

TRYPANOSOMA CRUZI-LIKE BLOODSTREAM TRYPOMASTIGOTES IN BATS FROM THE STATE OF PIAUÍ, NORTHEASTERN BRAZIL

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One-hundred and thirty-five bats from 12 species were examined for the presence of trypomastigotes by means of direct blood examination, xenodiagnosis, and hemoculture. Of those, 44 specimens (32.6%) from 8 species were infected with trypanosomes. *Phyllostomus discolor discolor* presented the highest rate of infection, being captured only in one locality, while *Phyllostomus hastatus hastatus* captured in four localities showed high rates. Two species, *Anoura geoffroyii* and *Pteronotus* (*Phyllodia*) *parnelli rubiginosa*, were found infected by *T. cruzi*-like trypanosomes, apparently described for the first time. Flagellates from *Artibeus jamaicensis jamaicensis* and *A. geoffroyii* were able to produce detectable parasitaemia in young mice. One triatomine bug was found infected in natural conditions, *Triatoma brasiliensis* was associated with a *P.h. hastatus* colony, in which six captured bats were also found infected.

Key words: *Trypanosoma cruzi*-like trypanosomes. Bat infections. Bloodstream trypomastigotes. Epidemiology.

Several bat species from South America are described as natural hosts for *Trypanosoma cruzi*, the etiological agent of Chagas' disease, as well as for *T. cruzi*-like trypanosomes^{1 2 3 7 8}. The latter display morphological characteristics similar to those of *T. cruzi*, but differ from it mainly for their inability to infect laboratory animals².

The present paper describes the prevalence of *T. cruzi*-like trypanosomes in bats from the State of Piauí, Northeastern Brazil.

MATERIAL AND METHODS

Capture of bats. The bats were captured mainly in caves in five different localities in the State of Piauí: Castelo do Piauí, Palmeiras, Picos, São João do Piauí, and Canto do Buriti (Figure 1). The bats were caught using either mist or hand-nets.

Search of trypanosomes. Three parasitological methods were used for detection of trypanosomes: a) Microscopical examination of blood – from each animal, three fresh preparations were examined. The bat was considered negative if no flagellates was found after 15 minutes observation; b) Xenodiagnosis – this technique, as used by Freitas⁶, consisted on feeding clean triatomines (4-5 instar), reared in the laboratory, on vertebrate's blood. It was performed using the

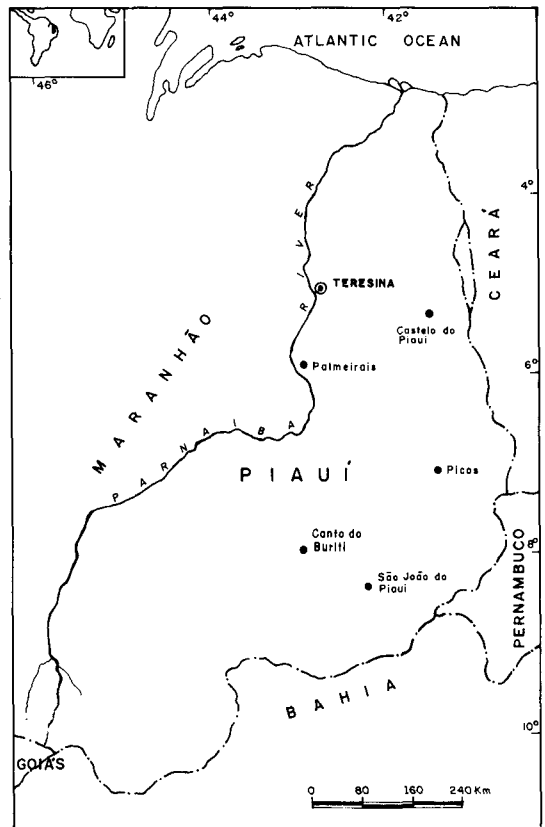


Figure 1. Map of the State of Piauí, showing the localities where the bats were captured and the State capital (Teresina).

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This work was supported by grants from CNPq.

Recebido para publicação em 29/8/1985.

species *Triatoma brasiliensis*, *Rhodnius prolixus* and *Dipetalogaster maximus*. About 10 insects were fed

on each bat, and 25-30 days later their feces were microscopically examined; c) Hemoculture – about 0.5 ml of cardiac blood of each bat was taken with a tuberculin syringe, previously rinsed with heparinized physiological saline and dispensed in three tubes containing 5.0 ml of NNN medium, added with penicillin (200 U/ml) and streptomycin (50 µg/ml). The tubes were kept at 28° C and examined daily for flagellate growth for up to one month.

From those bats that presented flagellates by fresh blood examination, thin blood and organ impression smears (heart, spleen, liver, stomach and kidney) were made, fixed with methanol and giemsa stained. The micrometry was carried out using a OSM Micrometric Olympus eyepiece and 100X immersion oil objective.

Infectivity of flagellates to young mice. Infected blood from 9 bats, positive bug faeces from xenodiagnosis in 25 bats and culture forms from 15 bats (Table 3), were inoculated intraperitoneally in groups of 3 young mice. The mice were later examined by fresh blood preparation and xenodiagnosis.

Natural infection of triatomines by trypanosome. Triatomines were searched in the bat colonies and examined for the presence of flagellates by abdominal compression. If flagellates were present, the feces were inoculated into mice, which were examined by fresh blood preparations and xenodiagnosis.

Identification of triatomine blood meal. The gut content of bugs was placed on filter paper and used later a precipitin test, as described by Siqueira¹¹. The following antisera were used: anti-human, anti-dog, anti-cat, anti-cattle, anti-pig, anti-rodent, anti-bird and anti-marsupial. All of them were prepared by three intra-muscular injections of sera with Freund's complete adjuvant, at intervals of three weeks.

RESULTS

Distribution of naturally infected bats in the localities studied. One hundred and thirty-five bats from 12 species were examined and 44 (32.6%) belonging to 8 species, were found to be naturally infected with flagellates. The infection rate ranged from 0 in 4 species to 66.7% in one species (Table 1). *P. d. discolor* presented the highest rate of infection, and was captured only in one locality, while *P. h. hastatus*, which was captured in four localities, showed also a high rate of infection.

Characteristics of natural infection of bats. The positive bats, determined by blood microscopic examination, showed a low grade of parasitaemia. Trypomastigotes in stained smears were observed only twice in bats of the species *P. d. discolor* and *P. h. hastatus* (Figure 2). No flagellate forms were observed in the organ impression smears.

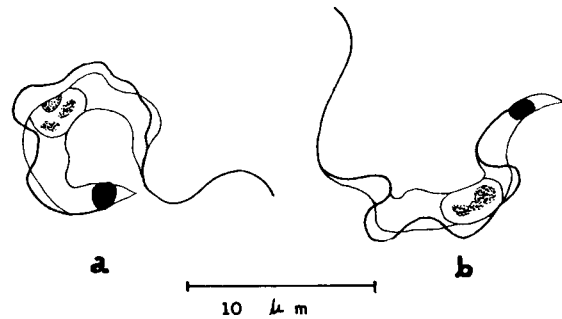


Figure 2. Drawing of bloodstream trypomastigotes observed in Giemsa stained smears (a, from *P. d. discolor*; b, from *P. h. hastatus*).

Table 1 – Rate of infection by *Trypanosoma cruzi*-like trypomastigotes in bats from five localities of the State of Piauí (Castelo do Piauí, Palmeirais, Picos, São João do Piauí and Canto do Buriti), Northeastern Brazil.

Species	Castelo do Piauí		Palmeirais		Picos		São João do Piauí		Canto do Buriti		Total	
	Exam-ined	Infected	Exam-ined	Infected	Exam-ined	Infected	Exam-ined	Infected	Exam-ined	Infected	Exam-ined	In-fected (%)
<i>Anoura geoffroyi</i>	—	—	—	—	—	—	—	—	13	3	13	3 (23.1)
<i>Artibeus jamaicensis jamaicensis</i>	—	—	—	—	5	2	4	1	—	—	9	3 (33.3)
<i>Carollia perspicillata perspicillata</i>	—	—	12	2	8	2	—	—	—	—	20	4 (20)
<i>Desmodus rotundus rotundus</i>	—	—	—	—	2	1	—	—	1	—	3	1 (33.3)
<i>Desmodus youngii</i>	—	—	—	—	1	—	—	—	—	—	1	— (0)
<i>Diphylla ecaudata</i>	—	—	—	—	—	—	—	—	9	—	9	— (0)
<i>Phyllostomus discolor discolor</i>	24	16	—	—	—	—	—	—	—	—	24	16 (66.7)
<i>Phyllostomus hastatus hastatus</i>	7	—	14	6	11	6	14	3	—	—	46	15 (32.6)
<i>Pteronotus (Chilonycteris) personatus personatus</i>	—	—	—	—	1	—	—	—	—	—	1	— (0)
<i>Pteronotus (Phyllostoma) pamellii rubiginosa</i>	—	—	—	—	—	—	1	—	5	1	6	1 (16.7)
<i>Pteronotus (Pteronotus) gymnotus</i>	—	—	—	—	—	—	—	—	1	—	1	— (0)
<i>Trachops cirrhosus cirrhosus</i>	—	—	2	1	—	—	—	—	—	—	2	1 (50)
Total	31	16(51.6%)	28	9(32.1%)	28	11(39.3%)	19	4(21.1%)	29	4(13.8%)	135	44 (32.6)

Comparative data of the diagnosis methods used. For comparative analysis of the results obtained by different methods employed see Table 2.

Infectivity of flagellates to mice. The data on the young mice inoculated with flagellates collected from different localities are shown in Table 3. Only flagellates from the species *A. j. jamaicensis* and *A. geoffroyii* were able to produce detectable parasitaemia in the inoculated young mice.

Triatomine associated with P. h. hastatus. One triatomine, *Triatoma brasiliensis*, was found associated with a *P. h. hastatus* colony, inside a chapel at the Picos, a place where eleven bats were also captured. The triatomine and six bats were found infected with flagellates that were unable to produce detectable parasitaemia in the young mice inoculated. The gut content of the insect was reactive only against the anti-bat antiserum.

DISCUSSION

The bat species which were found infected with trypanosomes in the present survey, were also described as infected in other regions of South America^{1 2 3 7 8}. Only the species *A. geoffroyii* and *P. (P.) parnellii rubiginosa* seem not to have found infected before. Although *P. d. discolor* had presented the highest rate of infection, it was captured only in one locality, while *P. h. hastatus*, which also showed a high infection rate, was captured in four localities. These data agree with those found in the literature with regard to this bat species from other regions^{1 2 3 8}.

The low levels of parasitaemia, as observed in the present study, and the low counts of tissue forms in bat species are well documented.^{2 7} The considerable natural resistance of bats to infection may be due to the high environmental temperature of roosting bats, which would reflect in the body temperature⁷.

Table 2 - Comparative data using different methods of diagnosis, to search trypanosomes in bats of different species from the State of Piauí, Northeastern Brazil.

Bat species	Number of bats examined	Methods of diagnosis and number of positive bats				Total	(%)
		Blood examination	Xenodiagnosis	Hemoculture			
<i>P. h. hastatus</i>	46	6	14	12	15	(32.6)	
<i>P. d. discolor</i>	24	10	16	16	16	(66.7)	
<i>C. p. perspicillata</i>	20	3	—	4	4	(20.0)	
<i>A. geoffroyii</i>	13	1	3	2	3	(23.1)	
<i>A. j. jamaicensis</i>	9	2	3	2	3	(33.3)	
<i>D. ecaudata</i>	9	—	—	—	—	—	
<i>P. (P.) parnellii rubiginosa</i>	6	—	—	1	1	(16.7)	
<i>D. r. rotundus</i>	3	1	1	—	1	(33.3)	
<i>T. c. cirrhosus</i>	2	—	1	—	1	(50.0)	
<i>P. (P.) gymnotus</i>	1	—	—	—	—	—	
<i>D. youngii</i>	1	—	—	—	—	—	
<i>P. (C.) p. personatus</i>	1	—	—	—	—	—	
Total	135	23(17.0%)	38(28.1%)	37(27.0%)	44	(32.6)	

Table 3 - Production of detectable parasitaemia in young mice inoculated with trypanosomes from six bat species captured in different localities of the State of Piauí, Northeastern Brazil.

Bat species	Origin of flagellates							
	Blood		Xenodiagnosis		Hemoculture		Total	
	Inoculated	Positive	Inoculated	Positive	Inoculated	Positive	Inoculated	Positive
<i>A. geoffroyii</i>	—	—	2	—	1	1	3	1
<i>A. j. jamaicensis</i>	1	—	2	1	1	—	4	1
<i>C. p. perspicillata</i>	1	—	—	—	2	—	3	—
<i>D. r. rotundus</i>	—	—	1	—	—	—	—	—
<i>P. d. discolor</i>	4	—	10	—	4	—	18	—
<i>P. h. hastatus</i>	3	—	10	—	7	—	20	—
Total	9	—	25	1	15	1	48	2

Xenodiagnosis showed, in general, the highest rate of positivity in relation to hemoculture. It could be due, in some cases, to the difficulty to obtain a suitable cardiac puncture in these small animals. On the other hand, all xenodiagnoses were negative in bats from the species *C. p. perspicillata*, found infected by fresh blood preparations. This fact has been observed in bats of the same and other species such as *Lonchoglossa ecaudata*, *Glossophaga soricina* and *Noctilio labialis* from other regions^{4 5}. In our study, xenodiagnosis was performed at same conditions with bats from the species *P. h. hastatus*, in which the test resulted positive. This event may reflect biological dissimilarities (e. g. vector restriction) among these flagellates. The triatomine species *T. brasiliensis* seems to be involved in trypanosome transmission between certain bat species, such as *P. h. hastatus* in Northeastern Brazil, as indicated in the present study. One infected specimen was found associated to a bat colony, from which six bats were also found infected.

We believe that the flagellates from the species *A. geoffroyii* and *A. j. jamaisensis*, that were able to produce detectable parasitaemia in young mice were similar to *T. cruzi*, since the latter was described as infected with *T. cruzi* in the bordering State of Ceará¹. On the other hand, after the classical work by Dias³ with trypanosomes from *P. h. hastatus*, this and other bat species, from different regions in the Americas, have been found infected with flagellates which show similarities with *T. cruzi* but differ from it mainly for their incapacity to infect laboratory animals^{1 2 3 7 8}. Although the taxonomy of these trypanosomes is still an open question, the possibility should be considered that they may be potentially infective to man².

Chagas' disease remains a serious public health problem in Brazil, particularly in the Northeast. Perhaps a more extensive survey in this and other localities from the State of Piauí about the role of bats as reservoirs of *T. cruzi* may prove to be rewarding.

RESUMO

Cento e trinta e cinco morcegos, pertencentes a 12 espécies diferentes, foram examinados quanto à presença de tripomastigotas sanguíneos, por meio do exame direto do sangue, xenodiagnóstico e hemocultura. Deles, 44 exemplares (32.6%), pertencentes a 8 espécies, estavam infectados por flagelados semelhantes ao *T. cruzi*. *P. d. discolor* apresentou a mais elevada taxa de infecção, sendo capturado em apenas uma localidade, enquanto que *P. h. hastatus*,

capturado em quatro localidades, apresentou também altas taxas de infecção. Duas espécies, *A. geoffroyii* e *P. (P.) parnellii* rubiginosa foram encontradas infectadas por flagelados semelhantes ao *T. cruzi*, aparentemente pela primeira vez. Flagelados provenientes das espécies *A. j. jamaisensis* e *A. geoffroyii* foram capazes de produzir parasitemia em camundongos jovens. Um exemplar de *T. brasiliensis*, infectado em condições naturais, foi encontrado associado a uma colônia de *P. h. hastatus*, na qual seis morcegos também foram encontrados infectados.

Palavras-chaves: Tripanossomas semelhantes ao *T. cruzi*. Infecção de morcegos. Tripomastigotas sanguíneos. Epidemiologia.

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