TRYPANOSOMATIDS IN PHYLLOSTOMIDS (CHIROPTERA, PHYLLOSTOMIDAE) FROM PEROBAS BIOLOGICAL RESERVE, PARANÁ, BRAZIL

Sandra Mara Milani Nishimura[1]
Henrique Ortêncio Filho[2]
Marcos Magalhães[3]

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ABSTRACT: The family *Trypanosomatidae* reunites a great number of species which parasite several organisms, among them, chiropterans, which may act as reservoirs. The present study aimed at demonstrating the occurrence of trypanosomatids in the blood of phyllostomids from Perobas Biological Reserve, Paraná, Brazil. The capture of the animals was performed with the aid of mist nets in July and August, 2008. The bats were contained manually and for the parasite analysis, a small drop of blood was collected in order to prepare imprint samples which were fixed in absolute methanol. In the laboratory, the slides were stained by the method of Giemsa, observed and photographed with the aid of a light microscope. Fifteen positive results were found out of 33 animals captured. Five of the species captured, *Artibeus lituratus*, *Artibeus fimbriatus*, *Artibeus planirostris*, *Artibeus obscurus* and *Sturnira lilium* presented trypanosomatids. Only two species did not have positive slides, *Carollia perspicillata* and *Pygoderma bilabiatum*. The species with positive slides have a diversified diet, including the ingestion of insects, which may facilitate the infection. These results contribute with information about the occurrence of these blood parasites in bats since studies about the subject in Brazil are scarce.
KEYWORDS: Trypanosomatids. Bats. Northwest of Paraná.

TRIPANOSSOMATÍDEOS EM FILOSTOMÍDEOS (CHIROPTERA, PHYLLOSTOMIDAE) DA RESERVA BIOLÓGICA DAS PEROBAS, PARANÁ, BRASIL

RESUMO: A família Trypanosomatidae reúne grande número de espécies que parasitam os mais diversos organismos, entre eles, os quirópteros, que podem atuar como reservatórios. O presente estudo teve por objetivo investigar a ocorrência de tripanossomatídeos no sangue de filostomídeos da Reserva das Perobas, Paraná. A captura dos animais ocorreu com o uso de redes de neblina, nos meses de julho e agosto de 2008. Os morcegos foram contidos manualmente, e, para a análise dos parasitas, uma gotícula de sangue foi retirada para a confecção do esfregaço e fixação com metanol absoluto. No laboratório as lâminas foram coradas pelo método Giemsa, observadas e fotografadas ao microscópio. Foram obtidos 15 resultados positivos dos 33 exemplares capturados, sendo que cinco das sete espécies capturadas, *Artibeus lituratus*, *Artibeus fimbriatus*, *Artibeus planirostris*, *Artibeus obscurus* e *Sturnira lilium*, apresentaram tripanossomatídeos sanguíneos. Somente em *Carollia perspicillata* e *Pygoderma bilabiatum*, não foram constatados esses parasitos. Os animais com resultados positivos têm uma dieta bastante diversificada, incluindo a ingestão de insetos, o que pode acarretar infecções, caso alimentem-se de insetos contaminados. Esses resultados contribuem com as informações acerca da ocorrência de parasitas sanguíneos em morcegos, haja vista a escassez de estudos sobre o assunto no Brasil.
PALAVRAS-CHAVE: Tripanossomatídeos. Morcegos. Noroeste do Paraná.

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RESUMEN: La familia Trypanosomatidae reúne gran número de especies que parasitan los más diversos organismos, entre ellos, los quirópteros, que pueden actuar como reservatorios. Esta investigación buscó la ocurrencia de tripanosomatídeos en la sangre de filostomídeos de la Reserva de las Perobas, Paraná. La captura de los animales ocurrió con el uso de redes de neblina, en los meses de julio y agosto de 2008. Los murciélagos fueron contenidos manualmente, y para el análisis de los parasitas, una gota de sangre fue retirada para fregado y fijación con metanol absoluto. En el Laboratorio las láminas fueron coloreadas por el método Giemsa, observadas y fotografiadas al microscopio. Se alcanzó 15 resultados positivos dos 33 ejemplares capturados, siendo que cinco de las siete especies capturadas, *Artibeus lituratus*, *Artibeus fimbriatus*, *Artibeus planirostris*, *Artibeus obscurus y Sturnira lilium*, presentaron tripanosomatídeos sanguíneos. Solamente en *Carollia perspicillata* y *Pygoderma bilabiatum*, no se constató esos parasitas. Los animales con resultados positivos tienen una dieta bastante diversificada, incluyendo la ingestión de insectos, lo que puede causar infecciones caso se alimenten de insectos contamina-

[1]Biologist and integrant of GEEMEA (Group of Studies in Ecology of Mammals and Environmental Education) from Universidade Paranaense, Campus Cianorte-PR. Address: Av. Brasil, 1123, CEP 87200-000 - Paraná, Brazil. E-mail: sandranishimura@yahoo.com
[2]Science Department Professor - Universidade Estadual de Maringá, Campus Goioerê - PR and Coordinator of GEEMEA. E-mail: henfilhobat@gmail.com
[3]Biological Sciences Department Professor - Universidade Paranaense and integrant of GEEMEA, Campus Cianorte-PR, E-mail: marcosmls@yahoo.com.br

dos. Esos resultados contribuyen con las informaciones acerca de la ocurrencia de parasitas sanguíneos en murciélagos, dada la escasez de estudios sobre el asunto en Brasil.
PALABRAS CLAVE: Tripanosomatídeos. Murciélagos. Noroeste de Paraná.

Introduction

The family *Trypanosomatidae* reunites a great number of flagellate protozoan which parasite several organisms, men including. Most of them have, as a permanent or temporary habitat, the digestive tract of insects (CIMERMAM; CIMERMAM, 2006). These invertebrate hosts, kissing bugs known in Brazil as barbeiros, chupanças, among other popular names, belong to the order *Hemiptera*, family *Reduviidae* which are infected when they ingest blood with trypomastigote forms (REY, 2001).

This family is composed by nine genera with obligatory parasites. Hosts may be other protozoans, plants, annelids, arachnids, insects, reptiles, amphibians, birds and mammals (NEVES, 2005).

Chagas disease or American trypanosomiasis is a broadly disseminated zoonosis in Latin America, presenting a strong incidence in Brazil, where there is an estimative of 15 to 20 million infected people (REY, 2001). The protozoan *Trypanosoma cruzi*, etiological agent of the disease, develops in the digestive tract of vectors and transmission occurs when these parasites are found in metacyclic trypomastigote form, by the contact with feces of the vector (FERREIRA; ÁVILA, 2001).

This zoonosis is involved in several ecological niches due to the great variety of vertebrate hosts which are susceptible to the infection, the number of triatomine bug species which host the parasite (REY, 2001) as well as the protozoans, which present a high degree of heterogeneity, which makes epidemiological, clinical, pathological, therapeutic and laboratory studies difficult to be performed (FERREIRA; ÁVILA, 2001).

T. cruzi is infective for a great number of species belonging to the orders *Marsupialia, Edentata, Carnivora, Rodentia, Artiodactyla, Perissodactyla, Primates* and *Chiroptera* (REY, 2001). The occurrence of *T. cruzi*-like organisms in bats is a problem to be taken into account in epidemiological studies about the agents of Chagas disease (SOUZA, 1999).

Chiropterans occupy, in Brazil, the second group in diversity among mammals in the neotropical region, both in number of species and individuals (TADDEI; PEDRO, 1998); are susceptible to several pathogens and have an important role as natural reservoirs (TAMSITT; VALDIVIESO, 1970; TADDEI, 1983). Several species are proven reservoirs of *T. cruzi*, specially the genera *Desmodus*, *Artibeus*, *Carollia*, *Phyllostomus* e *Eumops* (REY, 2001).

Bats use as shelters: caves, mines, rock clefts, cavities in hollow trees and branches, bushes and buildings (PERACCHI, et al. 2006). Thus, they are important, not only by the great mobility and easy adaption to human shelters, but also by the high rates of parasitism, as verified in the state of Pará, Brazil, where 80% of *P. hastatus* which were captured were hosts of *T. cruzi*. Some invertebrate vectors, such as the triatomine bug *Cavernicola pilosa* Barber, 1937, are found exclusively in wild habitats, cohabiting with bats in their shelters (REY, 2001).

Due to the scarceness of information about blood parasites in chiropterans, the present work aims at investigating the occurrence of trypanosomatids in phyllostomid bats from Perobas Biological Reserve, Paraná, Brazil.

Material and Methods

The present study was performed at Perobas Biological Reserve, from July to August, 2008. The conservational unit is located at 23° 52' 52,38''S, 52° 44' 08,65''W and 400 meters of altitude, in the cities of Tuneiras do Oeste and Cianorte, northwest of the state of Paraná, with an area of 8,716 hectares (Figure 1). The region is characterized by the contact between the Stational Semidecidual Sub-mountain Forest, with predominance of large timber trees (*Aspidosperma* spp.), cedars (*Cedrella fissilis*) and palmettos (*Euterpe edulis*) and Mixed Ombrophile Forest, distinguished by the occurrence of pine trees (*Araucaria angustifolia*) (CASTELLA; BRITEZ, 2004).

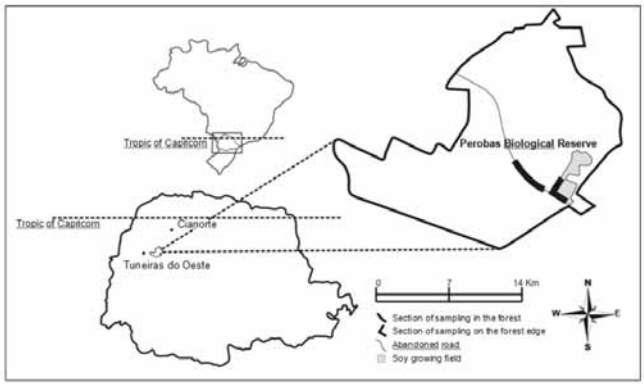

Figure 1. Location of Perobas Biological Reserve, Paraná, Southern Brazil and sites of sampling.

In order to capture the bats, 32 mist nets measuring 8,0 meters of length and 2,5 meters of height were used, set during 11 hours, from the sunset until the sunrise, beginning when the intensity of light was lower than 5 lux and ending when intensity was higher than this value. These values were determinated with the aid of a digital luximeter Minipa® model MLM-1010. The nets were set along a track of, approximately 1,5 km in an inactivated road, which occupies a track of 11 km inside the reserve.

Bats were contained manually with leather gloves and afterwards specimens were identified, according to criteria of Vieira (1942), Husson (1962), Vizotto and Taddei (1973), Jones and Carter (1976) and biometric data were recorded.

For blood collection, a venous puncture technique according to Baptista et al. (2006) was used. The technique consists of a perforation in the cephalic vein ventrally, with the aid of an hypodermic needle after antisepsis with 70% ethylic alcohol. A blood drop of about 10µl was used for preparing smears and fixed with absolute methanol. After the procedure, animals were released.

All the slides were identified and stored in a slide box. In the Universidade Paranaense Microbiological Labo-

ratory, Campus Cianorte, the slides were stained by the method of Giemsa, according to Neves (2005) and afterwards, analyzed with the aid of a binocular light microscope and photographed using the software Motic Images Version 1.3. Data were analyzed by means of descriptive statistics and represented in percentages.

Results

Samples were collected from 33 phyllostomid bats belonging to seven species, mostly *Sturnira lilium* (n=12) and *Artibeus lituratus* (n=11). According to results, 15 positive slides were found (table 1).

Table 1. Occurrence of trypanosomatids in bats captured at Perobas Biological Reserve, Paraná, Southern Brazil, from July to August, 2008, according to hematoscopy. NT – total number of individuals, PO – percentage of occurrence, NP – total number of parasitized individuals and NP(%) percentage of parasitized individuals related to the species.

Species	NT	PO (%)	NP	NP(%)
Family Phyllostomidae				
Subfamily Carolliinae				
Carollia perspicillata (Linnaeus,1758)	03	9,1	00	0,0
Subfamily Stenodermatinae				
Artibeus lituratus (Olfers,1818)	11	33,3	06	54,5
Artibeus fimbriatus Gray,1838	01	3,0	01	100,0
Artibeus planirostris Spix, 1823	02	6,1	02	100,0
Artibeus obscurus Schinz, 1821	01	3,0	01	100,0
Pygoderma bilabiatum (Wagner, 1843)	03	9,1	00	0,0
Sturnira lilium (E. Geoffroy, 1810)	12	36,4	05	41,7
Total	**33**	**100**	15	

The species *C. perspicillata* and *Pygoderma bilabiatum* did not present trypanosomatids in the blood, whereas the others revealed percentages between 41,7 to 100% of contamination. Protozoans presented a dimension of about 20 micrometers. It was also possible to evidence structures such as kinetoplast, nucleus and flagellum (Figure 2).

Figure 2. *Trypanosoma* sp. in trypomastigote form found in the blood of *Sturnira lilium* captured at Perobas Biological Reserve, from July to August, 2008.

Discussion

Among captured species, *C. perspicillata*, *P. bilabiatum* and *S. lilium,* have frugivorous feeding habits, whereas species belonging to the genus *Artibeus*, despite being frugivorous, also eat insects (GARDNER, 1977). As oral contamination, by means of ingesting infected triatomine bugs was demonstrated by Thomas, Rasweiler IV and Alessandro (2007), such fact may, to some extent, explain the high percentage of infected specimens of *Artibeus*, although this supposition may not be extended to the species *S. lilium*, which presented a relatively high percentage of infected individuals, despite not eating insects. It suggests that both oral transmission and the inoculation (by the insect bite) are important, because, according to Rey (2001), the oral infection by trypanosomatids is frequent among animals which hunt or kill insects, or leak contaminated fur in irritated bite spots.

Usually, invertebrate hosts get infected ingesting blood with trypomastigote forms, which are found in the blood of vertebrates, being some destroyed by digestive enzymes while others differentiate into amastigotes, which are ovoid and intracellular forms, which may degenerate or be taken to the medium intestine where they differentiate into epimastigotes, multiply and suffer a new metamorphosis, adopting the form of metacyclic trypomastigotes. This is the infective form for vertebrates, which is eliminated in the feces of the parasite or by the oral apparatus. According to the same author, blood trypomastigote forms are found in vertebrate hosts whereas amastigote forms are found in muscular tissues, where they multiply and go back to the circulatory system in trypomastigote forms (REY, 2001).

The family *Trypanosomatidae* contemplates the genus *Trypanosoma* with two sections, *Stercoraria* and *Salivaria*. In the section *Stercoraria,* three subgenera are found: *Megatripanum*, *Herpetosoma* and *Schizotrypanum* (REY, 2001). These three subgenera of trypanosomatids were already evidenced in bat blood, according to studies previously cited in the present work. However, according to Sousa (1999), the supposedly specificity of *Schizotrypanum* species in bat species need to be better investigated, by means of hemoculture from animals inoculated experimentally for longer periods, in order to confirm the infectivity of these parasites.

Some reports have been published focusing the

parasitism between trypanosomatids and bats. In Iraq, Marinkelle (1977) verified that bats of the species *Pipistrellus Kuhli* were infected by trypanosomatids of the genus *Schizotrypanum*. In *Taphozous nudiventris,* was evidenced the parasite of the subgenus *Megatripanum*, and in some individuals of this species, parasites of the subgenus *Herpetosoma* were found.

In Madagascar, Raharimanga et al. (2003) collected 440 bats, from which 93 presented at least one species of hemoparasite, with a prevalence of 0,7% of trypanosomatids.

Studies in Colombia about experimental transmission, bats, after ingesting triatomine bugs *Rhodimus prolixus* infected by *T. cruzi*, obtained the following rates of infection: from 10 bats of the species *C. perspicillata* three became infected, of eight *A. lituratus* two were infected, of 14 *Molossus molossus* two were infected and only *Glossophaga soricina* was not infected. From a total of 20 individuals of *P. hastatus*, seven became infected after eating contaminated mice. *Glossophaga soricina* and *C. perspicillata*, were infected with a subcutaneous and intragastric inoculation of a fecal suspension containing *T. cruzi* (THOMAS; RASWEILER IV; ALESSANDRO, 2007).

Studies focusing the infection by trypanosomatids are scarce in Brazil. Fábian (1991), recorded 15 bat species in the state of Ceará, and among them, *A. planirostris* (Spix, 1823), *P. hastatus* (Pallas, 1767), *P. discolor* (Wagner, 1843) and *S. lilium* (Geoffroy, 1810), presented natural infection by *T. cruzi*. Trypanosomatids isolated from *A. planirostris* (Spix, 1823) and some specimens obtained from *P. hastatus* (Pallas, 1767), were *T. cruzi*-like, due to its form, dimension and behavior. On the other hand, forms and dimensions found in protozoans obtained from *P. discolor* (Wagner, 1843) and the non infection in mice allowed the identification of *T. cruzi* variety marinkellei. The species *T. cruzi* marinkellei is a subspecies of *T. cruzi*, characterized with the aid of enzymatic studies by Baker et al. (1978), which used bats as reservoirs. The species *T. verspertilionis* parasitize bats and is morphologically similar to *T. cruzi* (REY, 2001).

In southern Brazil, Grisard, Sturm and Campbell (2003), isolated *T. desterrensis* sp., a new species and Vilar et al. (2004) demonstrated the presence of *T. pessoai* Deane and Sugay 1963 subgenus *Megatrypanum* in the hematophagous species *D. rotundus* (GEOFFROY, 1810) in the state of Rio de Janeiro.

Dias et al. (1942), in the state of Pará, verified several species of bugs, among them, the tryatomine bub *C. pilosa* in hollow trees cohabiting with colonies of bats. Researches were performed in the digestive tube of these bugs, but only specimens of the species *C. pilosa*, were infected with flagellates which were very similar to the subgenus *Schizotripanum*.

Deane (1961) examined blood samples of 49 bats in the state of Pará, with four animals positive, being two of the species *P. hastatus*, one *M. rufus* and one *M. obscurus*.

Dias et al. (1942), in Marajó island, Pará, examinated 588 morcegos belonging to 17 species with 27 positive individuals among seven species: *Eumops abrasus, Saccopteryx bilineata, Micronycteris megalotis, Dirias albiventer, Hemiderma perspicillatum, P. elongatum* and *G. soricina*, which were parasitized by trypanosomatids of the genus *Schizotrypanum*.

Conclusion

It was possible to conclude that the presence of trypanosomatids in bats is a great problem for epidemiological studies of Chagas Disease, due to difficulties in distinguishing species and subspecies, mainly the subgenus *Schizotryapnum* in which *T. cruzi* is included. On the other hand, there is a possibility that *T. cruzi*-like species may be used as models for selecting new medicines.

So far, there are not enough studies about the infectivity and pathogenicity potential of species found in bats for humans and other animals, in part due to the great variation of hosts and parasite lineages. We may consider, however, that many chiropteran species are quite common, with great populations, with habitats close to human residences and with high rates of parasitism.

Due to the amount of infected chiropterans, we suggest the need of other studies focusing reservoirs, vectors, species of trypanosomatids and infective potential for humans of the parasites found in bats captured at Perobas Biological Reserve.

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