Animal Reservoirs for *Trypanosoma cruzi* Infection in an Endemic Area in Paraguay

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**ABSTRACT.** Animal reservoirs for *Trypanosoma cruzi* infection were investigated in 5 communities in the Department of San Pedro, currently one of Paraguay's most highly endemic areas. A total of 112 domestic animals (37 cattle, 2 horses, 1 ass, 20 pigs, 44 dogs and 8 cats) and 4 wild animals (1 white-eared opossum, 2 yellow armadillos and 1 common long-nosed armadillo) were examined for blood. Although no trypomastigotes were found by 2 direct observation methods, the microhaematocrit and Giemsa stained thick and thin smears methods, several forms of trypanosoma flagellates morphologically identical to *T. cruzi* were detected in the liver infusion trypomero (LIT) medium from a single sample taken from a yellow armadillo, *Euphractus sexcinctus*. When serum samples of all the animals were examined for antibody to *T. cruzi* by direct agglutination (DA) test, 3 cattle, 2 pigs, 16 dogs and 3 cats had positive titers (1:32 or greater), but no wild animals showed positive reactions. *T. cruzi* was not found by culture nor microscopic examination of samples from any of the seropositive animals. However, domestic animals such as cattle, pigs, dogs and cats which were found to be seropositive in this study, possibly act as an animal reservoir in this endemic area as well as armadillos in which *T. cruzi* was observed.—**KEY WORDS:*** animal reservoir, direct agglutination test, Paraguay, *Trypanosoma cruzi.*

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Chagas’ disease, American human trypanosomosis, is a widespread endemic infection in Central and South America, and a zoonosis in most regions. *Trypanosoma cruzi* has been detected in many species of vertebrates, such as opossums and dogs, which are important animal reservoirs of the infection in North America [2, 7]. These animal species and the armadillo fill the same role in Central and South America [4, 11, 17, 18]. Few previous reports, however, have probed the role of large domestic animals such as pigs and cattle [8, 13].

The first human cases of this disease in Paraguay were reported by González and Rivarola [5], and Canese [3] reviewed Chagas’ disease in this country between 1940 and 1977. The latter report is the only one giving details of animal reservoirs in Paraguay, but the investigation was limited with regard to the area studied and timespan. Furthermore, environmental conditions and the distribution of this disease have changed since 1978 when that report was published.

In the present study, isolation and identification of *T. cruzi* from domestic animals kept in houses inhabited by *T. cruzi* infected patients, as well as wild animals live-trapped near these houses in the Department of San Pedro, Paraguay, are reported.

**MATERIALS AND METHODS**

**Study area:** This study was conducted in 5 communities on the outskirts of San Pedro de Ycuamanuy, capital of the Department of San Pedro: -Aguaray Seco (24 km from San Pedro de Ycuamanuyyá community), Pirí Pucú (30 km), Pataño (35 km), Barbero (56 km) and Chamorro Cué (60 km) (Fig. 1). The departmental capital is situated about 400 km north-east of the national capital Asunción. The study was carried out between December 1991 and March 1992. This area is known to be highly endemic for *T. cruzi* infection, with up to 12% of the inhabitants seropositive (Dra. G. Russomando, personal communication).

**Survey design:** Domestic animals examined in this study were kept in houses inhabited by people who were positive for *T. cruzi* infection by ELISA test, as previously analysed by our institute from 1989 to 1990. Wild animals were captured by hunters within 1 km of these patients' houses in each community. A total of 112 domestic animals (37 cattle, 2 horses, 1 ass, 20 pigs, 44 dogs and 8 cats) and 4 wild animals (1 white-eared opossum, 2 yellow armadillos and 1 common long-nosed armadillo) were examined.

Blood samples from domestic animals were collected aseptically by jugular vein puncture and transferred into sterilized tubes (Disposable centrifuge tube; Corning Glass Work, U.S.A.) with sodium citrate. Blood samples from the wild animals killed by exsanguination under ether anesthesia were collected by cardiac puncture into sterilized tubes as above, stored at 4°C, and transported to our laboratory for examination. The sera were frozen and retained for further studies.

**Detection of *T. cruzi* parasites:** *T. cruzi* parasites were examined in all domestic and wild animals by the following procedures. (i) Using the microhaematocrit method [16], five microhaematocrit tubes per sample were
examined. (ii) Thick and thin smears were air dried and stained with Giemsa stain by the routine method. (iii) About 4 ml of each sample mixed with sodium citrate was inoculated into the same volume of liver infusion tryptose (LIT) medium supplemented with 10% heat-inactivated (56°C, 30 min) fetal calf serum (FCS), 100 IU penicillin, and 100 μg streptomycin/ml of medium [9]. Cultures incubated at 28°C were examined for development of organisms at 2-week intervals for up to 20 weeks under an inverted and a conventional light microscopes. When flagellate parasites were detected in a culture, subcultures were made into fresh medium. Cultures were considered negative if no flagellates were observed within 20 weeks of culture.

Serological diagnosis: Serological examination of all animals was done by direct agglutination (DA) test for IgG anti- _T. cruzi_ antibody (Chagastest AD color: Wiener lab., Argentina) [15]. Sera with a titer of 1:32 or greater were considered positive.

RESULTS

Trypomastigotes were not detected by direct observation such as microhaematocrit centrifugation and Giemsa stained blood smears methods. However flagellate trypnosoma parasites such as trypomastigotes, epimastigotes and undeveloped flagellates, morphologically identical to _T. cruzi_, were observed in the LIT blood culture medium from a yellow armadillo, _Euphractus sexcintus_, captured in Pirí Pucú. Another type of flagellate parasite, differing from _T. cruzi_ in total length and flagellum length and width, was also found in cultures from 11 of 37 cattle. This species of parasite was morphologically identified as _Trypanosoma theileri_.

Table 1 shows the results of the DA test. All wild animals including the armadillo from which _T. cruzi_ was detected were DA test negative. However positive titers to _T. cruzi_ were detected in dogs (1:32-1:1024), pigs (1:64-1:128), cattle and cats (1:32-1:64). Three of 37 cattle (8.1%), 2 of 20 pigs (10.0%), 16 of 44 dogs (36.4%) and 3 of 8 cats (37.5%) examined were positive. One of 11 _T. theileri_ infected cattle showed a seropositive titer of 1:64. Almost all positive titers in cattle and cats were lower than 1:64. High positive rates in dogs (100% in both areas examined) and cats (46.2%) were observed in Pirí Pucú and Chamorro Cué, and pigs showed a seropositive titer were in only Chamorro Cué. No seropositive animals were found in Aguaray Seco, and only a few positive animals were found in Pátiño and Barbero in this study. The results of the serological examination did not fully correlate with those of other examinations.

DISCUSSION

Dogs, opossums ( _Didelphis_ spp.) and armadillos ( _Dasyurus_ spp. and _Euphractus_ sp.) are the principal reservoir hosts for _T. cruzi_ in Paraguay's neighboring countries [10, 12, 17]. In Paraguay, opossums ( _Didelphis azarae_) and dogs have been the only mammals found to be naturally infected [3].

In the present study, _T. cruzi_ flagellate parasites in cultured medium were found in only one armadillo, although the blood of other domestic and wild animals was also examined. Four species of domestic animals, i.e. cattle, pigs, dogs and cats showed seropositive by the DA test. Armadillos in which _T. cruzi_ was detected, are thought to act as an animal reservoir in this endemic area. This is consistent with previous reports [4, 12, 14].
Table 1. Results of direct agglutination (DA) test in domestic and wild animals examined in each community

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Aguaray Seco</th>
<th>Pirí</th>
<th>Pucú</th>
<th>Patiño</th>
<th>Barbero</th>
<th>Chamorro</th>
<th>Cué</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Domestic animals</td>
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<tr>
<td>Cattle</td>
<td>2/6</td>
<td>0/2</td>
<td>0/19</td>
<td>1/6</td>
<td>0/6</td>
<td>0/2</td>
<td>2/7</td>
<td>3/37</td>
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<td>Horse</td>
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<td>Ass</td>
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<tr>
<td>Pig</td>
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<td>2/7</td>
<td>2/0</td>
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<tr>
<td>Dog</td>
<td>6/13</td>
<td>4/17</td>
<td>0/1</td>
<td>1/6</td>
<td>16/44</td>
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<tr>
<td>Cat</td>
<td>0/2</td>
<td>1/1</td>
<td>0/3</td>
<td></td>
<td>3/8</td>
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<td>Wild Animals</td>
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<td>Yellow armadillo</td>
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<td><em>Euphractus sexcinctus</em></td>
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<td>Common long-nosed armadillo</td>
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<td><em>Dasypus novemcinctus</em></td>
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<td>White-eared opossum</td>
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However, since the four domestic animal species tested are susceptible to *T. cruzi* infection, their seropositive status may be considered as an indicator of *T. cruzi* infection, as *T. cruzi* was not conclusively detected from all these species. The range of such domestic animals indicated above as reservoirs is broad, and since houses in these communities are usually unfenced, the animals concerned can roam freely between inside houses and nearby bushland. They also have many chances to interact directly or indirectly with *T. cruzi* infected wild animals, such as armadillos, opossums and rodents. Such environmental conditions allow domestic animals greater exposure to *T. cruzi*.

There was no relation between the results of the LIT blood culture and the DA test in this study. The DA test used in this study is the most sensitive available for detecting antibodies during the acute stage of infection in the human [1, 14]. Furthermore, this serological method showed a high sensitivity in *T. cruzi* infected dogs [6, 8]. In light of these facts, it can be surmised that seropositive animals were in the late part of the acute phase when the intensity of parasitemia is low, or that other factors prevented development of *T. cruzi* parasites, despite such animals being infected. Although the highest titers are specifically observed with homologous *T. cruzi* organisms, some cross-reactivity at lower dilutions in the DA test is observed between *T. cruzi* antigens and heterologous sera such as American leishmaniasis, kala-azar and *Trypanosoma rangeli* infection in Central and South America [4]. However, there have been no reports of American leishmaniasis, kala-azar nor *T. rangeli* infection from patients and animals examined in these 5 communities to date. We consider the possibility of cross-reactivity with leishmaniasis, *T. rangeli* and *T. theileri* from cattle to be low, but acknowledge the necessity to establish reliable serodiagnosis of this disease in animals.

In order to implement effective control measures further epidemiological studies are required fully define the host range, distribution and source of human infection for *T. cruzi* in Paraguay. The current study highlights the possibility of transmission of Chagas’ disease among family members living in association with infected domestic animals.

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REFERENCES