

Blood Host Sources of *Mepraia spinolai* (Heteroptera: Reduviidae), Wild Vector of Chagas Disease in Chile

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ABSTRACT There are two vectors of the Chagas' disease in Chile: *Triatoma infestans* Klug the domestic vector and *Mepraia spinolai* Porter the sylvatic vector. The alimentary profile of *M. spinolai* has been poorly studied. In this work we study the participation of humans, goats, dogs, cats, rodents, rabbits, birds (hens), and reptiles in the diet of *M. spinolai* by analyzing the intestinal content through immunoradiometric assay. To put our results in a general context, we also compared the diet with that described for *T. infestans*. In decreasing order, we detected blood of rabbits, dogs, goats, rodents, humans, and birds (hens). There were 12.3% of insects infected with *T. cruzi*, but this fact was not significant for diet variance. In warm weather there was a larger diversity of alimentary sources than in a cold one. The niche breadth increased from 0.029 in cold weather to 0.464 in warm weather. The niche overlap of *T. infestans* and *M. spinolai* was 0.23.

KEY WORDS *Mepraia spinolai*, wild insects, feed sources, Chagas disease, Chile

SOME 60 OUT 126 Triatominae (Hemiptera: Reduviidae) have been reported as potential vectors of *Trypanosoma cruzi*, the agent of Chagas disease (Schofield 1994). Most of them are wild species (Maeckelt 1983) but some have invaded and colonized domestic and peridomestic habitats (Schenone et al. 1980, Schofield 1994). Wild vectors of *T. cruzi* are of relatively low epidemiological interest because of their low visitation frequency of human dwellings and neighboring places (Schenone et al. 1980). However, there is increasing interest to determine their behavior and ecology (Canals et al. 1997, 1999a). For example, *Triatoma rubrovaria* Blanchard and *M. spinolai* live mainly in sylvan ecotopes (Apt and Reyes 1990), but they have also been reported as human dwelling colonizers (Salvatella 1986, Salvatella et al. 1991, Canals et al. 1994).

Currently, only two species of Triatominae has been recognized as vectors of *T. cruzi* in Chile, *T. infestans* and *M. spinolai* (*T. spinolai*; Lent et al. 1994) both having similar distributions between 18 and 34° 30' S (Apt and Reyes 1990). However, *Mepraia gajardoi* Frías, Henry & González, a new species of triatomine, recently described in Chile, is restricted between 18 and 26° S. Thus, it is possible that the northernmost

limit of *M. spinolai* was 26°S, and its populations within 18 and 26° S correspond to the new species (Frías et al. 1998). Although *T. infestans* lives in domestic and peridomestic habitats, *M. spinolai* lives in sylvan ecotopes such as hills, stony grounds, bird nets, crevices, and holes (Apt and Reyes 1990). Occasionally, it has been found in rustic buildings (Schenone et al. 1980). Both species are strictly hematophagous (Lent and Wygodzinsky 1979, Maeckelt 1983): *T. infestans* is a nocturnal species and *M. spinolai* is diurnal (Canals et al. 1997). The feeding habits of *T. infestans* has been well studied. It feeds on the blood of endothermic animals, with a significant preference for human blood (Maeckelt 1983). In Chile, human blood accounts for 68% of its diet. It also obtains blood from cats, dogs, birds (hens), goats, and some rodents. (Schofield 1979, Maeckelt 1983, Schenone et al. 1985). In contrast, the feeding habits of *M. spinolai* are not well known. There have been reports, based on a few individuals (Knierim et al. 1976, Schenone et al. 1985, Apt and Reyes 1986), that show goats, birds (hens), cats, dogs, rodents, and humans as hosts (Apt and Reyes 1986).

The aim of this study was to determine the importance of humans, goats, dogs, cats, rodents, rabbits, birds (hens), and reptiles of sylvan, domestic and peridomestic habitats in the host-feeding profile of *M. spinolai* in a zone of epidemiological risk in Chile.

Materials and Methods

We collected 130 individual specimens of *M. spinolai* (15 adults, 38 fifth instars, 47 fourth instars, 26 third instars, and four second instars) in quarries of a peri-urban zone in Santiago, Chile (33° 17' S). The study area is located 15 km from the center of the city and

The experimental procedures using animals where in agreement with the International Guiding Principles for Biomedical Research Involving Animals (NIH) and comply with the "Principles of Animal Care" publication No. 86–23 (1995) of the National Institutes of Health.

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corresponds with a modified xeric steppe, where hawthorn (*Acacia caven*) is predominant. The ground cover is herbs and wild grasses, but stones and rocks from the quarries are dispersed elsewhere. The area is surrounded by farms and a sector of urbanized cottages. Climatic conditions include a warm summer and a cold winter. Annual rainfall reaches 250 mm, concentrated in the winter. Although a few humans who work in quarries were present, the area was inhabited mainly by rodents, rabbits, reptiles (*Liolaemus* sp.), some foxes (*Pseudoalopex culpaeus*), and domestic animals including cats, dogs, hens (chickens), and goats. Individual *M. spinolai* were captured exclusively on the stones of quarries at 1200 hours (noon). The following procedure was used to capture the insects: a person standing on the rocks was used as bait to attract the insects. When an insect climbed onto a stone near the sampler, the insect was pushed (using a brush) into a collecting jar. Captures were done between December 1995 and October 1996. For data analysis, the study period was divided into cold and warm weather: *cold weather* (from 21 March to 21 June, corresponding to the autumn season, with an average temperature of $18.8 \pm 4.17^{\circ}\text{C}$) and *warm weather* (from 21 September to 21 March, corresponding to spring and summer, with an average temperature of $27.15 \pm 2.82^{\circ}\text{C}$).

The intestinal contents of each insect were removed by means of abdominal compression using the methodology of Schenone et al. (1980, 1985), and inspected by microscopy for the presence of *T. cruzi*. Each intestinal sample was mixed with 50 μl of PBS-azide buffer, centrifugated at $10,000 \times g$, and frozen at -20°C until analysis.

To analyze the presence of blood in the intestinal content of the insects, lapine antiserum antiseric antigens were used. These antiseric antigens were generated in seven New Zealand white rabbits that had been immunized following a modified Coligan protocol (Coligan et al. 1995). Protein content in serum (Ag) was determined using the method published by Bradford (1976). The antibodies resulting from rabbit infections were measured by immunoradiometric assay (IRMA) as reported by Catt and Tregear (1967). The intestinal contents were observed for the presence of seric antigens of: humans, mice (rodents), dogs, cats, goats, hens, and reptiles (*Liolaemus* sp.). The antiserum was also used to determine the occurrence of cross-reactions among hosts; several confirmability assays were performed on insects that had fed on a human (M.C.) and a hen (M.C.M., unpublished data). All of the antigen-antibody reactions were analyzed by immunoradiometric assay. In this assay we used the IgG of goat anti-IgG of rabbit purified by affinity and radiolabeled with ^{125}I (Fraker and Speck 1978). Pre-immune serum and PBS-Soya were used as negative controls and the intestinal contents of an insect that had been fed on hen blood were the positive control. A sample of positive results with a pre-immune serum indicated the presence of seric Ag of rabbit in the intestinal contents of the insect analyzed,

increasing to eight the possible alimentary sources (species) of *M. spinolai*.

To compare the proportion of individuals in which we detected their host blood source (P) between cold and warm weather periods and between insects infected and not infected by *T. cruzi*, we used the chi-square test. In addition, we used the same test to compare the different host blood source under the different weather and infection conditions.

To put our results in a general context, the dietary niche breadth was estimated by using Levin's index ($B = 1/\sum p_i^2$) modified by Colwell and Futuyma (1971):

$$B_{\text{sta}} = (B_{\text{obs}} - B_{\text{min}}) / (B_{\text{max}} - B_{\text{min}})$$

and the dietary overlap with the index described by Pianka (1974):

$$O_{ij} = \sum (p_{ik} \cdot p_{jk}) / \sqrt{(\sum p_{ik}^2 \cdot \sum p_{jk}^2)},$$

We compared our results with the data on *T. infestans* published by Schenone et al. (1985). In these indexes, p_i is the proportion of the source i in the diet of a species, B_{obs} , with B_{min} and B_{max} are the observed minimum and the maximum values possible for the B , respectively. The p_{ik} and p_{jk} were the proportion of the source k in the diet of the species i and j , respectively.

Results

Fifty-six out of 130 *M. spinolai* showed a positive reaction with one or more antiserum (43.1%): 29 with only one, 11 with two, three with three, and three with four antiserum (Table 1). In decreasing order, the prevalence of feeding sources were rabbits, dogs, goats, rodents, humans, and birds (hens). Reptilian blood was not detected in the intestinal content of any insects collected. Twenty-six of the individuals (12.3%) were infected with *T. cruzi*.

Differences in the host blood-source among the different seasons were found to be significantly different ($\chi^2 = 12.43$, $P < 0.01$; Table 2). In warm weather, a greater diversity in host blood-source occurred than during cold weather. The niche breadth increased from 0.029 in cold weather to 0.464 in warm weather. However, the proportion of individuals in which we identified its host blood-source (P) was neither significantly affected by temperature ($\chi^2 = 2.61$, $P > 0.05$) nor by infection with *T. cruzi* ($\chi^2 = 0.28$, $P > 0.05$). Infection with *T. cruzi* did not significantly affect the quality of the diet ($\chi^2 = 2.65$, $P > 0.05$) (Table 2).

The standardized niche breadth of *M. spinolai* was $B_{\text{sta}} = 0.29$; nearly two times greater than $B_{\text{sta}} = 0.15$ of *T. infestans* (Table 2). The niche overlap of these two species was $O_{is} = 0.23$.

Discussion

Because the antiserum group used to screen most of the fauna within the study zone (rodents, rabbits, dogs, foxes, reptiles, birds (hens) and some other do-

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

	Weather		Total Bugs	%T N = 130	%i+ N = 56	%m+ N = 81	<i>T. cruzi</i> infection	
	Cold	Warm					+	-
Feed (-)	18	56	74	56.9			16	58
Feed (+)	21	35	56	43.1			10	46
Total bugs	39	91	130	100			26	104
H	0	6	6	4.62	10.71	7.41	0	6
R	0	8	8	6.15	14.28	9.88	2	6
Rb	20	23	43	33.02	76.79	53.09	8	35
C	0	3	3	2.31	5.36	3.7	0	3
D	1	9	10	7.69	17.86	12.35	1	9
G	0	10	10	7.69	17.86	12.35	1	9
L	0	0	0	0	0	0	0	0
B	1	0	1	0.77	1.79	1.24	0	1
Samples (+)	22	59	81				12	69

Feed (-): the number of bugs negative; Feed (+): the number positive to some antiserum. The following rows show the number of samples positives to: humans (H), rodents (R), rabbit (Rb), cat (C), dog (D), goat (G), reptiles (L) and birds (B). The symbols %T, %i+ and %m+ represent percentage of the total number of bugs, percentage of the total number of positive individuals (absolute frequencies), and percentage of the total number of positive samples (relative frequencies) respectively. 56 bugs were (+); but 81 samples were (+). 17 bugs were (+) to more of one antiserum: 8 bugs (+) to R and Rb, 2 (+) to D and G, 1 (+) to Rb and G, 3 (+) to H, G, and D, and 3 (+) to H, C, D, and G.

mestic animals) we found that 43.8% (negative reactions) of the insects had not fed on any animal within the last 30 d. This was not surprising in triatomines, which have been reported to endure long periods of starvation (Schofield 1994). Triatomines also have a low rate of digestion, e.g., that in *T. infestans* may take 40 d.

The human blood index for *T. spinolai* shows that this insect is not an important vector for *T. cruzi*. Previous data reported 2.4% of these insects feeding on human blood (Apt and Reyes 1990), whereas here we recorded 4.6%. To assess the relative effect of different vectors on an animal population the most commonly used measure is the vectorial capacity (Garret-Jones et al. 1980); however, Canals et al. (1993) proposed addressing the following questions: (1) What is the probability that a susceptible host could become infected after being bitten by a vector insect (vectorial efficiency)? (2) How many potential infecting bites can a population of a vector insect distribute as a result of a bite on one infected host (vectorial capacity)? (3) Of a population of infected animals, what proportion has been infected by a given vector (vectorial effectiveness)? Canals et al. (1999b) evaluated their utility by using them on a published data set of *T. infestans* and *M. spinolai*. Results of this assessment showed that *T. infestans* has a vectorial efficiency 32 times the vectorial efficiency of *M. spinolai*. According to Canals et al. (1999b) the elimination of *T. infestans* implies

that the future of *T. cruzi* would depend on the extent to which *M. spinolai* human blood index and capability to colonize human dwellings increased.

It has been suggested that diurnal insects, such as *M. spinolai* (Canals et al. 1997), feed on reptiles (Schofield 1979, Salvatella et al. 1994). Moreover, Apt and Reyes (1990) reported that *Hepatozoon triatominae*, a protozoan parasite of reptiles, occurred in the intestinal content of an individual *M. spinolai*. However, our results did not show a reptilian host in the diet of *M. spinolai*. Schenone et al. (1985) using double diffusion in gel found 10 individuals that had fed on reptiles. Thus, it is probable that reptile feeding occurs infrequently.

The 12.3% of the insects that were infected by *T. cruzi* was within the range reported by several authors, which was near the average Chilean infection index of *M. spinolai* (11.4%, Apt and Reyes 1990).

Parasites may alter the feeding behavior of the vector insects and, therefore, enhance their chances of entering the vertebrate host (Molyneaux and Jefferies 1986). It has been reported that tse-tse flies, *Glossina* sp., probe the host more frequently and feed more voraciously when they are infected with *Trypanosoma brucei* (Jenni et al. 1980) and *T. congolense* (Roberts 1981). However, we found no differences in the proportion of fed insects, whether they were infected (16/26) or not infected (58/104) by *T. cruzi*. To our knowledge, there are no reports of the possible be-

Table 2. Host blood source profile and standardized niche breadth (Bs) of *M. spinolai* and *T. infestans*, and niche overlap (O) between both species

Species	W	H	C	D	Rb	R	A	B	L	An	B _s	O
<i>M. spinolai</i>	Cold	0.0	0.0	0.045	90.9	0.0	0.0	0.045	0.0	X	0.029	
	Warm	10.2	0.05	15.3	39.0	13.6	16.9	0.0	0.0	X	0.464	
	Both	7.4	3.7	12.3	53.1	9.9	12.3	1.2	0.0	X	0.29	
<i>T. infestans</i>	X	68.4	6.1	3.2	5.9	1.6	0.3	7.2	X	0.5	0.15	0.229

Weather (W), Humans (H), rodents (R), rabbits (Rb), cats (C), dogs (D), reptiles (L), birds (B), artiodactyles (A) (goats, caws, etc . . .) and amphibians (An). The results correspond to percentages of samples positives. X, unknown data. Data of *T. infestans* from Schenome et al. (1985).

havioral effects of *T. cruzi* on triatomines. There was no relationship between frequency of infected insects and feeding from a particular host. Feeding behavior of insects depends on the prey availability. Because of this it is possible to consider the relative abundance of a host as an indicator of epidemiological importance. Thus, the greater relative abundance of rabbits in the samples makes this mammal an important reservoir in the wild cycle of *T. cruzi*. The rabbit's ability for dispersion is very high; this fact explains why this leporid has been able to colonize a wide variety of habitats in central Chile.

We did not find statistically significant differences in the proportion of fed insects collected during the different seasons. At moderate temperatures, the insects showed similar proportions (21/39 versus 35/91). *M. spinolai* is a diurnal species, which is active from 0800 to 2000 hours, preferring temperatures that range at 25°C (Canals et al. 1997). When exposed to temperatures <20°C, *M. spinolai* hematophagous activity decreases and development is delayed (Ehrenfeld et al. 1998). A similar figure has been reported for *T. infestans* and several other triatomines (Jorg 1960, 1989; Garcia da Silva 1985; Schofield 1994). Our results suggest that *M. spinolai*, like other triatomines, is able to find warm and stable micro-environments for nests, and holes where it can feed on several animals (Zeledon 1983, Schofield 1994).

Nevertheless, differences in the host blood sources alimentary profile in different seasons were observed. Rodents, *Octodon degus*, and rabbits, *O. cuniculi*, are mammals that have their holes in the quarries, which are part of the study site, or in the immediate vicinity of the area, thus being ideal hosts for *M. spinolai* because they are available all the year. In warm seasons, *M. spinolai* takes blood meals on a wider range of hosts. Sinantropic animals such as cats and dogs were used for longer periods in the warm than in the cold seasons, exposing them to more insect bites. Also in warm seasons, the pasturing of goats and the number of humans working in the quarries increased. In addition, the larger dietary diversity of the warm season may have been the result of increased insect dispersion.

The insect population increased in the spring and summer (Pinochet 1997), when temperatures also increased. It is known that the temperature affects insect dispersion (Lehane et al. 1992). Another factor responsible for insect dispersion is hunger (Zeledon 1983, Lehane et al. 1992). When the population size of *T. infestans* increases, its nutritional condition decreases (Schofield 1994). Also during the summer, when it requires more blood, *T. protracta* moves to human dwellings from the nests of rodents (Zeledon 1983). We found no reports for *M. spinolai* dispersion, but it is possible that the following three factors, high temperatures, increased population, and hunger, were determinants of its dispersion pattern and a wider range of hosts.

When compared with *T. infestans* it appeared that *M. spinolai* was more of a general feeder. However, Zeledon and Rabinovich (1984) showed a wide range

of hosts for *T. infestans* based on individuals collected from domestic/peridomestic and wild habitats of two countries. In Chile, *T. infestans* occurred in human dwellings and in peridomestic habitats in which the potential hosts were mainly humans. Thus, the differences in host blood sources may simply reflect host availability in their different habitats. However, our data indicate that the standardized niche breadth of *M. spinolai* is nearly two times greater than the niche of *T. infestans*, suggesting that the wild insect may become a vector of a greater epidemiological importance, considering that the success of the control program for *T. infestans* implies that this latter vector has practically disappeared from human dwellings.

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