Low probability of transmission of *Trypanosoma cruzi* to humans by domiciliary *Triatoma sordida* in Bolivia

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Abstract

The role of *Triatoma sordida* in the domestic transmission of *Trypanosoma cruzi* was assessed in 7 rural localities in Velasco Province, Department of Santa Cruz, Bolivia. *Tri. sordida*, the only triatomine species identified in these localities, was found inside 58·0% of houses but not in large numbers (3·1 bugs per infested house on average). A total of 220 faecal samples from domiciliary bugs was examined microscopically and by the polymerase chain reaction for the presence of trypanosomes: 21·4% were infected. Analysis of blood meals of domiciliary *Tri. sordida* showed that humans were the commonest host (70·4%), followed by chickens and dogs. Four of 418 persons tested were seropositive for *Tryp. cruzi*. Only 2 of a second group of 62 persons living in dwellings infested by *Tri. sordida* were seropositive. *Tryp. cruzi* infection was demonstrated in dogs and domestic rats. Three other species of small mammals were found to be infected with trypanosomes. In our study area, domestic *Tri. sordida* are mainly incriminated in the transmission of *Tryp. cruzi* to synanthropic animals, whereas transmission to humans is very rare. The presence in houses of small populations of *Tri. sordida* infected with *Tryp. cruzi* is therefore currently insufficient for this insect to constitute a major epidemiological risk factor.

Keywords: trypanosomiasis, American trypanosomiasis, Chagas disease, *Trypanosoma cruzi*, transmission, *Triatoma sordida*, Bolivia

Introduction

Among over a hundred species of Triatominae (Hemiptera: Reduviidae) described in the New World, *Triatoma sordida* is considered of epidemiological significance as a vector of *Trypanosoma cruzi* because of its wide distribution area and its tendency to invade domestic environments (SCHOFIELD, 1994). It is present throughout central Brazil, eastern and central Bolivia, the Chaco region of Paraguay and north-western Argentina (LENT & WYGODZINSKY, 1979). Although *Tri. sordida* occurs in the sylvatic environment, it is often found in peridomestic habitats and can also form domiciliary colonies (BARRETO, 1976; CARCAVALLO *et al.*, 1988). *Tri. sordida* is currently considered as a possible substitute for the present domestic vector *Tri. infestans* in the transmission of *Tryp. cruzi*. Nevertheless, data on the risk of transmission of *Tryp. cruzi* to humans by *Tri. sordida* are scarce (ROJAS DE ARIAS *et al.*, 1993; DIOTAIUTI *et al.*, 1995).

In various localities of Velasco Province, eastern Bolivia, *Tri. sordida* is the only triatomine species reported to colonize human dwellings (NOIREAU *et al.*, 1996). Because *Tri. infestans* has never been found in these localities, the domestication of *Tri. sordida* may be considered as primary (i.e., not consequent upon the eradication of *Tri. infestans*). The Velasco area thus provided exceptional conditions to assess the domestication of *Tri. sordida* and to study its role in the transmission of *Tryp. cruzi*.

Material and Methods

Study area

The field work was carried out in Velasco Province in the north of the Department of Santa Cruz, Bolivia. This Province forms part of the Chiquitania region and consists of a mosaic of semi-humid woodland and wooded savannah (*cerrado*). The mean annual rainfall is 1200 mm, including a dry season between May and September, the mean annual temperature is 26°C, and the relative humidity fluctuates between 57% and 78%.

Seven villages situated in the rural area around the small town of San Ignacio de Velasco (16° 23′ S; 60° 58′ W; altitude 413 m) were studied from February 1995 to September 1996: Recreo, San Juan Bautista, Cochabambita, Cerrito, Guapomocito, Tacoigo and Cotoca. The inhabitants belong to the Chiquitano ethnic group. The dwellings are mainly of wattle and daub construction and roofed with straw. A preliminary study in 1994 showed that *Tri. sordida* was the only triatomine species infesting houses in these localities (NOIREAU *et al.*, 1996). The houses had never been sprayed with insecticide.

Triatomine studies

Manual collections of triatomines (0·5 person–hours each) were carried out in 88 houses in the 7 localities during February, August and October 1995. The houses in Recreo, San Juan Bautista and Tacoigo were visited once. In the other 4 localities, 40·7% of houses were visited once, 42·4% twice and 16·9% 3 times. Bugs were caught in the sleeping quarters (on walls or in beds). In San Juan Bautista and Cotoca triatomines were also looked for in outhouses located 5–10 m from the houses, including kitchens and storerooms (mainly for maize). The captured insects were placed in plastic bottles containing filter paper and transported to the laboratory for morphological identification according to LENT & WYGODZINSKY (1979). To confirm the identification of nymphal instars, we used enzyme electrophoresis at 5 diagnostic loci (unpublished data).

Faeces were obtained by gently squeezing the live insects. The faecal droplets were then mixed with phosphate-buffered saline and examined for the presence of flagellates by direct microscopical observation at 400× magnification. Aliquots of faeces from most of the insects were stored at –20°C for subsequent confirmation of *Tryp. cruzi* infection by polymerase chain reaction (PCR) amplification according to BRENIÈRE *et al.* (1992).

The following indices were calculated (WHO, 1991): infestation and colonization index (see Table 1, footnotes), crowding index (no. of *Tri. sordida* captured/no. of houses with *Tri. sordida*), and infection rate.

Identification of blood meals

The gut contents of engorged insects were expressed on to filter paper, dried and stored at –20°C. The origin of blood meals of bugs collected from houses and outhouses was identified by double diffusion in agar (WISNIVESKY-COLLI *et al.*, 1982), using 8 antisera: anti-

horse, anti-pig, anti-cat, anti-dog, anti-human, anti-chicken, anti-murid rodents, and anti-*Didelphis*. Blood meals of human origin were confirmed by the presence of continuous precipitation bands between the gut contents and human serum placed in an adjacent well.

Serological survey

In March 1995, 5 of the villages were selected for a serological survey: Recreo, Guapomocito, Cotoca, Cochabambita and Cerrito. Capillary blood samples were taken from 418 residents who gave voluntary consent (319 schoolchildren 5–15 years old and 99 adults) from unselected houses and 62 people belonging to 14 families living in dwellings infested by *Tri. sordida*; at least one *Tryp. cruzi* positive specimen of *Tri. sordida* infected with *Tryp. cruzi* was detected in each house. Movement or residence of participants outside the area was noted. Sera derived from the blood samples were stored at –20°C until tested simultaneously for anti-*Tryp. cruzi* immunoglobulin G (IgG) antibodies using indirect immunofluorescence (IF) and enzyme-linked immunosorbent assay (ELISA) as described by BRENIÈRE *et al.* (1984). A sample was considered positive when both serological tests gave positive results.

Studies on animals

Dogs and cats were examined in 3 villages (Cochabambita, Guapomocito and Cotoca) in February 1996. All the animals living in 31 households were recorded (name, sex and age) but only those ≥3 months were examined. Xenodiagnosis was performed using 14 third-instar nymphs of *Tri. infestans* divided between 2 boxes. After 30 and 60 d, the faeces of these bugs were examined for the presence of flagellates. Blood samples were obtained by venipuncture from household dogs and sera were stored at –20°C. They were tested for anti-*Tryp. cruzi* IgG antibodies using ELISA. Sera from 6 dogs in a non-endemic area (city of La Paz) were tested as negative controls.

Small mammal trapping was carried out in February and September 1996 in Cochabambita. Thirty Sherman traps baited with a mixture of oat, pineapple and tuna fish were placed in maize fields, outhouses and houses. Xenodiagnosis of captured animals was carried out using 7 third-instar nymphs of *Tri. infestans*.

The PCR was performed on faecal samples from some bugs with positive xenodiagnoses to confirm the presence of *Tryp. cruzi* (see BRENIÈRE *et al.*, 1992).

Results

Collection and identification of Triatominae

A total of 236 triatomine bugs was caught in sleeping quarters. Adults and nymphs accounted for 41·1% (97/236) and 58·9% (139/236), respectively. *Tri. sordida* was the only species collected; all nymphs showed the characteristic allelic banding on electrophoresis. Fifty-one of the 88 houses examined (58%) contained triatomines and the total colonization index reached 90·2% (Table 1). Among the 35 houses visited 2 or 3 times, 82·9% were finally found to be infested, compared with only 68·6% after the initial search. Although no bug was found in the remaining 37 houses, in 24·3% of them indirect evidence of infestation, such as faecal streaks, dead eggs or exuviae, was found.

The crowding index was very low (3·1 bugs per infested house): 85·7% of houses had one to 5 bugs, while less than 3% had more than 10 bugs.

Sixty-one bugs were collected from outhouses in San Juan Bautista and Cotoca in February 1995. All were identified as *Tri. sordida* by their morphology and enzyme electrophoresis; 59% were nymphs.

Prevalence of Tryp. cruzi *in* Tri. sordida

Faecal samples from 220 domiciliary *Tri. sordida* originating from the 7 localities were examined microscopically for the presence of flagellates; 165 samples were also examined by PCR. Trypanosomes were identified by at least one method in 47 (21·4%) of the samples (Table 2). The percentage of infected triatomines varied greatly according to the locality (from zero in 2 villages to over 32% in 3 others). The infection rate did not differ significantly between adults and nymphs (28·0% and 17·4%, respectively; $P>0·05$). Thirty-three of the 165 faecal samples analysed simultaneously for the presence of parasites by microscopy and PCR (Table 3) were found to contain trypanosomes by microscopy, compared with 32 by PCR. The 2 techniques agreed on 93·3% of the faecal examinations. The overall infection rate of triatomines collected from outhouses was 4·9%.

Blood meals

Seventy-eight *Tri. sordida* (67 from houses and 11 from outhouses) had their blood meals examined. Forty-eight meals (61·5%) could be identified: 41 (85·4%) were single feeds on human, chicken or dog blood and the remainder had fed on 2 hosts (human/chicken, human/dog or dog/chicken). Among the *Tri. sordida* nymphs and adults collected inside houses, humans were the commonest hosts, followed by chickens and dogs (Table 4). Blood meal identification of 10 *Tri. sordida* collected from outhouses showed that chickens were the predominant host (90·9%), followed by dogs (9·1%).

Seroprevalence of Tryp. cruzi *in humans*

The overall seroprevalence of *Tryp. cruzi* infection was 0·96% among the sampled residents (4/418). Only 2 of the 62 persons living in dwellings infested by *Tri. sordida* were seropositive (3·3%). Only 2 male adults among these 6 seropositive persons were thought to have been infected locally; the others had been living in known *Tryp. cruzi* endemic areas where the vector was *Tri. infestans*.

Reservoir hosts of Tryp. cruzi

Three of the 39 dogs subjected to xenodiagnosis (7·7%) were infected with *Tryp. cruzi* and 7 of the 44 dogs examined by ELISA (15·9%) had antibodies to *Tryp. cruzi*. The 3 cats examined by xenodiagnosis gave negative results.

Table 1. Prevalence of *Triatoma sordida* in houses in Velasco Province, Santa Cruz Department, Bolivia

Locality	No. of houses Examined	No. of houses With *T. sordida*	Infestation index[a] (%)	No. of houses with nymphs of *T. sordida*	Colonization index[b] (%)
Recreo	10	7	70·0	7	100
San Juan Bautista	12	4	33·3	2	50·0
Cochabambita	14	10	71·4	9	90·0
Cerrito	24	10	41·7	9	90·0
Guapomocito	11	5	45·4	5	100
Tacoigo	7	7	100	6	85·7
Cotoca	10	8	80·0	8	100
Total	88	51	58·0	46	90·2

[a](No. of houses infested by *T. sordida* adults and/or nymphs/no. of houses examined)×100.
[b](No. of houses with *T. sordida* nymphs/no. of houses with *T. sordida*)×100.

Table 2. *Trypanosoma cruzi* infection rates in domiciliary *Triatoma sordida* in Velasco Province, Santa Cruz Department, Bolivia[a]

Locality	No. of nymphs Examined	No. of nymphs With *T. cruzi*	No. of adults Examined	No. of adults With *T. cruzi*	Total no. examined	No. with *T. cruzi*
Recreo	23	0	4	0	27	0
San Juan Bautista	4	0	2	0	6	0
Cochabambita	22	6 (27·3%)	18	7 (38·9%)	40	13 (32·5%)
Cerrito	17	7 (41·2%)	19	6 (31·6%)	36	13 (36·1%)
Guapomocito	26	3 (11·5%)	14	3 (21·4%)	40	6 (15·0%)
Tacoigo	29	3 (10·3%)	8	1 (12·5%)	37	4 (10·8%)
Cotoca	17	5 (29·4%)	17	6 (35·3%)	34	11 (32·4%)
Total	138	24 (17·4%)	82	23 (28·0%)	220	47 (21·4%)

[a]Examined by direct microscopy and/or polymerase chain reaction.

Table 3. Detection of *Trypanosoma cruzi* in faeces of domiciliary *Triatoma sordida*: comparison of direct microscopical observation and polymerase chain reaction

Locality	No. of *T. sordida*	Microscopy Positive PCR+[a]	Microscopy Positive PCR–[a]	Microscopy Negative PCR+[a]	Microscopy Negative PCR–[a]
Recreo	23	0	0	0	23
San Juan Bautista	6	0	0	0	6
Cochabambita	35	7	3	1	24
Cerrito	28	10	0	2	16
Guapomocito	32	2	2	0	28
Tacoigo	16	1	0	1	14
Cotoca	25	7	1	1	16
Total	165	27	6	5	127

[a]PCR, polymerase chain reaction; +, positive result; –, negative result.

Table 4. Identified blood meals from 67 domiciliary *Triatoma sordida* nymphs and adults in Velasco Province, Santa Cruz Department, Bolivia

Blood source	No. of blood meals[a] Nymphs	Adults	Total
Human	27	4	31 (70·4%)
Dog	5	0	5 (11·4%)
Chicken	6	2	8 (18·2%)
Total no. identified	38	6	44 (100%)
No. non-reactive	21	8	29
Total	59	14	73

[a]Some bugs had mixed blood meals.

Rattus rattus was the predominant rodent captured in houses and outhouses; 5 of the 13 specimens examined by xenodiagnosis (38·5%) were infected. The presence of trypanosomes was also demonstrated in 3 other species of mammals: *Calomys callosus* (2/13), *Oligoryzomys microtis* (1/1) and *Monodelphis domestica* (2/3). The only *Akodon* sp. examined was not infected. The PCR technique confirmed infection by *Tryp. cruzi* in the faeces of bugs used to xenodiagnose the 3 dogs, 5 *R. rattus*, and 2 *M. domestica*.

Discussion

Tri. sordida was commonly found inside houses and was able to form domestic colonies. The high infestation and colonization indexes observed suggest long-standing colonization. Domiciliation may result from increased environmental pressure (destruction of the natural vegetation for agriculture) and may also reflect invasion of domestic habitats from which the main vector, *Tri. infestans*, has been eliminated (BARRETTO, 1976; SCHOFIELD, 1994). In our study area, where there had been no previous infestation by *Tri. infestans*, colonization by *Tri. sordida* could have occurred as a result of environmental disturbance brought about by humans, especially deforestation and burning of the savannah. *Tri. sordida* probably invaded the human dwellings searching for more stable ecotopes and more readily available blood sources. Moreover, the invasion of houses would have been favoured by the absence of buildings housing domestic animals (in particular, the virtual absence of chicken coops). As reported by FORATTINI *et al.* (1973) and SCHOFIELD (1994), *Tri. sordida* does not form large colonies in houses, with the mean number of bugs captured per infested house being only 3·1 in our study area. This inability to build up significant domestic populations may be due to the fact that *Tri. sordida* rarely completes more than one generation per year (SCHOFIELD, 1994).

Despite the evidence of a great diversity of blood sources (human and domestic animals), analysis of the intestinal content of domiciliary bugs captured in the Velasco area showed that *Tri. sordida* was highly anthropophilic (taking 70% of its blood meals from humans). So humans have become the most important hosts in this area for the maintenance of domestic *Tri. sordida* colonies. Previous studies on the feeding habits of domiciliary/peridomestic *Tri. sordida* revealed a variety of hosts, among which birds were generally the preferential blood source (BARRETTO, 1968; FORATTINI *et al.*, 1982). The comparatively low rate (18%) of feeds from birds in our area may have been due to the near absence of chicken coops, as the chickens roost in trees at night.

Tryp. cruzi infection rates in domiciliary *Tri. sordida* were high in the Velasco area compared with previous rates reported in Brazil, which ranged from 0·3 to 1·3% (FORATTINI *et al.*, 1982; GRACIA ZAPATA & MARSDEN, 1992). The PCR technique performed on the faecal samples proved that the bugs really were infected with *Tryp. cruzi* (see BRENIÈRE *et al.*, 1992). Moreover, the presence of nymphs infected with *Tryp. cruzi* inside houses confirmed the occurrence of domestic transmission.

In spite of the presence of infected colonies of *Tri. sordida* in human dwellings in the Velasco area, transmission of *Tryp. cruzi* to humans was rare, as proven by the very low rate of human seropositivity. Four of the 6

seropositive persons had lived in other known endemic areas with domiciliary *Tri. infestans*. The prevalence of human infection with *Tryp. cruzi* in our area of domestic *Tri. sordida* is lower than that reported in the Paraguayan Chaco, where *Tri. sordida* was identified as the vector of *Tryp. cruzi* and the human seropositivity rate was 12% (ROJAS DE ARIAS *et al.*, 1993). However, individuals could have become infected in neighbouring communities infested by *Tri. infestans*. Our observations are in agreement with a serological survey carried out in Brazil after control measures had been introduced against *Tri. infestans*, followed by reinfestation of the houses with *Tri. sordida*; the survey showed a marked drop in the incidence of human infection with *Tryp. cruzi* (see DIOTAIUTI *et al.*, 1995).

Trypanosome infection was found in several synanthropic and wild mammals. The PCR technique confirmed that at least some of these infections were undoubtedly due to *Tryp. cruzi*. Dogs and the black rat (*R. rattus*) were the main domestic hosts. The gray short-tailed opossum (*M. domestica*) and 2 species of cricetid mice (*C. callosus* and *O. microtis*) were also found to be infected by flagellates. Because these species commonly occupy peridomestic areas, they may be considered as possible secondary hosts of *Tryp. cruzi*. The lack of a human reservoir suggests that contamination with the faeces of infected *Tri. sordida* is not an efficient route of transmission, and supports the idea that animals have acquired the infection by the oral route through ingestion of infected triatomines. This is to be expected with insectivorous rodents and marsupials (EDGCOMB & JOHNSON, 1970; SCHWEIGMANN *et al.*, 1995), and it has also been demonstrated in dogs by DIAZ-UNGRIA (1966). Because of the nocturnal habits of rodents, *Tryp. cruzi* infection of domiciliary *Tri. sordida* may result mainly from feeds on dogs.

Whether or not *Tri. sordida* is an important vector is still uncertain. Laboratory work on feeding behaviour and defaecation patterns seems to indicate high vectorial competence (CROCCO & CATALÁ, 1996). Nevertheless, the low rate of metacyclogenesis in *Tri. sordida*, and especially its inability to form large colonies, could explain its poor vectorial capacity within houses (PERLOWAGORA-SZUMLEWICZ & DE CARVALHO MOREIRA, 1994; SCHOFIELD, 1994). It is a fact that the *Tri. infestans* and *Rhodnius prolixus*, the most effective vectors of Chagas disease, occur in higher density inside houses than other triatomine species (ZELEDÓN, 1976). In Velasco, the density of domestic bugs was presumably below the critical level for the transmission of *Tryp. cruzi* to humans. So, in spite of its wide distribution and facility for colonizing domestic habitats, *Tri. sordida* may be considered a minor vector of Chagas disease, probably due to its inability to build up a significant domiciliary population.

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References

Barretto, M. P. (1968). Estudos sôbre reservatórios e vectores silvestres do *Trypanosoma cruzi*. XXXI. Observações sôbre a associação entre reservatórios e vectores, com especial referência a regiao nordeste do Estado de São Paulo. *Revista Brasileira de Biologia*, **28**, 481–494.

Barretto, M. P. (1976). Possible role of wild mammals and triatomines in the transmission of *Trypanosoma cruzi* to man. In: *New Approaches in American Trypanosomiasis Research*, Scientific Publication no. 318, Pan-American Health Organization, Washington, pp. 307–316.

Brenière, S. F., Carrasco, R., Miguez, H., Lemesre, J. L. & Carlier, Y. (1984). Comparisons of immunological tests for serodiagnosis of Chagas' disease in Bolivian patients. *Tropical and Geographical Medicine*, **37**, 231–238.

Brenière, S. F., Bosseno, M. F., Revollo, S., Rivera, M. T., Carlier, Y. & Tibayrenc, M. (1992). Direct identification of *Trypanosoma cruzi* natural clones in vectors and mammalian hosts by polymerase chain reaction amplification. *American Journal of Tropical Medicine and Hygiene*, **46**, 335–341.

Carcavallo, R. V., Canale, D. & Martinez, A. (1988). Habitats de Triatomíneos argentinos y zonas ecológicas donde prevalecen. *Chagas (Buenos Aires)*, **5**, 8–17.

Crocco, L. B. & Catalá, S. S. (1996). Feeding and defaecation patterns in *Triatoma sordida*. *Memórias do Instituto Oswaldo Cruz*, **91**, 409–413.

Diaz-Ungria, C. (1966). Transmission du *Trypanosoma cruzi* chez les mammifères. *Annales de Parasitologie Humaine et Comparée*, **41**, 549–571.

Diotaiuti, L., Ribeiro de Paula, O., Falcao, P. L. & Dias, J. C. P. (1995). Avaliação do programa de controle vectorial da doença de Chagas em Minas Gerais, Brasil, com referência especial ao *Triatoma sordida*. *Boletin de la Oficina Sanitaria Panamericana*, **118**, 211–219.

Edgcomb, J. H. & Johnson, C. M. (1970). Natural infection of *Rattus rattus* by *Trypanosoma cruzi* in Panama. *American Journal of Tropical Medicine and Hygiene*, **19**, 767–769.

Forattini, O. P., Ferreira, O. A., de Souza, J. M. P., Rabello, E. X., da Rocha e Silva, E. O. & Rodrigues, F. W. (1973). Medida da infestação domiciliar por *Triatoma sordida*. *Revista de Saúde Pública, São Paulo*, **7**, 241–250.

Forattini, O. P., Barata, J. M. S., Ferreira Santos, J. L. & Silveira, A. C. (1982). Hábitos alimentares, infecção natural e distribuição de triatomíneos domiciliados na região central do Brasil. *Revista de Saúde Pública, São Paulo*, **16**, 171–204.

Garcia Zapata, M. T. A. & Marsden, P. D. (1992). Control of the transmission of chagas' disease in Mambaí, Goias, Brazil (1980–1988). *American Journal of Tropical Medicine and Hygiene*, **46**, 440–443.

Lent, H. & Wygodzinsky, P. (1979). Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. *Bulletin of the American Museum of Natural History*, **163**, 127–520.

Noireau, F., Brenière, F., Cardozo, L., Bosseno, M. F., Vargbas, F., Peredo, C. & Medinacelli, M. (1996). Current spread of *Triatoma infestans* at the expense of *Triatoma sordida* in Bolivia. *Memórias do Instituto Oswaldo Cruz*, **91**, 271–272.

Perlowagora-Szumlewicz, A. & de Carvalho Moreira, C. J. (1994). *In vivo* differentiation of *Trypanosoma cruzi*—1. Experimental evidence of the influence of vector species on metacyclogenesis. *Memórias do Instituto Oswaldo Cruz*, **89**, 603–618.

Rojas de Arias, A., de Guillen, I., Inchausti, A., Samudio, M. & Schmeda-Hirschmann, G. (1993). Prevalence of Chagas' disease in Ayoreo communities of the Paraguayan Chaco. *Tropical Medicine and Parasitology*, **44**, 285–288.

Schofield, C. J. (1994). *Triatominae: Biology and Control*. Bognor Regis, UK: Eurocommunica Publications.

Schweigmann, N. J., Pietrokovsky, S., Bottazi, V., Conti, O. & Wisnivesky-Colli, C. (1995). Interaction between *Didelphis albiventris* and *Triatoma infestans* in relation to *Trypanosoma cruzi* transmission. *Memórias do Instituto Oswaldo Cruz*, **90**, 679–682.

Wisnivesky-Colli, C., Gürtler, R. E., Solarz, N. D., Salomon, D. & Ruiz, A. (1982). Feeding patterns of *Triatoma infestans* (Hemiptera, Reduviidae) in relation to transmission of American trypanosomiasis in Argentina. *Journal of Medical Entomology*, **19**, 645–654.

WHO (1991). *Control of Chagas Disease*. Geneva: World Health Organization, Technical Report Series, no. 811.

Zeledón, R. (1976). Effects of triatomine behavior on trypanosome transmission. In: *New Approaches in American Trypanosomiasis Research*. Washington, DC: Pan American Health Organization, Scientific Publication no. 318, pp. 326–329.

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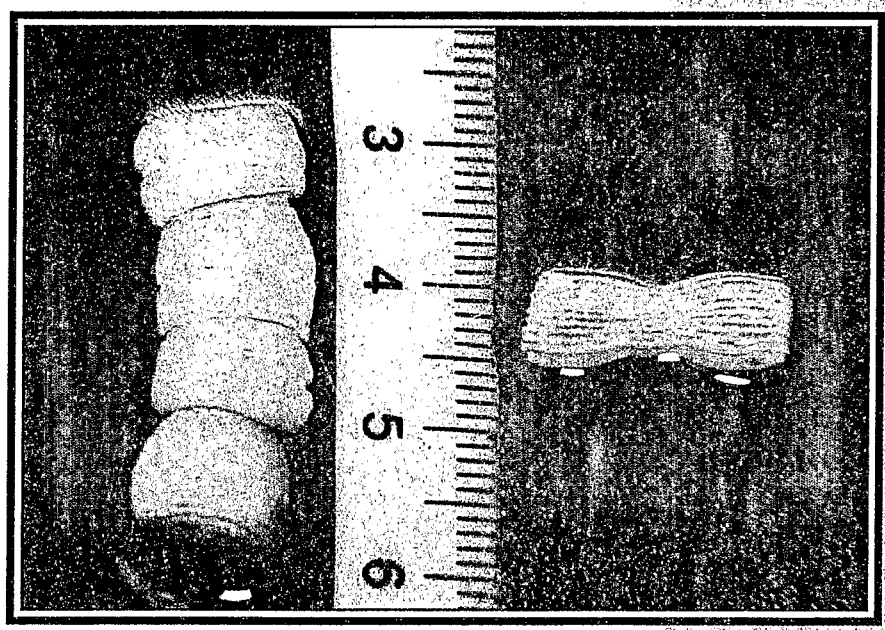

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