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A simple immunometric assay to assess the feeding habits of *Meprai spinolai*, a *Trypanosoma cruzi* vector

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Abstract We propose a simple assay to assess the importance of seven vertebrate species as food sources for *Mepria spinolai*, a wild arthropod vector of *Trypanosoma cruzi* (the agent of Chagas' disease). Rabbits were immunized with serum proteins from one of each of the seven species. After titration, a consensus 1/100,000 dilution of the immune sera detected vertebrate serum proteins in the intestinal contents of 48.9% of 131 insects tested. The high proportion of negative samples is consistent with previous information indicating that these insects can withstand prolonged fasting periods. Alternatively, they may have fed on a different animal species than those used to produce the antisera. In about 70% of the positive samples, only one species of serum protein was detected. All pre-immune sera were negative. In 67% of the positive vectors, rabbit immunoglobulins were detected directly by means of a specific goat antibody. Thus, rabbits may play a role in *T. cruzi* transmission.

Introduction

Chagas' disease (American trypanosomiasis) affects about 18 million people in Latin America (WHO 1995). It is caused by *Trypanosoma cruzi*, a flagellate parasite infecting both wild and domestic mammals, including humans (Chagas 1909). The most common and epidemiologically significant way of transmission is initiated

by metacyclic trypomastigotes present in the faeces of Triatominae, hematophagous arthropod insect vectors (Hemiptera: Reduviidae). Upon invading host cells, the parasites differentiate into intracellular amastigotes (Brenner 1973; Salvatella 1986; Schenone et al. 1991; Brenner and Gazzinelli 1997; De Souza 2002). From the 60 *Triatominae* species reported as potential vectors of *T. cruzi*, two are present in Chile: *Triatoma infestans*, Klug, the intradomiciliary vector, and *Mepraia spinolai*, Porter, the wild vector, increasingly found in peridomiciliary or domiciliary environments (Lent and Wygodzinsky 1979; Apt and Reyes 1986; Lent et al. 1994; Schenone et al. 1995; Schofield et al. 1998).

Knowledge of the feeding habits of potential *T. cruzi* vectors is necessary both from the epidemiological and epizootiological points of view in order to obtain relevant information with regard to potential wild and domestic parasite reservoirs. Until a recent report from our laboratories (Canals et al. 2001), the feeding profile of *M. spinolai* was poorly understood. Based on those findings, here we propose a simple immunometric assay that allows the detection of the feeding sources of *M. spinolai*. The assay relies on the specificity and sensitivity of rabbit polyclonal antibodies generated against serum components from a variety of vertebrate species, to detect the corresponding antigens present in the intestinal contents of the arthropod vectors.

Materials and methods

Generation of rabbit polyclonal antibodies against serum proteins from several vertebrate species

Blood from goats, dogs, cats, mice, chickens, and reptiles (*Lioalemus* sp.) was the source of serum proteins used as experimental immunogens. All experimental animals were handled by trained veterinary surgeons, following internationally accepted guidelines. Blood from healthy human volunteers was also obtained by standard procedures by trained medical personnel. Whole sera were obtained from all blood samples, using conventional methods. Four-month-old New Zealand White female rabbits were bled from the central ear artery, as a source of pre-immune sera. After 4 days, the animals were immunized 3 times, at weekly intervals, each one

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with approximately 50, 300 and 400 µg of serum proteins (Bradford 1976) respectively, from one of the vertebrate species. The first immunization was administered s.c., with complete Freund's adjuvant, while for the last two immunizations incomplete adjuvant was used with serum proteins partially depleted of BSA by precipitation with $(\text{NH}_4)_2\text{SO}_4$ at 50% saturation. The first two immunizations were administered s.c., and the last one i.p. After the last immunization, rabbits were bled 4 times at weekly intervals.

Antigens

M. spinolai vectors (131 individuals) were collected in the hills of the Pan de Azúcar mountain (Colina, Metropolitan Región, Chile). Only insects with some evidence of abdomen enlargement were used. Intestinal contents (5 µl) were extracted from the midgut, according to a standard xenodiagnostic technique (Minter-Goedbloed et al. 1978, Schenone et al. 1980). This material was dissolved with 50 µl PBS-azide 0.01% w/v. After centrifugation at 10,000 g, the protein contents of the supernatants were determined and the samples were stored at -20°C .

Immunoradiometric assay (Catt and Tregear 1967)

PVC microtitration plates were sensitized overnight, at 4°C , with 50 µl per well containing 1 µg total serum proteins. Alternatively, the intestinal contents of *M. spinolai*, diluted 1/50, in sodium carbonate buffer (0.005 M Na_2CO_3 /0.0035 M NaHCO_3), pH 9.6, was used. The intestinal content of *M. spinolai*, fed with chicken blood, was used as a positive control. The wells were blocked with 100 µl/well of PBS containing 0.5% w/v soybean proteins (PBS-SBP) (Aguillón et al. 1992), for 2 h at 37°C . The plates were washed 4 times with PBS containing 0.05% v/v Nonidet-P40. After titration of all rabbit pre-immune and immune sera against the corresponding antigens, a consensus 1:100,000 dilution was chosen. At this dilution, cross-reactivity was decreased to a minimum. Fifty microlitres of the antisera, diluted in PBS-SBP, was added to the microtitration wells in triplicate and incubated at 37°C for 2 h. The wells were washed, followed by addition of 25 µl/well (10^4 counts per minute) of affinity purified goat anti rabbit IgG (Sigma, Montana) (Fraker and Speck 1978), labelled with ^{125}I . After a 2-h incubation and five washes, the radioactivity associated with the wells was measured in a Ten-Detector gamma counter. The intestinal content of *M. spinolai* that had fed on rabbits was detected directly with a radio-labelled goat immunoglobulin anti rabbit IgG.

A sample was considered positive for a given source of serum proteins when the signal generated by the immune serum in the immunoradiometric assay (IRMA) was significantly higher than that generated by the corresponding pre-immune serum, +2 SDs.

Controls

In order to determine the persistence of blood proteins in the midgut of *M. spinolai*, 30 insects, corresponding to instars IV–V (more prevalent in the wilderness) were fed once with chicken blood. The intestinal content of two insects was extracted daily, for 15 days, and tested with the corresponding rabbit polyclonal antisera, as described above.

Statistics

Paired *t*-tests were used in all statistical analysis.

Results

None of the seven pre-immune sera reacted with the serum proteins, regardless of the donor species. When

immune sera were tested against serum proteins from each of the seven species used, the responses against a given immunizing species was always clearly significantly higher ($P < 0.0032$) than the cross-reactive response against proteins from the other six sources (Fig. 1).

Chicken proteins in the midgut of *M. spinolai* were detected by IRMA (Fig. 2). Out of 30 midgut samples, 14 were highly positive ($P < 0.0079$) for chicken proteins, while four others were positive to a lesser degree ($P < 0.0365$). No correlation was detected between the radioactive signal and the time elapsed since the blood meal. Thus, chicken serum proteins are present in the intestinal content of some vectors up to 15 days after a single controlled feeding opportunity, while in other insects, as early as 2 days after the meal, no detectable amounts of bird proteins were found.

Table 1 summarizes the results obtained with 131 *M. spinolai* collected as described in the Materials and methods. Vertebrate serum proteins were detected in about 50% of the intestinal contents of the insects tested. While approximately 70% of these samples were positive for serum proteins from only one host species, the rest of the positive samples contained proteins from two or more vertebrate species.

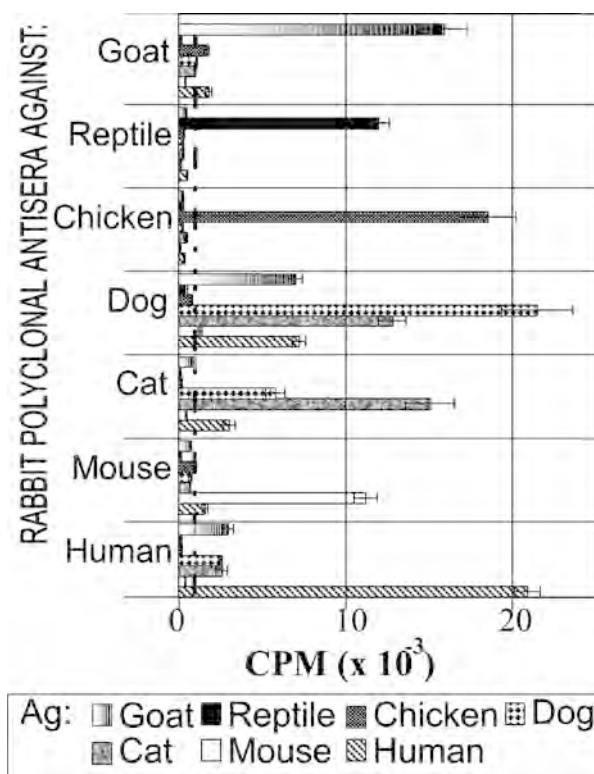


Fig. 1 Immunometric assay to evaluate rabbit antisera generated against serum proteins from several vertebrate species. Seven rabbit polyclonal antisera against serum proteins from goats, reptiles, chickens, dogs, cats, mice and humans were tested in homologous and heterologous combinations. The assays were performed in triplicate and average values of one representative experiment are shown. Immune and pre-immune sera were used at a consensus 1/100,000 dilution. – Represents the mean value of all pre-immune sera + SDs. CPM Counts per minute

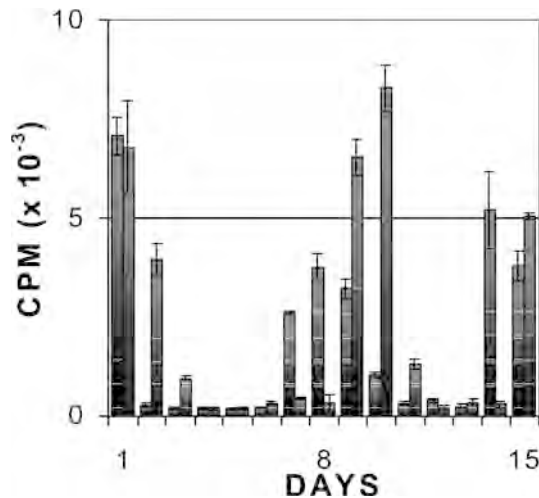


Fig. 2 Detection of chicken proteins in the midgut of 30 *Meprai spinolai*. Insects were fed once with chicken blood. Two insects were dissected daily for extraction of intestinal contents. Each bar represents the value obtained with the intestinal content of each arthropod. The immunometric assay was performed in triplicate and the average values of one representative experiment are shown. The immune sera were used at a consensus 1/100,000 dilution

Discussion

Although *T. cruzi* infections have decreased during the last years, Chagas' disease continues to be a serious

Table 1 Origin of the intestinal contents of 131 *Meprai spinolai* individuals, determined by polyclonal antisera generated against serum proteins from seven vertebrate species

	Total	% of samples	% positive
Positive	64	48.86	
Negative	67	51.14	
Samples positive for one vertebrate species	44	33.59	68.75
Human	2	1.53	3.12
Mouse	3	2.29	4.69
Goat	1	0.76	1.56
Rabbit	35	26.72	54.69
Dog	3	2.29	4.69
Reptile	0	0.00	0.00
Chicken	0	0.00	0.00
Samples positive for two hosts species	15	11.45	23.44
Mouse and rabbit	8	6.11	12.50
Mouse and dog	2	1.53	3.13
Goat and dog	4	3.05	6.25
Cat and goat	1	0.76	1.56
Samples positive for more than two host species	5	3.82	7.81
Dog > cat and human	1	0.76	1.56
Goat > cat, human and dog	1	0.76	1.56
Goat > human and dog	3	2.39	4.69

health and economic problem in Latin America, in particular in poor rural areas (Schmunis et al. 1996; Segura et al. 2000; Cohen and Gürtler 2001).

Here we propose a simple immunometric assay for the detection of vertebrate serum proteins in the intestinal content of *M. spinolai*, a wild *T. cruzi* arthropod vector, increasingly found in domiciliary habitats in endemic areas. This assay is both sensitive and specific, and is most likely useful for other vector species.

The rabbit polyclonal antibodies generated against serum proteins of different species reacted with their homologous antigens with a highly significant degree of specificity. The antibodies generated against feline and canine serum proteins showed some cross-reactivity (Fig. 1). In particular, the anti-dog serum protein antibodies significantly cross-reacted with human, cat, and goat serum proteins. In spite of these cross-reactions, recognition of the homologous antigens was easily assessed (Table 1) with a high degree of statistical confidence ($P < 0.0032$) in all cases. The cross-reactivity detected is not surprising, given the complexity of the specimen used for immunization. Indeed, at low serum dilutions the magnitude of cross-reactivity observed was higher with some loss of specificity. The proposed 1/100,000 consensus dilution of the rabbit polyclonal polyspecific antisera represents a compromise between sensitivity and specificity and contributes to the low cost of the assay.

In an experiment designed to assess the persistence of serum proteins in the vectors, it was surprising to detect these proteins up to 15 days after a controlled feed (Fig. 2). Although all insects used in this experiment were at comparable stages of development, and they were given the opportunity to simultaneously feed on a chicken, it was not possible to control the volumes of blood consumed by each individual, nor the frequency and amount of defecation. Although these issues may account for the variability in the signal generated by the different intestinal contents, they do not alter the main conclusion of this experiment (i.e. the assessment of the persistence of serum proteins in the vectors).

Table 1 summarizes our conclusions with regard to the presence of vertebrate serum components in the intestinal content of the 131 insects studied. It should be noted that, besides the cross-reactivity issue already discussed, it is possible that a given rabbit antiserum could recognize not only serum proteins from the species used as the immunogen source, but also other closely related vertebrate species prevalent in the geographical area where the vectors were collected. Thus, polyclonal antisera against serum proteins from dogs (*Canis familiaris*), mice (*Mus musculus*), rabbits (*Oryctolagus cuniculus*) and cats (*Felis domesticus*) may respectively recognize serum components from foxes (*Pseudalopex culpaeus*), from a variety of rodents (such as *Phyllotis darwini*), from hares (*Lepus capensis*) and from other felines (such as *Lynchailurus colocolo* or *Puma concolor*).

It is important to emphasize that, of those samples positive for vertebrate serum proteins, 67% were po-

sitive for rabbit serum proteins (Table 1). The frequent detection of proteins from this species in the intestinal content of *M. spinolai*, suggests that rabbits may be epidemiologically relevant carriers of Chagas' disease. It should be pointed out that the presence of rabbit serum proteins in the intestinal content was directly determined using the goat anti-rabbit immunoglobulin (as described in Materials and methods). Therefore, if there are rabbit proteins in the vector intestinal content, a strong positive signal will be observed in the absence of rabbit antiserum. With regard to the presence of human blood in the midgut of captured vectors, we found 1.5% (Table 1) positive only to human serum components, suggesting an epidemiological relevance for this vector. This proportion is comparable with that given by another study (Apt and Reyes 1990). Moreover, 11.5–25.5% of *M. spinolai* captured between the Third and Metropolitan Chilean regions are infected with *T. cruzi* (Canals et al. 1998). Although it has been suggested that *M. spinolai* feed on reptiles (Schofield 1979; Salvatella et al. 1994), in the 131 insects tested, we did not find reactivity with the antiserum specific for reptiles. It is possible that reptiles are not abundant in the area where the insects were collected.

Knowing the sources of the blood meal for the relevant vectors with reasonable accuracy makes an important contribution to the study of the life cycle of the parasite. Since these vectors can undergo prolonged fasting periods (Canals et al. 1998), specific and sensitive immunometric assays are necessary. Previously, double diffusion gels, indirect haemagglutination, and precipitin assays have been used (Salvatella et al. 1994; Gurtler et al. 1996; Gonçalves et al. 2000; Sagua et al. 2000). The immunometric assay proposed here detects blood proteins in the intestinal content of *M. spinolai*, up to 15 days after their controlled single blood meal (Fig. 2). Fifty-one percent of the samples were negative for serum proteins, suggesting that the insects can withstand prolonged fasting periods or, alternatively, they may have fed on a different animal species than those providing the sera to produce the polyclonal rabbit antisera.

The fact that most of the insects fed on rabbits (Table 1) is not surprising since these animals live in caves in the area where *M. spinolai* were collected. Of the total number of samples, 11.45% were from vectors that had fed on more than one host, specifically, mice and rabbits, mice and dogs, and goats and dogs. In this last case, the recognition by the immune serum was equally intense, indicating that the reactivity was specific for serum proteins from both species. In the samples recognized by antisera to cat and goat serum proteins, dog serum proteins were also recognized, but with less intensity, suggesting cross-reactivity (Fig. 1). The vectors providing the remaining five positive samples (3.82%) most likely fed on dogs or goats. Other proteins were weakly recognized.

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