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Neutralizing Antirabies Antibodies in Urban Terrestrial Wildlife in Brazil

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ABSTRACT: The prevalence of rabies neutralizing antibodies (NA) in sera of wild animals from São Paulo City (Brazil) was investigated using the Rapid Fluorescent Focus Inhibition Test between 1994 and 1997. Sera from 547 specimens were examined. Marsupials represented 45% of the sample and primates 37%; carnivores, rodents, deer and edentates represented 6, 6, 3 and 2%, respectively. The overall prevalence of NA was 14%. The prevalence of NA was 18% in primates; whereas in marsupials, carnivores, edentates and rodents it was 13, 9, 8 and 6%, respectively. The stratification according to sex, age, and site of capture of the marsupials and primates showed a small predominance in males versus females and a large predominance of adults versus juveniles. The same relationship was seen in specimens captured near human habitations versus specimens captured in their own habitat. It is evident that there is circulation of rabies virus in wild animals, which are not recommended as pets since they represent a potential risk of exposure to rabies virus for both humans and domestic animals.

Key words: Antibody, marsupials, primates, rabies virus, wildlife reservoir.

Dogs are the most frequently affected animals with urban rabies in Brazil. There are 1,000 to 1,500 cases per year. They also were responsible for 1,001 of the 1,256 cases (80%) of rabies in humans between 1980 and 1998. During that period, 117 cases of human rabies were transmitted by wild animals; 81 by bats, 17 by foxes, 13 by primates, four by wild felines and two additional cases with one each in the "caititu" (a wild pig) and the opossum (Brazilian Ministry of Health, 1980–98).

In 1988 bats assumed the second most important vector for transmission of rabies to humans in Brazil, a rank traditionally occupied by cats. This phenomenon also is happening in other American countries where bats are already responsible for 11% of rabies in humans. Between 1994 and 1997, 170 cases of rabies in wild animals occurred in Brazil, in which 80 cases were in bats, 65 in foxes, 10 in monkeys, two in "quatis" (*Nasua nasua*) and 14 cases in other species (Organización Panamericana de la Salud, 1994–97).

In 1981 and 1983, the Zoonosis Control Center (São Paulo City, Brazil; CCZ-SP) reported the last case of rabies in humans and pet animals, respectively. Thereforward, eight isolated cases of rabies have been reported including one caprine, one ovine, and six insectivorous bats.

The purpose of the present study was to investigate the prevalence of rabies antibodies in wild animals living in São Paulo City (23°32′36″S; 45°37′59″W) between January 1994 and December 1997. Knowledge of the prevalence of rabies antibodies among wild animals is important because 9% of all rabies cases in humans reported in the period between 1980 and 1997 were caused by contact with wild animals. Also, there are a number of these animals living in parks and woodlands of urban centers and they have frequent contact with the human population. In addition, an increasing prevalence of rabies-infected bats has been reported in São Paulo State. These bats may act as vectors of rabies, acquiring the infection from wild animals and casually transmitting it to humans and other domestic hosts (Secretariat of Health of the State of São Paulo, 1999).

The specimens of our sample included the order Primata (*Cebus apella, Allouata fusca, Callithrix jacchus* and *C. penicillata*); Marsupialia (*Didelphis marsupialis, D. albiventris,* and *Lutreolina crassicaudata*);

Rodentia (*Hydrochaeris hydrochaeris, Myocastor coypus* and *Sciureus ingrami*); Artiodactyla (*Mazama gouazoubira*); Edentata (*Dasypus novemcinctus, Bradypus variegatus* and *Euphractus sexcinctus*) and Carnivora (*Nasua nasua, Procyon cancrivorous, Felis tigrinus, F. pardalis, F. wiedii, Panthera leo; Gallactis sp., Eira barbara*; and *Cannis alous*).

All the animals of this sample were brought to the CCZ-SP either after dwelling invasion or history of aggression to humans or after being captured by the police authorities due to illegal commerce. In all cases, the capture methods applied tried to avoid unnecessary suffering of the animals. Despite the fact that all of the animals were living in São Paulo City, some were not native to the city. None of the aggressor animals analyzed were positive to rabies virus. The nonaggressive animals were reintroduced into the parks and woodlands of the city after the blood sampling.

Data were stratified to study the relationship between factors such as sex, age (juveniles or adults) and site of capture and the presence of NA (neutralizing antibodies) in the serum. Statistical analysis was performed by the chi-square and Fisher exact tests (Armitage and Berry, 1994).

The animals captured near or inside human habitation were considered "residents" and the animals captured in areas like parks, woodlands or surrounding the urban area were considered "native".

Blood samples were collected by vein puncture after immobilization with an intramuscular injection of Ketamine hydrochloride (Ketalar-Parke-Davis, Ache Laboratorio, São Paulo State, Brazil). All the ethical procedures to avoid suffering were guaranteed.

Serum samples were tested by Rapid Fluorescent Focus Inhibition Test (RFFIT) (Smith et al., 1973) in the rabies laboratory of the CCZ-SP. Sera were inactivated at 56 C and diluted in microplates beginning with 1:5 dilution to 1:160. Rabies virus suspension, containing 32–64 FFD50 was added to all dilutions (Pasteur Virus strain, Pasteur Institute, Paris, France) and incubated for 1 hr in CO_2 incubator at 37 C. After the incubation, a BHK.21 cell suspension (Baby Hamster Kidney-c.13, American Type Culture Collection, Manassas, Virginia, USA) was added and the microplates were reincubated at 37 C for 24 hr in a CO_2 incubator.

The growth medium (MEM-Minimum Essential Medium Eagle-Sigma M1018, St. Louis, Missouri, USA) containing 10% of bovine fetal serum (Sigma F4135, St. Louis, Missouri, USA) was removed and the cells were fixed with cold acetone 80% for 10 min. After this, fluorescein isothiocyanate conjugated anti-rabies serum, a dilution determined by titration, was added (Immunology Laboratory of CCZ-SP) and the microplates were incubated for 1 hr at 37 C.

The microplates were analyzed under an immunofluorescence microscope. The titer of the serum was the dilution at which a reduction of 50% in the number of the fields with fluorescent cells was obtained. The comparison between the titer of the serum test and the standard serum one (hyperimmune antirabies serum, Pasteur Institute) served as a base to convert the titers into International Units. Test controls for the RFFIT included negative and positive samples. The cut-off value considered was 0.5 IU/ml.

Marsupials represented 45% of the sample with 246 specimens, and primates 37% with 203 specimens. Carnivores, rodents, deer and edentates represented 6, 6, 3 and 2% of the sample, respectively. The overall prevalence of neutralizing antibodies to rabies was 14%. Table 1 summarizes the results stratified by order.

Stratification according to sex showed a small predominance in the number of males among the marsupials (n = 132 versus n = 114) as well as among the primates (n = 107 versus n = 96). The prevalences of NA observed among females of the primates and marsupials were not significantly higher than those observed among

TABLE 1. Prevalence of rabies neutralizing antibodies and geometric mean titers of rabies neutralizing antibody titers of NA in São Paulo 1994–97.

Order	Total sera[a]	Reagents[b]	GMT total[c]	GMT reagents[d]	Prevalence[e] (%)
Marsupialia	246	32	0.2	1.2	13
non human Primates	203	36	0.2	0.9	18
Carnivorora	34	03	0.1	0.7	9
Rodentia	33	02	0.2	1.6	6
Artiodactyla	18	0	0	0	0
Edentata	13	01	0.1	0.8	8
Total	547	74	0.2	1.0	14

[a] Total number of sera examined.
[b] Number of sera with rabies neutralizing antibody titers > 0.5 UI/ml.
[c] Overall geometric median of rabies neutralizing antibody titers.
[d] Geometric median of rabies neutralizing antibodies of reagent samples.
[e] Prevalence of rabies neutralizing antibodies.

males of the primates (19 versus 17%, $P = 0.469$) and opossums (16 versus 11%, $P = 0.864$).

With regard to age there was a predominance in the number of adults over the juveniles of primates ($n = 137$ versus $n = 28$) and marsupials ($n = 179$ versus $n = 31$). The largest NA prevalence among the marsupials occurred in the youngest specimens (23%). In primates the NA prevalence was slightly higher among the juveniles (21%) and adults (22%). These differences, however, were not significant ($P = 0.39$ in primates and $P = 0.78$ in marsupials).

Considering the site of capture, 49% of the primates and 73% of the marsupials were captured in residences. Forty-two percent of the primates captured belonged to species from other parts of the country. A large proportion of these animals (31%) displayed a typical captivated animal behavior, which indicates that they were illegally introduced in São Paulo City as pets.

Opossums, not attractive as pets, maintained their original geographical distribution. Most of the animals were *D. marsupialis,* native to the study area. Only 4% ($n = 10$) were from other regions of the country.

Primates are attractive as pets while they are young. However, when they reach sexual maturity, followed by behavioral changes (which makes them less adapted to domestic life), their owners tend to abandon them in the parks and woodlands of the city. Most of them cannot survive and invade residences searching for food and shelter.

Sera from twenty-four animals (four monkeys, three marmosets and seventeen opossums) showed very high titers of NA (1,1-3.2 IU/ml). Apparently healthy wild animals with such high titers of NA were observed by Rosatte and Gunson (1984) and Hill et al. (1992). These high titers were attributed to subclinical disease (Greenwood et al., 1997); sublethal infection (McLean, 1975; Gascoyne et al., 1993); localized replication of the virus; ingestion or inhalation of infected material (Beran, 1981; Hall, 1978 *in* Carey 1985); infection with other strains of rabies virus (Rosatte and Gunson, 1984), and latent virus activated by stress caused by change in the habitat, high population density, migration, and fights to establish their own territories (Zumpt, 1969; Ballard and Krausman, 1997).

Another possibility could be that some of the animals illegally kept as pets would have been vaccinated. However, this is unlikely since these animals are not routinely vaccinated in São Paulo City. According to Andrade (1997) several doses of the rabies vaccine used in the campaign against rabies in Brazil (processed strains in mouse

brain tissue), are needed to induce high titers of antibodies in marmosets.

The NA prevalence was higher among the specimens of primates captured in residences, suggesting vaccination. The higher prevalence among the marsupials occurred among the natives, suggesting natural infection by contact with infective animals, like bats, with which they share shelter. Opossums are less susceptible to rabies than other species (World Health Organization, 1973), although they can casually get the infection and, therefore, produce antibodies. The NA prevalence among juvenile primates may indicate the presence of the maternal antibodies (transplacental transmission and transmammary route) but the opossums are aplacental and certainly not vaccinated.

The high prevalence of the opossums in this study (246/547) reflects a drastic reduction of their natural predators in the native environment. The ecosystem of urban areas is obviously not adequate for the typical predators of opossums. In addition, the omnivorous opossums are a more adaptable species, so they prevail (Emmons and Feer, 1990; Fonseca et al., 1994). Opossums are largely present in urban areas like São Paulo City. Although opossums rarely bite humans, accidental bites occur. Considering that 15% of the captured opossums came from residences (school, cabinet, swimming pool, kitchen appliances, electric equipment or captured by dogs), it seems clear the potential risk of rabies transmission represented by that species.

Nineteen animals with multiple fractures as a consequence of trampling or wound caused by dogs were submitted to euthanasia. These animals also were bled to determine their rabies serostatus and they were checked for the presence of rabies virus (they were all negative).

The low number of edentates, rodents, deer, and carnivores in our study does not permit any inference about the results, but suggests that rabies virus is circulating among these species.

Our sample consisted of animals captured during the routine procedures of the CCZ-SP, therefore this was not a random sample but biased towards animals brought in due to their contact with humans and the observed prevalence of NA tends to be overestimated. Every bite from a wild animal is considered a risk of disease by the Secretariat of Health of the State of São Paulo (1999) and the post-exposure prophylaxis must be initiated, except when the laboratory test is negative. The population must, therefore, be oriented not to keep wild animals as pets.

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