**INTRODUCTION**

Rabies is an anthropozoonosis that affects the Central Nervous System (CNS), causing acute infectious encephalomyelitis and, in most cases, death. The virus that causes this disease belongs to the genus *Lyssavirus*, Rhabdoviridae family, Mononegavirales order, which has a number of different variants that may be maintained in one or more species, acting as regional hosts. In Latin America, domestic dogs have always been considered to be the primary reservoir of the classic rabies virus (RABV), but since 2004, the common vampire bat, *Desmodus rotundus* (E. Geoffroy 1810), has become the principal vector and reservoir of this zoonosis. This shift in the epidemiological profile of the disease has been particularly relevant in northern Brazil, where outbreaks of human rabies in 2004 and 2005 were caused by hematophagous bats in rural areas of the states of Para and Maranhão, reflecting the widespread deforestation and associated environmental impacts that affect this region.

In urban areas, the number of cases of human rabies caused by dogs declined considerably in the Americas, as a result of the Pan-American Health Organization initiatives, meanwhile the number of cases caused by bats increased, because of the aforementioned rural cases. However, a number of South American studies have confirmed cases of rabies, in both bats and humans, in urban environments in countries such as Chile and Colombia, as well as Brazilian cities, including Rio de Janeiro, Ubatuba in São Paulo, and Campo Grande in Mato Grosso do Sul.

The outbreaks of rabies in humans caused by *D. rotundus* that occurred throughout much of the northeast of the state of Para, made this region an important area for the study of the virus and its vectors, although the researches were restricted to rural areas. Given this, our study presents the first virological and serological diagnosis of RABV in bats, in an urban zone in the Brazilian Amazon region. The main aim of this study is to better understand the epidemiology of the zoonosis in this environment through the identification of potential vector species that may contribute to the transfer of RABV between rural and urban environments.

**METHODS**

**Ethical statement:** The collection and transportation of bat specimens and biological samples (brain tissue and blood) for scientific
purposes was authorized by the Federal Chico Mendes Institute for the Conservation of Biodiversity (ICMBio) through license number 23151-1, obtained on June 15, 2010.

Study site: The present study was conducted in the town of Capanema (1.19° S, 47.18° W), which is located in the northeastern mesoregion of the Brazilian State of Para, in the eastern Amazon basin (Fig. 1). This municipality covers an area of 614,693 km², at an altitude of 24 m a.s.l. 19.

The region’s climate is of Köppen’s Am subtype, that is, equatorial hot and humid, with rainfall throughout the year, but distributed in a

Fig. 1 - Map of Brazil (A) showing the municipality of Capanema (B), located in the northeast of the state of Para, and the urban area of Capanema with the eight points of sampling (C).
wet season, between February and July, and a dry or less rainy season, between September and December. Annual precipitation is generally between 2,300 mm and 2,500 mm, and mean annual temperatures range from 26.5 °C to 31.5 °C90.

**Specimen collection:** The specimens were collected in May/June and October/November, 2011, covering the dry and wet seasons, respectively. Ten mist nets (3 m x 7 m, with a 20 mm mesh) were employed at different sample points located next to wooded areas inside the town, likely to provide bats with resources (e.g., refuges, food). The nets were set 0.5 m above ground level between 6 p.m. and 1 a.m. during the waning and new moon. They were set up on two consecutive nights at each sampling point during each season, with a total of 16 nights of specimen collection in the dry season and 16 in the wet season. The captured bats were maintained in cotton bags prior to manipulation and the collection of blood samples, and were subsequently released at the capture site.

**Identification of specimens and preparation of the voucher specimens:** The bats captured in the mist nets were identified to species using a number of different dichotomous keys8,13,15,16,22 and the species were classified as in REIS et al.9. The age of the specimens was determined by the degree of epiphyseal discs fusion in the articulations of the hand, between the metacarpals and the phalanges1.

The specimens that had to be sacrificed for extraction of the brain tissue samples were labeled, fixed in 10% formalin and then conserved in 70% ethanol for subsequent taxonomic analysis. These specimens constitute the reference collection for the present study, and were deposited in the Braganca Zoological Collection at the Braganca campus of the Federal University of Para.

**Collection and processing of serum samples:** Blood samples of 0.2-0.3 mL were collected through cardiac puncture (in the case of the voucher specimens) using a 1 mL insulin syringe. In all other specimens, the blood samples were extracted from the propatagial vein, which was perforated with a hypodermic needle for the collection of drops of blood in a hematocrit tube4.

These samples were initially preserved at a temperature of 15 °C for transportation to the laboratory, where they were centrifuged at 4,000 rpm for five minutes. The serum was removed and poured into sterilized microtubes and identified with individual codes prior to being stored at temperatures of 2-8 °C for subsequent transportation to the Laboratory for Zoonoses and Vector-transmitted Diseases of the Zoonosis Control Center (CCZ-SP) in Sao Paulo, Brazil. The samples were tested in this laboratory for the presence of neutralizing antibodies for RABV, using the Rapid Fluorescent Focus Inhibition Test (RFFIT), with a cutoff point of 0.5 IU/mL14.

**Collection and processing of brain tissue samples:** Prior to fixing the specimens in 10% formalin, samples of brain tissue were collected in the laboratory under aseptic conditions, using 170 mm polypropylene Pasteur-type pipettes with 3 mm-diameter tips and a capacity of 3 mL, which were introduced into the cranial cavity through the foramen magnum to retrieve the material by suction16. This method enables the brain tissue to be removed without damaging the cranium, which is an essential diagnostic criterion for the identification of some chiropteran species and storage in scientific collection.

The samples of brain tissue were conserved at -70 °C, and then sent to the Rabies Research and Diagnosis Laboratory of the Arbovirology and Hemorrhagic Fevers Sector at the Evandro Chagas Institute in Belem, Para, Brazil, where the samples were tested for rabies antigen using Direct Immunofluorescence (DIF) and Intracerebral Inoculation in Mice (IIM), following the recommendations of the World Health Organization99.

**Data analysis:** A Chi-square ($\chi^2$) test was used to evaluate deviations in the proportion of seropositive specimens by age, sex or season. A multiple logistic regression was used to evaluate the probability of obtaining a seropositive specimen, according to its sex, age and the climatic season during which it was captured. The analyses were run in BioEstat 5.0.

**RESULTS**

A total of 441 bat specimens were captured, representing 10 different species (none of which were hematophagous), with RABV-neutralizing antibodies being detected in nine of these species (Table 1). The diagnosis of the serum samples nevertheless indicated that just over half (50.34%, 95% CI: 45.67-55.01%) of the specimens captured were seropositive for the disease, with statistically equal proportions of seropositive specimens being captured ($\chi^2 = 0.05$, d.f. = 1, $p = 0.88$) (Table 1). A total of 153 samples of brain tissue were analyzed from the 441 bats tested serologically, although none of these samples tested positive for RABV. This is despite the fact that more than half the specimens (53.59%, 95% CI: 45.69-61.49%) were seropositive (Table 1).

The most common species collected during the study was *Artibeus planirostris* (Table 1), with almost four fifths of all the specimens captured (n = 345, 78.2% of the total). While a slightly higher proportion (52.46%, 95% CI: 52.31-52.60%) of these specimens were seropositive in comparison with the overall mean (50.34%); once again, there was no significant difference in the proportions of seropositive individuals ($\chi^2 = 0.38$, d.f. = 1, $p = 0.38$). The second most common species, *Carollia perspicillata*, was represented by a much smaller proportion of the specimens collected (n = 38, 8.6%), with a much lower proportion (32.21%, 95% CI: 30.88-33.12%) of seropositive individuals. By contrast, *Phyllostomus discolor*, which represented less than 5% (n = 22) of the specimens captured, had the highest proportion of seropositive individuals (63.64%, 95% CI: 54.09-71.91%). The next most common species was *Glossophaga soricina*, with 16 specimens captured, exactly half of which were seropositive. However, the main bat species to carry and transmit RABV, *D. rotundus*, was not captured in the sampling plots of the city of Capanema.

Statistically equal proportions of seropositive individuals were recorded in the two seasons, with only a slightly higher proportion (50.92%, 95% CI: 44.28-57.56%) being recorded during the dry season ($\chi^2 = 0.057$, d.f. = 1, $p = 0.88$) (Table 2). A significantly higher proportion of males were seropositive (55.96%, 95% CI: 48.96-62.96%), in comparison with the females (45.97%, 95% CI: 39.77-52.17%) ($\chi^2 = 4.334$, d.f. = 1, $p = 0.04$), and a significantly higher proportion of adults (52.37%, 95% CI: 47.35-57.39%) were seropositive in comparison with the juveniles (37.70%, 95% CI: 25.54-49.86%) ($\chi^2 = 4.521$, d.f. = 1, $p = 0.04$) (Table 2).

Significant differences were found in the distribution of seropositive
Table 1
Number of seropositive bats analyzed for RABV antibodies (serum) in the urban zone of Capanema, Para (Brazil)

| Taxon                     | Serum n | n+ | % (CI)       | Partial Serum 1 n | n+ | % (CI)       |
|---------------------------|---------|----|--------------|--------------------|----|--------------|
| Family Emballorunidae     |         |    |              |                    |    |              |
| Rhynchonycteris naso (Wied-Neuwied, 1820) | 1 0     | 0 |              | 1 0                | 0 |              |
| Family Phyllostomidae     |         |    |              |                    |    |              |
| Subfamily Glossophaginae  |         |    |              |                    |    |              |
| Glossophaga soricina (Pallas, 1766) | 16 8    | 50.00 (25.50-74.50) | 5 2 | 40.00 (02.94-82.94) |
| Subfamily Phyllostominae  |         |    |              |                    |    |              |
| Lophostoma brasiiliense Peters, 1866 | 2 1    | 50.00 (iss) | 2 1 | 50.00 (iss) |
| Phyllostomus discolor Wagner, 1843 | 22 14 | 63.64 (54.09-71.91) | 6 5 | 83.33 (iss) |
| Subfamily Carolliniae     |         |    |              |                    |    |              |
| Carollia perspicillata (Linnaeus, 1758) | 38 13   | 32.21 (30.88-33.12) | 12 6 | 50.00 (21.71-78.29) |
| Subfamily Stenodermatinae |         |    |              |                    |    |              |
| Dermanura cinerea (Gervais, 1856) | 1 1    | 100 (iss) | 1 0 | 0 |
| Artibeus planirostris Spix, 1823 | 345 181 | 52.46 (52.31-52.60) | 118 65 | 55.08 (46.10-64.05) |
| Sturnira lilium (E. Geoffroy, 1810) | 5 2    | 40.00 (02.94-82.94) | 2 0 | 0 |
| Uroderma bilobatum Peters, 1866 | 2 1    | 50.00 (iss) | 2 1 | 50.00 (iss) |
| Family Molossidae         |         |    |              |                    |    |              |
| Molossus molossus (Pallas, 1766) | 9 1    | 11.11 (09.33-31.55) | 4 2 | 50.00 (1.00-99.00) |
| Total                     | 441     | 222 | 50.34 (45.67-55.01) | 153 82 | 53.59 (45.69-61.49) |

n: number of individuals analyzed; n+: number of seropositive bats; CI: 95% confidence intervals; iss: insufficient sample size; Partial Sample 1: Serum of bats analyzed also by brain tissue.

Table 2
Prevalence of antibodies for the RABV in the bat specimens captured in Capanema, Para (Brazil) by season, age, and sex

|            | Dry season |                             | Rainy season |                             | Total |                             |
|------------|------------|-----------------------------|--------------|-----------------------------|-------|-----------------------------|
|            | n  | n+   | % (CI)   | n  | n+   | % (CI)   | n  | n+   | % (CI)   |
| Females    | 120 | 60   | 50.00 (41.06-58.94) | 128 | 54   | 42.19 (33.63-50.75) | 248 | 114  | 45.97 (39.77-52.17) |
| Males      | 98  | 51   | 52.04 (42.15-61.93) | 95  | 57   | 60.00 (50.15-69.85) | 193 | 108  | 55.96 (48.96-62.96) |
| Adults     | 203 | 104  | 51.23 (44.35-58.11) | 177 | 95   | 53.67 (46.32-61.02) | 380 | 199  | 52.37 (47.35-57.39) |
| Juveniles  | 15  | 7    | 46.67 (21.42-71.92) | 46  | 16   | 34.78 (21.02-48.54) | 61  | 23   | 37.70 (25.54-49.86) |
| Total      | 218 | 111  | 50.92 (44.28-57.56) | 223 | 111  | 49.78 (43.22-56.34) | 441 | 222  | 50.34 (45.67-55.01) |

n: number of individuals analyzed; n+: number of seropositive bats; CI: 95% confidence intervals.

specimens in relation to sex, age, and season ($\chi^2 = 9.294$, d.f. = 3, $p = 0.02$). In fact, the probability that an adult male was seropositive during the dry season (67.79%) was close to double that (41.06%) of a juvenile female during the wet season (Table 3). It is important to emphasize that, according to the multiple logistic regression, just the variable season showed no significant difference in relation to serology ($z = -0.2511, p = 0.8017$) (Table 4). While there was no significant difference between seasons in the number of seropositive specimens, the highest probabilities (56.82-67.79%) were recorded during the dry season (Table 3).

**DISCUSSION**

Just over half of the bats captured during the present study in the urban zone of Capanema were seropositive for RABV. This result was consistent with that of a previous study in a rural area, also on the east coast of Para, in the Brazilian Amazon region, where half of the bat specimens were also seropositive for this zoonosis8. Together with the findings of COSTA et al., from a rural area, the results of the present urban study indicate that the proportion of seropositive bats in the population may not vary significantly between urban and rural areas in the region of the eastern coast of Para.
The overall rate recorded in the urban and rural areas of the eastern coast of Para was relatively high, especially in comparison with results of studies conducted in other countries of Central and South America. In Colima, Mexico, for example, only 37% of non-hematophagous bats were seropositive, while in Grenada and Trinidad, seropositive rates were 7.6% and 12.8%, respectively, in hematophagous and non-hematophagous bats. In Peru, SALMON-MULANOVICH et al. recorded antibodies in 10.3% of hematophagous and non-hematophagous bats.

Regardless of the proportion of seropositive individuals found in the population, all studies throughout the world have found antibodies for rabies virus in non-hematophagous bats. In the present study, virtually all (90%) of the species analyzed had seropositive individuals, indicating that most of the bat species found in this urban area may be exposed to RABV, even though some of these species are not included in the updated list of bats that have tested positive for rabies in Brazil. Though rare, these species may contribute to the transmission of RABV in both rural and urban environments.

The presence of RABV in a number of different bat species inhabiting urban environments has been recorded in a number of previous studies. In the present study, A. planirostris was the most common species in terms of the number of specimens captured, and presented a high proportion (52.46%) of seropositive individuals. Rabies positive individuals of this species have been captured in other urban areas, and urban environments. These species may contribute to the transmission of the virus, especially in relation to roost structure and the behavioral characteristics of the species that form maternity colonies, although this question was not analyzed in the present study.

None of the specimens we analyzed for infection, however, based on the analysis of brain tissues that tested positive none of the bats were actually infected with RABV. In spite of this, the number of seropositive specimens indicates that at least half of the bats of the different species captured had had some contact with RABV during the course of their lives, which presumably reflects the circulation of the virus within this urban area. The fact that none of the specimens captured was infected with RABV is consistent with the findings of RUPPRECHT, who recorded a 1% infection rate in active bats, and concluded that infection alters...
the foraging behavior of these animals, making them less likely to fly at night, and thus less vulnerable to be captured in mist nets. Ultimately, our results may also indicate that, given the known cases of human and animal rabies transmitted by *D. rotundus* in the surrounding rural areas, the urban zone may provide a transmission route for the virus between different rural areas.

**RESUMO**

Diagnóstico virológico e sorológico de raiva em morcegos de uma área urbana na Amazônia Brasileira

Os surtos de raiva em humanos transmitida por *Desmodus rotundus* em 2004 e 2005 no nordeste do estado do Pará, Brasil, Amazônia Oriental, fizeram desta uma área prioritária para estudos sobre essa zoonose. Diante disso, o presente estudo fornece dados sobre esse fenômeno em contexto urbano, afim de avaliar uma possível circulação do vírus clássico da raiva (RABV) entre espécies de morcegos em Capanema, cidade localizada na bacia Amazônica. Os morcegos foram coletados em 2011, com auxílio de redes de espera durante as estações seca e chuvosa. Amostras de encefálo e de sangue foram coletadas para o diagnóstico virológico e sorológico, respectivamente. Das 153 amostras de encefálo analisadas, nenhuma encontrou-se positiva para infecção pelo RABV, porém, 50,34% (95% CI: 45,67-55,01) das amostras de soro analisadas estavam soropositivas. *Artibeus planirostris* foi a espécie mais comum, e seu percentual de indivíduos soropositivos foi bem elevado (52,46%, 95% CI: 52,31-52,60). Porções estatisticamente iguais de soropositivos foram registrados nas estações (*χ²* = 0,057, d.f. = 1, *p* = 0,88). Uma porção significativamente elevada de machos (55,96%, 95% CI: 48,96%-62,96%), e adultos (52,37%, 95% CI: 47,35%-57,39%) foram soropositivos. Apesar de nenhuma das amostras de encefálo terem sido positivas para raiva, a alta proporção de espécimes soropositivos indica uma possível circulação do RABV nessa área urbana.

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