Acta Tropica 162 (2016) 171–173

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Feeding profile of *Mepraia spinolai*, a sylvatic vector of Chagas disease in Chile

F. Chacón[a], A. Bacigalupo[b], J.F. Quiroga[a], A. Ferreira[c], P.E. Cattan[b,*], G. Ramírez-Toloza[a,*]

[a] Departamento de Medicina Preventiva Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Avenida Santa Rosa 11735, La Pintana, Santiago, Chile
[b] Departamento de Ciencias Biológicas Animales, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Avenida Santa Rosa 11735, La Pintana, Santiago, Chile
[c] Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Avenida Independencia 1027, Independencia, Santiago, Chile

ARTICLE INFO

Article history:
Received 10 December 2015
Received in revised form 14 June 2016
Accepted 23 June 2016
Available online 25 June 2016

Keywords:
Sylvatic triatomine
Serum antigens
Food source
Chagas disease

ABSTRACT

American trypanosomiasis is a chronic disease transmitted mainly by vectors. The hematophagous triatomine vectors transmit *Trypanosoma cruzi* to a wide variety of mammals, which usually are their food source. This study determined the feeding profile of *Mepraia spinolai*, a sylvatic triatomine vector, present in endemic areas of Chile. Vectors were captured in the north-central area of Chile. Samples of intestinal contents were analyzed by an Enzyme-linked immunosorbent assay (ELISA) that identifies and discriminates the presence of serum antigens from *Homo sapiens* and nine animal species (*Canis familiaris, Felis catus, Capra hircus, Mus musculus, Gallus gallus, Octodon degus, Thylamys elegans, Phyllotis darwini* and *Oryctolagus cuniculus*). Our data indicate the most frequent feeding source in this area was *P. darwini*, followed by *O. degus*, *O. cuniculus*, *M. musculus*, *G. gallus*, *T. elegans*, *C. familiaris*, *F. catus* and *C. hircus*. Mixed food sources were also identified.

1. Introduction

Chagas disease, or American trypanosomiasis, is a potentially fatal parasitic disease caused by *Trypanosoma cruzi*. There are 6–7 million people infected worldwide, mostly in Latin America, where the disease is considered endemic (Toso et al., 2011; WHO, 2015). In Chile, the traditional endemic area encompasses the Region of Arica y Parinacota to the O'Higgins Region, including the Metropolitan Region (Apt et al., 2008). *T. cruzi* infects a wide host range, including around 150 species of both wild and domestic mammals, and humans (Schofield, 2000). It has been described in different wild mammals, including: *Octodon degus*, *Thylamys elegans* and *Phyllotis darwini* (Lent and Wygodzinsky, 1979).

The protozoan is transmitted to humans by food contaminated with the parasite, transfusion of infected blood, transmission from infected mother to her child during pregnancy or childbirth, organ transplant from an infected person, and laboratory accidents, although the most epidemiologically significant is the vectorial transmission, achieving 80% of the total incidence (WHO, 2015).

There are four triatomine species in Chile: *Triatoma infestans*, *Mepraia spinolai*, *Mepraia gajardoi* and *Mepraia parapatrica*, distributed in the extreme north of the country (*M. gajardoi* and *T. infestans*), in the north (*T. infestans*, *M. parapatrica* and *M. spinolai*) and in the north-central area (*T. infestans* and *M. spinolai*) (Apt et al., 2008; Frias et al., 1995; Schofield, 2000).

Mepraia spinolai, principally involved in the wild cycle, lives in sylvatic ecotopes such as hills, stony grounds, bird nests, crevices, holes (Apt and Reyes, 1990), bromeliads (Bacigalupo et al., 2006) and occasionally, in rustic buildings (Schenone et al., 1980). This vector is mainly diurnal (Canals et al., 1998) and strictly hematophagous (Lent and Wygodzinsky, 1979; Maekelt, 1983). They consume blood from any available animal, showing a general sit-and-wait strategy for host finding (Botto-Mahan et al., 2005; Schofield, 1994). A strict relationship among vector, environment and possible blood sources has been proposed (Apt and Reyes, 1990; Schenone et al., 1985). Moreover, *M. spinolai* has the potential to invade human dwellings when its habitat has been intervened (Canals et al., 1994), mainly by human encroachment into wild environments (Canals et al., 1994; Schenone et al., 1985, 1980).

The feeding profile of *M. spinolai* in the wilderness has been poorly described. Some studies indicate that goats, birds, cats, dogs, rodents and humans are important feeding sources to *M. spinolai* (Knierim et al., 1976; Schenone et al., 1985). According to samples

* Corresponding authors.
E-mail addresses: pcattan@uchile.cl (P.E. Cattan), galiaram@uchile.cl, galiaramirez@gmail.com (G. Ramírez-Toloza).

http://dx.doi.org/10.1016/j.actatropica.2016.06.027
0001-706X/

from 1995 to 1996, the bugs had ingested blood from rabbits, dogs, goats, rodents, humans and birds – arranged in decreasing order (Canals et al., 2001).

Herein, we aim at describing the feeding profile of *M. spinolai* in an endemic area of Chile. By using an Enzyme-Linked Immunosorbent Assay (ELISA), we identified serum antigens from 10 specific species in the intestinal contents of these insects. A panel of 9 anti-sera, raised against serum antigens from each of the relevant animal species, was used. Thus, important information has been obtained with regard to the feeding profile.

2. Materials and methods

2.1. Capture and collection areas

One-hundred seventy-three *M. spinolai* were captured during the 2011–2013 period in the following Chilean North-Central communes: Monte Patria (30°41′S), and Illapel (31°37′S) (Coquimbo Region); Petorca (32°15′S) and Putaendo (32°38′S) (Valparaíso Region); and Til–Til (33°05′S) (Metropolitana Region), using yeast or mouse baited traps.

Then, we obtained 5 μL of intestinal contents by abdominal compression of each insect as described by Schenone et al. (1985, 1980) and Canals et al. (2001). The intestinal contents were stored in about 50 μL of buffer Na_2CO_3 0.1 M pH 9.6 at −20 °C until analysis. According to Bradford analyses (Bradford, 1976), protein concentration in the samples ranged between 0.4–2.2 μg/μL.

2.2. Identification of the triatomine feeding sources

Our study covered domestic, peridomestic and sylvatic host species, which were present in previous studies (Canals et al., 1998). To identify the triatomine feeding sources we used rabbit antisera against serum antigens, generated and basically used as described in Canals et al. (2001) and Molina et al. (2004). These antisera were generated against serum proteins from 7 mammals (*Homo sapiens, Canis familiaris, Felis catus, Capra hircus, Mus musculus, Octodon degus, Thylamys elegans* and *Phyllotis darwini*) and one bird species (*Gallus gallus*). The antigen-antibody reactions were analyzed by ELISA. To define the antisera dilution that specifically recognizes serum antigens from a given species, we performed an ELISA using serial dilutions of each antiserum against the sera from each animal species until determining a specific dilution that reacted only with the homologous species. The specific antisera dilution were: anti-*C. familiaris*: 1:800,000; anti-*F. catus*: 1:100,000; anti-*H. sapiens*: 1:512,000; anti-*C. hircus*: 1:64,000; anti-*M. musculus*: 1:50,000; anti-*G. gallus*: 1:56,000; anti-*O. degus*: 1:40,000; anti-*T. elegans*: 1:64,000 and anti-*P. darwini*: 1:120,000. As a secondary antibody, we used a polyclonal goat anti-rabbit immunoglobulin/HRP (P0448, Dako®) diluted 1:2000. This antibody was also used to identify serum antigens from *Oryctolagus cuniculus*. The substrate azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was used. The reaction was measured at 405 nm in a plate reader (Bio-Rad®). The standardized ELISA detected approximately 10 pg IgG/mL of, highly sensitive for our purposes.

To analyze the presence of serum antigens from different animals in the intestinal contents, we used 96-well, flat-bottomed, polyvinylchloride (PVC) microtiter plates for ELISA. Eleven wells were sensitized with 100 μL of triatomine intestinal contents diluted in Phosphate Buffered-Saline (PBS) (1:25). The plate was incubated at 37 °C for two hours and then washed four times with PBS-Tween 20, 0.05% v/v. The wells were blocked with 200 μL of PBS- 3% bovine serum albumin (BSA) and incubated for 2 h at 37 °C. One-hundred microliters of each specific antiserum were added in the dilutions indicated above. Each intestinal content was confronted with the entire panel of anti-sera. After incubation, the secondary antibody and ABTS were added, and the plate was read at 405 nm in an ELISA reader (Bio-Rad ®). In order to assess a positive reaction, a cut-off value was determined, at two standard deviations above the mean for negative sera, corresponding to an O.D. of approximately 0.2 nm.

Table 1
Host feeding profile of *M. spinolai*.

Species	Common name	Positive reactions	
		n	%
Canis familiaris	Dog	1	2.4
Felis catus	Cat	1	2.4
Homo sapiens	Human	0	0.0
Capra hircus	Goat	1	2.4
Mus musculus	Mouse	3	7.3
Gallus gallus	Chicken	3	7.3
Octodon degus	Degu	9	22.0
Thylamys elegans	Elegant Fat-tailed Opossum	2	4.9
Phyllotis darwini	Darwin's Leaf-eared Mouse	14	34.2
Oryctolagus cuniculus	Rabbit	7	17.1
Total		41	100

3. Results and discussion

In 38 out of the 173 *M. spinolai* (21.9%), the hosts were identified. Most samples reacted with one antiserum (92.1%), three with two (7.9%), indicating that these insects fed, at least on 2 different animal species. These three individuals were alternatively positive to blood components from the following couple of mammal species: *P. darwini* and *C. hircus*; *P. darwini* and *M. musculus*; and *O. degus* and *G. gallus* (Table 1).

However, it is likely that individuals with negative reactions had consumed blood from species (*i.e.* from reptiles) not included in this panel (Sagua et al., 2000). Concomitantly or alternatively, since triatomines are able to endure long periods of fasting (Canals et al., 2001; Schofield, 1994), had passed all blood from the storage region to the digestive part, having digested the proteins that had induced the antibody synthesis. The samples studied in this report came from wild insects, therefore the time between last feeding and sample collection of intestinal contents could be quite broad, thus justifying the low number of positive samples.

The most frequent animal species used by these parasites corresponds to *P. darwini* (34.1%), followed by *O. degus* (22%), *O. cuniculus* (17.1%) and *T. elegans* (4.8%). The dominance of *P. darwini* and *O. degus* as hosts of *M. spinolai*, either alone or in combination with other species, could be explained by an increase in their populations or by their higher proportion, compared to other mammals in endemic areas (Jimenez et al., 2015; Lent and Wygodzinsky, 1979; Oda et al., 2014). On the other hand, these mammals share habitats with triatomine insects in rocky environments and on slopes of northern exposure (Jimenez and Lorca, 1990). Thus, *T. cruzi* infected *O. degus* travels shorter distances than uninfected ones. In contrast, infected *P. darwini* increases their displacement area (Jimenez et al., 2015), thus amplifying the contact between this rodent and *M. spinolai*. This fact makes the *P. darwini* and *O. degus* a readily available food supply (Schofield, 1994). Moreover, an increased presence of *T. cruzi* in wild rodents has been described (Botto-Mahan et al., 2010; Lent and Wygodzinsky, 1979). *O. cuniculus* was expected to be a frequent blood source for *M. spinolai* because they had been found infected with *T. cruzi* in previous studies, and they share the sylvatic habitat with *M. spinolai* (Botto-Mahan et al., 2009; Botto-Mahan et al., 2005). Therefore, it was expected that they appear prominently in a study of food profile in triatomines. In this regard, the low participation of *T. elegans* in the diet of wild triatomines, that share habitat with these insects (Canals et al., 2001), can be

explained by its low population density compared to rodents such as the *P. darwini* and *O. degus* (Lima et al., 2001; Lobos et al., 2005). Also, these animals have an insectivorous diet (Palma, 1997) and probably, only a few triatomine insects escape their predatory habits.

Serum antigens from *M. musculus* were detected in 3 bugs (7.3%). This species is synanthropic, which may represent a nexus between the wild and domestic cycles of Chagas disease. Also, this species behaves as an invasive rodent that remains relatively close to human dwellings within the sylvatic habitat (Lobos et al., 2005). Domiciliary and peridomiciliary species such as *C. familiaris*, *F. catus*, *C. hircus* and *G. gallus* were less frequently detected in intestinal contents. Three samples were positive for *G. gallus* (7.3%) and only one for *C. familiaris* (2.4%), *F. catus* (2.4%) and *C. hircus* (2.4%) (Table 1).

No sample was positive to *H. sapiens*, in contrast with a previous study where 4.62% of samples were positive (Canals et al., 2001). This result was expected, since *M. spinolai* individuals are often captured in stony hills, rock crevices, nest of birds and mammals, and only occasionally are found in human dwellings (Canals et al., 1998; Frias et al., 1995; Lent and Wygodzinsky, 1979). The fact that sampling was conducted in rural sylvatic sites could explain the low detection of domiciliary animals and the missing detection of human blood. On the other hand, Chile has controlled successfully the domiciliary vector *T. infestans* by means of intensive house spraying (Cattan et al., 2002). This control may have an effect over *M. spinolai* in peridomestic areas, keeping this vector away from domiciliary sources.

Knowledge of the feeding profile of sylvatic triatomines can help us to understand their potential peridomestic habitats, their possible incursions into human habitats (Salvatella et al., 1995) and a variety of other relationships between vectors and *T. cruzi* hosts. This is important to generate preventive strategies and focus efforts to control Chagas disease.

In this study, we provide original data regarding the feeding profile of a sylvatic *T. cruzi* vector *M. spinolai*, which is composed mainly by sylvatic hosts, although some synanthropic species are included, probably because of their intrusion to the sylvatic environment.

Acknowledgements

This work was supported by Grants from the 'Fondo Nacional para el Desarrollo Científico y Tecnológico', FONDECYT, CHILE: 11110251 (G.R-T) and 1100339, 1140650 (P.E.C).

References

Apt, W., Reyes, H., 1990. Algunos aspectos de la Enfermedad de Chagas en Latinoamérica. Parasitol Día 14, 23–40.

Apt, W., Heitmann, I., Jercic, M.I., Jofré, L., Muñoz. del, V.P., Noemí, I., San Martín, A.M., Sapunar, J., Torres, M., Zulantay, I., 2008. Guéas clénicas de la enfermedad de Chagas: parte I. Introducciñn y epidemiologéa. Rev. Chil. Enfectol. 25, 189–193.

Bacigalupo, A., Segura, J.A., García, A., Hidalgo, J., Galuppo, S., Cattan, P.E., 2006. Primer hallazgo de vectores de la enfermedad de Chagas asociados a matorrales silvestres en la Región Metropolitana, Chile. Rev. Med. Chil. 134, 1230–1236.

Botto-Mahan, C., Cattan, P.E., Canals, M., Acuna, M., 2005. Seasonal variation in the home range and host availability of the blood-sucking insect *Mepraia Spinolai* in wild environment. Acta Trop. 95, 160–163.

Botto-Mahan, C., Acuña-Retamar, M., Campos, R., Cattan, P.E., Solari, A., 2009. European rabbits (*Oryctolagus cuniculus*) are naturally infected with different *Trypanosoma cruzi* genotypes. Am. J. Trop. Med. Hyg. 80, 944–946.

Botto-Mahan, C., Campos, R., Acuna-Retamar, M., Coronado, X., Cattan, P.E., Solari, A., 2010. Temporal variation of *Trypanosoma cruzi* infection in native mammals in Chile. Vector Borne Zoonotic Dis. 10, 317–319.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.

Canals, M., Cattan, P., Ehrenfeld, M., 1994. Sobrevivencia de *Triatoma spinolai* en ambiente habitacional. Parasitol Día 18, 82–87.

Canals, M., Bustamante, R.O., Ehrenfeld, M.H., Cattan, P.E., 1998. Assessing the impact of disease vectors on animal populations. Acta Biotheor. 46, 337–345.

Canals, M., Cruzat, L., Molina, M.C., Ferreira, A., Cattan, P.E., 2001. Blood host sources of *Mepraia spinolai* (Heteroptera: reduviidae), wild vector of Chagas disease in Chile. J. Med. Entomol. 38, 303–307.

Cattan, P.E., Pinochet, A., Botto-Mahan, C., Acuna, M.I., Canals, M., 2002. Abundance of *Mepraia spinolai* in a periurban zone of Chile. Mem. Inst. Oswaldo Cruz 97, 285–287.

Frias, D., Solari, A., Gonzáles, C., Henry, A., Alviña, A., 1995. Índices de infección de *Mepraia spinolai* con *Trypanosoma cruzi*, su invasión a ambientes domésticos e interacción con *Triatoma infestans*. Parasitol Día 19, 195.

Jimenez, J., Lorca, M., 1990. American Trypanosomiasis in wild vertebrates and its relation to the vector *Triatoma-spinolai*. Arch. Med. Vet. 22, 179–183.

Jimenez, C., Fonturbel, F.E., Oda, E., Ramirez, P.A., Botto-Mahan, C., 2015. Parasitic infection alters rodent movement in a semiarid ecosystem. Mamm.Biol. 80, 255–259.

Knierim, F., Castro, M., Villarroel, F., Schenone, H., 1976. Preliminary studies on *Triatoma infestans* and *Triatoma spinolai* source of feeding by the use of gel double diffusion test (author's transl). Bol. Chil. Parasitol. 31, 34–36.

Lent, H., Wygodzinsky, P.W., 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. Bull. Am. Mus. Nat. Hist. 163, 130–138.

Lima, M., Stenseth, N.C., Yoccoz, N.G., Jaksic, F.M., 2001. Demography and population dynamics of the mouse opossum (*Thylamys elegans*) in semi-arid Chile: seasonality, feedback structure and climate. Proc. Biol. Sci. 268, 2053–2064.

Lobos, G., Ferres, M., Palma, R., 2005. Presencia de los géneros invasores *Mus* y *Rattus* en áreas naturales de Chile: un riesgo ambiental y epidemiológico. Rev. Chil. Hist. Nat. 78, 113–124.

Maekelt, G., 1983. La epidemiología de la enfermedad de Chagas en relación con el ecosistema domiciliario. Interciencia 8, 353–360.

Molina, M.C., Cattan, P., Canals, M., Cruzat, L., Aguillon, J.C., Ferreira, A., 2004. A simple immunometric assay to assess the feeding habits of *Meprai spinolai*, a *Trypanosoma cruzi* vector. Parasitol. Res. 92, 375–379.

Oda, E., Solari, A., Botto-Mahan, C., 2014. Effects of mammal host diversity and density on the infection level of *Trypanosoma cruzi* in sylvatic kissing bugs. Med. Vet. Entomol. 28, 384–390.

Palma, R., 1997. *Thylamys elegans*. Mamm. Species, 1–4.

Sagua, H., Araya, J., Gonzalez, J., Neira, I., 2000. *Mepraia spinolai* in the Southeastern Pacific Ocean coast (Chile) – first insular record and feeding pattern on the Pan de Azucar island. Mem. Inst. Oswaldo Cruz 95, 167–170.

Salvatella, R., Rosa, R., Basmadjian, Y., Puime, A., Calegari, L., Guerrero, J., Martinez, M., Mendaro, G., Briano, D., Montero, C., et al., 1995. Ecology of *Triatoma rubrovaria* (Hemiptera, Triatominae) in wild and peridomestic environments of Uruguay. Mem. Inst. Oswaldo Cruz 90, 325–328.

Schenone, H., Villarroel, F., Rojas, A., Alfaro, E., 1980. Factores biológicos y ecológicos en la epidemiología de la Enfermedad de Chagas en Chile. Bol. Chil. Parasitol. 35, 42–45.

Schenone, H., Christensen, H., Vásquez, A.M., González, C., Méndez, E., Rojas, A., Villarroel, F., 1985. Fuentes de alimentación de triatomas domésticos y su implicancia epidemiológica en relación a Enfermedad de Chagas en áreas rurales de siete regiones de Chile. Bol. Chil. Parasitol. 40, 34–38.

Schofield, C.J., 1994. Triatominae: Biology & Control. Eurocommunica Publications.

Schofield, C., 2000. Trypanosoma cruzi ¾ the vector-parasite paradox. Mem. Inst. Oswaldo Cruz 95, 535–544.

Toso, A., Vial, F., Galanti, N., 2011. Transmisión de la enfermedad de Chagas por vía oral. Rev. Med. Chil. 139, 258–266.

WHO, 2015. La enfermedad de Chagas (tripanosomiasis americana). Organización Mundial de la Salud.