions between 24° 36′- 26° 51′ S in an area intermediate between the northern boundary of the distribution of *M. spinolai* and the southern boundary of *M. gajardoi* (Frías, 2010). The distribution in wild and peri-domestic habitats, the opportunistic feeding behavior and human settlements in risky areas are features of

high epidemiological significance that may make *Mepraia* species be important *T. cruzi* vectors (González et al., 2015; Toledo et al., 2013). The infective status of *Mepraia* species was shown in Campos-Soto et al. (2016). Sagua Franco et al. (2000) reported an insular population (Pan de Azucar Island, Atacama Region) of *Mepraia* that feeds mainly on seabirds, marine mammals and reptiles, but no *T. cruzi* infection was detected. Later a few cases of *T. cruzi* infection were reported in *Mepraia* from a coastal zone of the Antofagasta Region (Botto-Mahan et al., 2008). This report corresponds to zones where *M. parapatrica* is currently distributed, an endemic area where the most frequent vertebrates inhabiting are lizards, seabirds and a few micromammals. In an area with similar ecological features, *M. gajardoi* infection near fishermens' dwellings was reported (Toledo et al., 2013); the main infected vertebrate hosts reported in this zone are dogs (Gonzalez et al., 2015). Another study suggested that the infected mammalian host density surrounding the vector colonies is relevant to determine vector infection risk (Oda et al., 2014). For these reasons it is important to know the infective status of sylvatic vector population. The taxon *T. cruzi* is divided into six discrete typing units (DTUs) named TcI-TcVI

(Zingales et al., 2012). Both TcI and TcII, but also TcV and TcVI were found in a few *M. parapatrica* and in several *M. gajardoi* of coastal areas (Botto et al., 2008; Toledo et al., 2013; Campos-Soto et al., 2016). Infected *T. cruzi* vectors from insular areas have not been reported. The aim of this study is to determine the *T. cruzi* DTU infection prevalence in *Mepraia* species from two insular zones of Chile: Pan de Azucar Island and Santa Maria Island of the Atacama and Antofagasta Regions, respectively. Also two zones in the continent opposite the islands were studied. Both areas (island and continent) are inhabited by sea birds, reptiles, marine mammals and a few rodents. We discuss *T. cruzi* detection in these insects and *T. cruzi* presence, suggesting the possible reservoirs that are interacting in this habitat.

2. Materials and methods

2.1. Study areas and insect collection

Individuals of *Mepraia* were collected during the austral summer of 2015 and 2016 in four coastal zones from northern Chile: Santa Maria Island (N = 30), Santa Maria continent (N = 15), Pan de Azucar Island (N = 6) and Pan de Azucar continent (N = 7). The last two are included in Pan de Azucar National Park (Table 1). Insects were manually collected by trained people as described in Campos-Soto et al. (2015). The insects were fed individually with *Mus musculus* and maintained in a climate chamber at 27 °C with a relative humidity of 70% and a 14:10 h light:dark photoperiod. Triatomines were fed 1 or 2 days after collection, and fecal/urine samples were obtained from each individual after a second feeding 4–5 weeks later to assess the infective status.

2.2. T. cruzi detection and genotyping

T. cruzi infection was evaluated by detection of the variable region of minicircle kinetoplast DNA by polymerase chain reaction (PCR) using primers 121 and 122 to amplify a product of 330 base pairs (Wincker et al., 1994). Each specimen sample was assayed twice. Samples with two positive or negative results were considered positive and negative, respectively. The significance of *T. cruzi* detection between insects from insular and continental areas was estimated with a Chi-squared test. For genotyping, PCR DNA blots were prepared using 10 μL of each PCR product. Four *T. cruzi* corresponding to Tc I, Tc II, Tc V and Tc VI were used to generate specific DTU probes. The construction of minicircle probes was performed as described Campos-Soto et al. (2016). The PCR products were transferred onto Hybond N + nylon membranes (Amersham, Piscataway, NJ), and cross-linked by ultraviolet light for DNA fixation. After transferring the PCR products, four membranes were pre-hybridized for at least 2 h at 55 °C. Each membrane was then hybridized with a DTU-specific probe labeled with adenosine triphosphate by the random priming method (^{32}P-α-dATP 1×10^6 cpm/membrane). Four copies of identical membranes containing DNA blots were hybridized against a panel of the DNA probes that recognize the specific DTUs Tc I, Tc II, Tc V and Tc IV, which are the main DTUs circulating in Chile as described in Campos-Soto et al. (2016). After hybridization, membranes were washed under high-

stringency conditions and then exposed in a Molecular Imager FX (Bio-Rad Laboratories, Hercules, CA).

3. Results

A new insular zone of the genus *Mepraia* from Santa Maria Island (Antofagasta Region) is reported (Table 1). The positive insects out of the total analyzed from the studied zones were: Santa Maria island 10/30, Santa Maria continent 9/15, Pan de Azucar island 3/6 and Pan de Azucar continent 5/7. The geographical localities and *T. cruzi* infection rates are described in Table 1. Insect form continental areas showed higher *T. cruzi* infection than insular areas (Chi-squared = 4.16, $P = 0.041$). Five and seven positive samples were genotyped from Santa Maria Island and Santa Maria continent, respectively. Prevalent *T. cruzi* DTUs from the studied zones are shown in Table 1; mixed infection was observed in samples from insular and continental zones (see lanes 5, 10, 12 and 13 Fig. 1). Both TcI and TcII were present in Santa Maria Island and continent, and additionally TcV in the continent. In Pan de Azucar Island TcII, TcV and TcVI were present while in the continent only TcVI was observed (Table 1, Fig. 1).

4. Discussion

Sagua Franco et al. (2000) reported an insular population of the genus *Mepraia* in Pan de Azucar Island (Atacama Region). This zone is currently assigned to the *M. parapatrica* lineage (Frías, 2010). Insects from Santa Maria Island are located between the distribution of *M. parapatrica* and *M. gajardoi*; because this is a new report, the phylogenetic clustering of these insects is unknown. However, the morphological characters of these insects agree with *M. gajardoi* lineages according to Frías (2010). Insects form Pan de Azucar Island fed mainly on seabirds (78%), reptilians (7%) and a few marine mammals (5%); no *T. cruzi* infection was found by light microscopy (Sagua Franco et al., 2000); in this study the non-infection in these zones was explained because birds and reptiles are refractory to the *T. cruzi* infection (Kierszenbaum et al., 1976; Urdaneta-Morales and McLure, 1981). However, another explanation is the poorly sensitive technique performed to detect *T. cruzi*. We demonstrated *T. cruzi* infection by the more sensitive PCR technique, which increases the probability of *T. cruzi* detection at least four-fold (Botto-Mahan et al., 2005). It has also been demonstrated *T. cruzi* infection is less likely to be detected in insects in starved conditions (most wild insects). Detection increases after artificial feeding (Egaña et al., 2016, 2014). For this reason we performed *T. cruzi* detection in insects after a second feeding 4–5 weeks after arrival, which explains the high rate of infection detected. TcII is the lineage that circulates most in both insular zones, and this can be explained as it is an ancient lineage associated with the sylvatic cycle (Campos-Soto et al., 2016). Unidentified *T. cruzi* DTUs were observed in Santa Maria Island and continent (see lanes 1, 2, 3, 4, 7, 8, 9 and 11 in Fig. 1).This may be due to TcI or TcII variants that do not hybridize with the probes used, or that they are other DTUs (TcIII or TcIV) not analyzed. Mixed infections were present in both insular zones. This is a relevant result considering that mixed infections are more prevalent when the infection rate is high or there is a high diversity of mammals

Table 1
Geographical coordinates locality, *Trypanosoma cruzi* infection and discrete typing units (DTUs) in *Mepraia* specimens from insular and continent zones.

Locality	Latitude/ longitude	N°positives/ N° captured	*Trypanosoma cruzi* DTUs				
			TcI	TcII	TcV	TcVI	NN
Santa Maria Island	23°25'51"S/70°36'31"W	10/30 (33.3%)	1	1	0	0	4
Santa Maria Continent	23°28'3"S/70°37'2"W	9/15 (60%)	2	1	2	0	4
Pan de Azucar Island	26° 9'6"/70°41'7"W	3/6 (50%)	0	2	1	1	0
Pan de Azucar Continent	26°10'27"S/70°39'58"W	5/7 (71,4%)	0	0	0	5	0

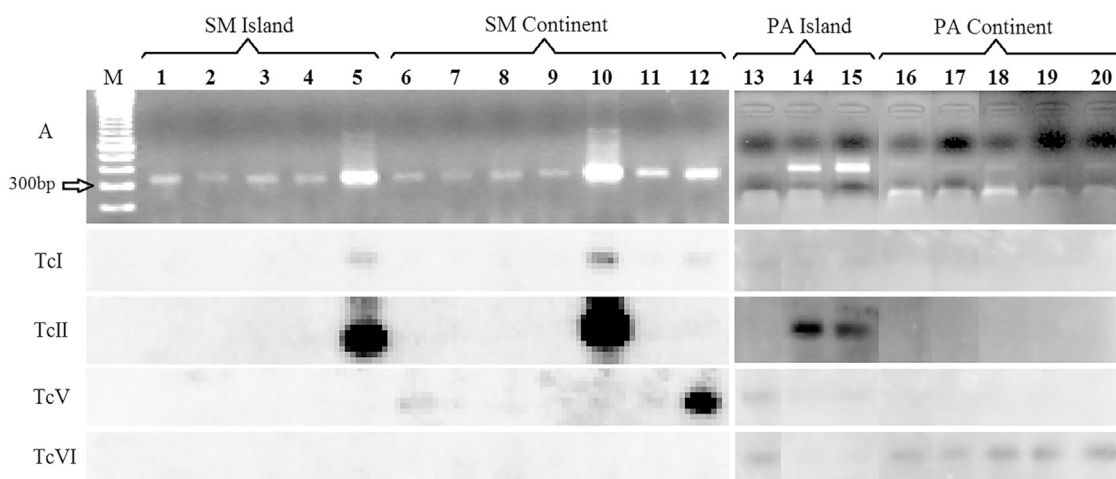(%), percentage of insects infected in each locality. NN, unidentified DTUs.

Fig. 1. *T. cruzi* DTUs detection by means of hybridization tests of *Mepraia* samples. A: Agarose gel electrophoresis of amplified kDNA minicircles stained with ethidium bromide (lanes 1–21). SM: Santa Maria locality. PA: Pan de Azucar locality. TcI, TcII, TcV and TcVI: Hybridization with TcI, TcII, TcV and TcVI probes respectively. Lane M: 100-bp DNA ladder.

carrying different *T. cruzi* DTUs (Campos-Soto et al., 2016). Mixed infections suggest that there are reservoirs maintaining a permanent infection in these habitats. The fauna of the islands is composed mostly of birds, reptiles and marine mammals, however several rodents and a cave inhabited by bats of the genera *Myotis* and *Desmodus* were described in Pan de Azucar National Park by Rundel et al. (1996). These mammals could be the reservoir hosts who maintain the infection cycle. It has been described infection with *T. cruzi* in wild bats associated with infected *Rhodnius prolixus* in a sylvatic habitat (Añez et al., 2009). The insects captured in Pan de Azucar Island were near to cave inhabited by bats. It has been suggested that the infected host density surrounding the vector colonies is a relevant variable for vector infection (Oda et al., 2014). Therefore in these ecotypes new approaches are necessary to reveal the role of bats and rodents in *T. cruzi* infection.

We found higher infection in continental areas which may be explained by a higher abundance and/or biodiversity of mammals which may host *T. cruzi*. In fact, several taxa such as the rodents *Oligoryzomys*, *Abrothrix* and *Phyllotis*, the mouse opossum *Thylamys*, the camelid *Lama*, and the fox *Dusicyon* were reported in the Pan the Azucar National Park (Rundel et al., 1996).

Sagua Franco et al. (2000) suggested that *Mepraia* presence in an insular zone could be explained through passive transportation by marine birds or prey birds. It can be inferred that birds can transport infected vectors to island zones. New studies should evaluate these hypotheses, and determine the divergence and gene flow between these vector populations as well as the origin of insular *Mepraia* populations.

5. Conclusion

This study demonstrated *T. cruzi* infection in *Mepraia* specimens from insular zones and described the main *T. cruzi* DTUs that are circulating in the sylvatic cycle. The occurrence of mixed and unidentified infections suggests that reservoir interaction in these habitats is complex and unknown. Therefore new approaches are necessary to reveal the role of bats and rodents as *T. cruzi* reservoirs in these habitats as well as approaches that reveal the origin of insular *Mepraia* populations.

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