Chagas Disease in the Amazon Basin: Association of *Panstrongylus geniculatus* (Hemiptera: Reduviidae) with Domestic Pigs

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J. Med. Entomol. 35(2): 99–103 (1998)

ABSTRACT Just over 100 autochthonous cases of Chagas disease are reported from the Brazilian Amazon Basin. *Panstrongylus geniculatus* (Latreille) occurs throughout the region and is the known vector of *Trypanosoma cruzi*, principal zymodeme 3 (Z3) to the armadillo *Dasypus novemcinctus*. In the small riverine community of Furo do Rio Pau Grande, pigsties adjoining houses were heavily infested with *P. geniculatus*, which repeatedly attacked local inhabitants. Palm trees in the immediate vicinity were also infested. *T. cruzi* principal zymodeme 1 (Z1) was isolated from *P. geniculatus*, domestic pigs, and opossums, but no human infections were detected. The threat of endemic Chagas disease to the Amazon Basin from either domiciliation of local silvatic triatomine species, or from migration of domestic vectors, demands a program of vigilance and plans of action to eliminate household triatomine colonies.

KEY WORDS *Panstrongylus geniculatus*, *Trypanosoma cruzi*, triatomine bugs, Amazon Basin, Chagas disease, zymodemes

TRIATOMINE BUGS (HEMIPTERA: REDUVIIDAE) are the arthropod vectors of *Trypanosoma cruzi*, the protozoan agent of Chagas disease (South American trypanosomiasis). In Pará or Amazonas States of the Brazilian Amazon Basin, 13 species of triatomine bug have been reported [*Belminus herreri* Lent & Wygodzinsky, *Cavernicola lenti* Barrett & Arias, *C. pilosa* Barber, *Eratyrus mucronatus* Stål, *Microtriatoma trinidadensis* (Lent), *Panstrongylus geniculatus* (Latreille), *P. lignarius* (Walker), *P. rufotuberculatus* (Champion), *Rhodnius brethesi* Matta, *R. paraensis* Sherlock, Guitton & Miles, *R. pictipes* Stål, *R. robustus* Larrousse, and *Triatoma rubrofasciata* (De Geer)]. With the exception of *Belminus* (lizard feeder) and *Cavernicola* (associated with bats), all 13 species have been found to be infected with *T. cruzi*. *R. pictipes* and *R. robustus* are also vectors of the nonpathogenic trypanosome *T. rangeli* (*Herpetosoma*); and *T. rubrofasciata* transmits *T. conorhini* (*Megatrypanum*) to the rat (*Rattus rattus*) (Miles et al. 1981c, 1983b; Coura et al. 1994b).

Many mammal species in Amazonian forests are reservoir hosts of *T. cruzi*, including marsupials such as the opossums *Didelphis marsupialis* and *Philander opossum*; edentates like the armadillo *Dasypus novemcinctus* and the ant-eater *Tamandua tetradactyla*, and rodents, including porcupines, *Coendou prehensilis*, and spiny rats, *Echimys chrysurus*.

Between 16 and 18 million people are thought to be infected with *T. cruzi* in Latin America, ≈5 million of whom live in Brazil. Despite the vastness of the Amazon Basin and the abundance of natural hosts and vectors of *T. cruzi*, Chagas disease is sporadic in the region. One hundred and 30 cases in humans are known, of which 103 were acute (with 4 deaths) and 27 chronic. Sixty-one of these occurred in Pará State, 14 in Amazonas, 45 in Amapá, 7 in Acre, and 3 in Maranhão (Fraiha Neto et al. 1995; V.C.V. and S.A.S.V., unpublished data).

Descriptions of the habitats of Amazonian triatomine bugs, aided by spool-and-line tracking of mammalian hosts, have produced an explanation for the absence of endemic Chagas disease: none of the local species are domestic vectors, apart from *T. rubrofasciata*, which seldom feeds on human blood (Miles et al. 1981b, c). Thus, the sporadic cases of Chagas disease in the Amazon are attributable to adult bugs, such as *R. pictipes* and *P. geniculatus*, flying into houses and transmitting infection by contamination while taking a blood meal, or orally through contamination of food. Oral transmission to multiple simultaneous acute cases has been implicated in 12 family outbreaks in the region, encompassing 57 of the recorded acute cases: an alternative source for oral transmission is anal gland secretions of the opossum *D. marsupialis*, in which

All procedures used in the collection and examination of animals, and in the collection of human blood samples, were in accord with the approved methods specified by relevant staff of the Instituto Evandro Chagas, Fundação Nacional de Saude, Brazil.

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Table 1. Triatomine bugs from Muana, Pará State, Brazil

Habitat	No. infested/No. examined	Species captured (*T. cruzi* infected/examined)			
		P. geniculatus	*P. lignarius*	*R. pictipes*	*E. mucronatus*
Houses	10/15	21 (1/5)	4	7 (1/4)	—
Pigsties	15/15	418[a] (20/118)	2	12 (0/3)	1
Palms (*S. martiana*)	3/4	7 (2/7)	—	10 (3/10)	—
Palms (*M. regia*)	1/2	2 (0/2)	1 (0/1)		—

[a] Females, 207; males, 168; 2 stage V, 7 stage III, 16 stage II, 28 stage I, nymphs and, in addition, 385 unecloded eggs.

infective metacyclic forms of *T. cruzi* equivalent to those in the vector may occur (Deane et al. 1986, Asin and Catala, 1991, Shikanai-Yasuda et al. 1991, Calvo-Méndez et al. 1994). In addition, one bug species, *R. brethesi*, may transmit *T. cruzi* to forest workers who harvest fronds from bug-infested palm trees (*Leopoldinia piacaba*) (Coura et al. 1994a).

Here we describe hundreds of *P. geniculatus* infesting pigsties annexed to human dwellings, repeatedly attacking local inhabitants, and exposing them to the risk of *T. cruzi* infection.

Materials and Methods

Study Area. Furo do Rio Pau Grande is a scattered riverine community with a population of ≈400 in the Muana Region (latitude, 1° 31′; longitude, 49° 13′; altitude 22 m) on the island of Marajo, in the mouth of the Amazon River. The area has tidal-type vegetation and seasonally flooded forest (varzea) with innumerable small islands. Annual rainfall is heavy (3,740 mm in 1994) and intense during the first 6 mo of the year, followed by a humid summer. Annual mean monthly maximum and minimum temperatures are 32 and 23°C, respectively, with little variation throughout the year. The local economy is dependent on cattle, pig, buffalo, and poultry production with additional income from fishing and harvesting of palm hearts from the acai palm, *Euterpe oleracea*. Access to the communities of Furo do Rio Pau Grande is only by boat; houses are widely scattered and several kilometers apart.

Collection of Triatomine Bugs. Bugs were mainly collected at night (between 1900 and 2300 hours) using a flashlight and forceps. No pyrethroid irritants were used to generate movement in either the houses or peridomestic pigsties. Palms were cut down and dissected as described by Miles et al. (1983a). In addition, 2 UV traps were used for 15 d to capture light-attracted triatomine bugs. Bugs were stored in plastic containers and a proportion dissected and examined microscopically for the presence of *T. cruzi*, which when found was inoculated into biphasic blood agar culture medium (Miles et al. 1981a).

Examination of Domestic and Silvatic Mammals. We examined 105 domestic pigs, *Sus scrofa*, 5 domestic dogs, and 4 domestic cats. The animals were held immobile and sedated with acepromazine (Acepram, UNIVET, São Paulo, SA) before collection of blood for fresh film microscopy and for inoculation into biphasic blood agar medium. Xenodiagnosis was attempted with 5 fifth-stage nymphs of either *T. infestans* or *R. prolixus*. Silvatic mammals were captured in collapsible wire traps, as described by Miles et al. (1981b). Five traps were set at distances up to 500 m from each house and examined daily for up to 15 d.

Serology. A large proportion of the local inhabitants (253 individuals) volunteered to provide venous blood samples, which were cultured on biphasic blood agar and also examined by microscopy of fresh blood films and Giemsa stained thin films. Serum samples were prepared and used for indirect immunofluorescence tests (IFAT). Xenodiagnosis was also performed with 5 fifth-stage nymphs of *T. infestans*.

Results

Fifteen houses, 15 pigsties, and 6 adjacent palms were examined for triatomine infestation in Furo do Rio Pau Grande, and all were of similar construction, with wooden or palm walls and palm or tiled roofs. Pigsties either adjoined houses or were up to 10 m distant and constructed of bound palm trunks or of planks, with floors and roofs of palm fronds.

In total, 495 triatomine bugs were collected of the 4 species *P. geniculatus*, *R. pictipes*, *P. lignarius*, and *E. mucronatus*, all of which are known to be vectors of *T. cruzi* in the Amazon region (Table 1). *P. geniculatus* was most abundant in houses, with a total of 21 adults collected from 10 of the 15 houses examined. All 15 pigsties were infested and a large number of *P. geniculatus* were collected, including nymphs. Twenty of 118 *P. geniculatus* dissected were infected with *T. cruzi;* however, only a small proportion of the bugs captured could be examined because of the long distance between the houses studied and because of the lack of laboratory facilities in Muana. Five of the 6 palms [*Scheelea martiana* and *Maximilliana regia*] were found to be infested with *P. geniculatus*. No bugs were captured in light traps. Three of 105 domestic pigs were infected with *T. cruzi*, along with 4 of 4 *Didelphis marsupialis* and 1 of 2 *Philander opossum*. Silvatic mammals were scarce, possibly because of the constant deforestation around the houses.

Both *P. geniculatus* and pigs carried the principal zymodeme 1 (Z1), suggesting that the bugs were feeding in palms, possibly on *Didelphis*, and not on armadillos (Povoa et al. 1984). Xenodiagnoses and serological tests on all human volunteers were negative.

Fig. 1. Upper, a palm roofed, wooden house in Furo do Rio Pau Grande, where inhabitants were repeatedly attacked by *P. geniculatus;* lower left, rear view of the house above, showing pigsty infested with *P. geniculatus;* lower right, adult *P. geniculatus.*

Discussion

Our attention was drawn to the study locality in August 1993 (Valente et al. 1993) by one of the local inhabitants (A.C.C.V.) describing attacks by "bruxas," identifiable as *P. geniculatus* from examples brought to us preserved in alcohol. The house concerned was found to be on the banks of the river Pau Grande. It was built of timber with a palm frond roof and a wooden floor, with gaps between the boards, 1.5 m above ground level to protect against seasonal flooding. Immediately to the rear of the house was a rough plank enclosure for pigs, which spent the day in the forest and returned to the house at night (Fig. 1). The inhabitants had noted 1 or 2 *P. geniculatus* in the house each month from January until August, when the numbers increased to around 25. Five of these bugs were captured (blood-fed) on the 2nd of 2 nights when the inhabitants suffered numerous bites.

Subsequent inquiry revealed that *P. geniculatus* infestation was commonplace in the local community. The number of *P. geniculatus* encountered in this small group of houses was striking, because usually only single adult bugs, attracted by the light, are reported flying into houses. As far as we know there is also no record of large numbers of this species colonizing pigsties adjoining or adjacent to houses, with numerous adult bugs attacking the in-

habitants of a single house. *P. geniculatus* is frequently associated with the armadillo *D. novemcinctus*, infesting its burrows and transmitting *T. cruzi* principal zymodeme 3 (Z3) (Povoa et al. 1984). In Furo do Pau Grande, *P. geniculatus* was also found in palms and this habitat may in part be responsible for its introduction to pigsties, which were often constructed with trunks or fronds of the same palm species. The occurrence of *P. geniculatus* carrying *T. cruzi* indicated that the local inhabitants were exposed to a significant risk of acquiring Chagas disease, although none were shown to be infected. Although *T. cruzi* was only detected in 3 pigs, we thought that this was a gross under-estimate because of the severe difficulties encountered using anesthesia and xenodiagnosis for these animals. Small numbers of bugs were used, and many cultures were lost to contamination.

Triatomine bugs are reclusive insects that are seldom seen in forests. Silvatic populations are extremely difficult to locate and study, although the introduction of spool-and-line mammal tracking led to the detailed description of habitats of several Amazonian species (Miles et al. 1981c). Occasionally *P. lignarius* descends tree trunks toward forest workers, presumably attracted by movements or noise, or both (Miles et al. 1981c), and *R. brethesi* may attack harvesters of the Piacava palm (Coura et al. 1994a). *E. mucronatus* nymphs have occasionally been reported in peridomestic and domestic habitats (Noireau et al. 1995). *R. robustus* is morphologically very similar to the domestic vector *R. prolixus*, but we have no records of domiciliation by *R. robustus* in the Amazon region: a recent study indicates that the 2 species are closely related but may be genetically distinct (Garcia et al. 1998).

Miles et al. (1981c) considered that the Amazon Basin had so far been protected against endemic Chagas disease because none of the local silvatic triatomine bug species could readily adapt to houses. *P. geniculatus* was observed to require a constant high humidity, and laboratory colonies were initially only successful in Belém if they were housed in aquaria with 100% RH. However, laboratory colonies appeared to have adapted to ambient humidities. *P. geniculatus* is extremely widespread throughout the Amazon region: we have recorded its presence in >30 municipal regions of Pará State and infestation of pigsties in 1 other locality (Barcarena, V.C.V., unpublished data). If *P. geniculatus* becomes domestic it could lead to the introduction of both *T. cruzi* strain groups, Z1 and Z3, which rarely cause human infections, into domestic transmission cycles. It seems probable that at least one Amazonian species will eventually become an important domestic vector.

The states bordering the Amazon Basin to the south (Maranhao, Piaui) have 2 established domestic vector species (*T. infestans, T. braziliensis*) and to the north *R. prolixus* is the principal vector in Venezuela (Lent and Wygodzinsky 1979, Costa Bento et al. 1992, Schofield 1994). An even greater risk of the spread of endemic Chagas disease to the Amazon region is the passive transport of *T. infestans, T. braziliensis*, and *R. prolixus* from endemic areas. Domestic species such as *T. infestans* and *R. prolixus* are kept as laboratory colonies in the Amazon region for xenodiagnoses and for research purposes. Such colonies must be rigorously maintained to prevent escape into local communities. The scattered population of riverine and roadside communities suggest that control of endemic Chagas disease in the vast Amazon region would be supremely difficult.

Control programs based on spraying with pyrethroid insecticides, health education, community participation, and home improvement in well-organized preparation, attack and vigilance phases have been successful in eliminating domestic transmission of *T. cruzi* from many endemic areas in Brazil (Dias 1987). The Southern Cone Programme of cooperation among Argentina, Bolivia, Brazil, Chile, Paraguay, and Uruguay proposes to eliminate domestic *T. infestans* from South America. The observations here, however, guard against complacency. It seems clear that the threat of endemic Chagas disease to the Amazon region demands a special program of vigilance (Dias 1987). This should include training of public health personnel to recognize both triatomine bugs and *T. cruzi;* to examine suspect patients parasitologically and serologically, and to alert local populations to the risk and need to report any domestic bug populations. Local public health authorities should have a prepared plan of action for rapid implementation in the event of the discovery of domestic triatomine colonies, whether of an Amazonian or introduced species (Miles et al. 1981c, Amunarriz et al. 1991, Valente and Valente 1993).

Acknowledgments

We especially thank H. Fraiha Neto and M. M. Povoa for their encouragement and support, and J.E.A. Araujo, J.A.N. Lima, F. S. Gomes, R. M. Almeida, J. J. Viana, and A. Figueiredo for technical assistance. This research was funded by the Fundaçao Nacional de Saude (CCDTV-DF, Brazil), the Consejo Nacional de Investigaciones Cientificas y Tecnologicas (CONICIT, Venezuela), the Wellcome Trust (United Kingdom), and the European Commission (STD3) Contract No. TS3*CT92-0092, in collaboration with the Wellcome Parasitology Unit (Brazil) and the municipal authorities of Muaná.

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Received for publication 2 December 1996; accepted 7 July 1997.