

Evidence of hantavirus infection in wild rodents captured in a rural area of the state of São Paulo, Brazil¹

Elba Regina S. de Lemos², Paulo S. D'Andrea³, Cibele R. Bonvicino³, Kátia M. Famadas⁴, Paula Padula⁵, Aduino A. Cavalcanti⁶ and Hermann G. Schatzmayr²

ABSTRACT: Lemos E.R.S., D'Andrea P.S., Bonvicino C. R., Famadas K. M., Padula P., Cavalcanti A.A. & Schatzmayr H.G. 2004. **Evidence of hantavirus infection in wild rodents captured in a rural area of the state of São Paulo, Brazil.** *Pesquisa Veterinária Brasileira* 24(2):71-73. Depto Virologia, Instituto Oswaldo Cruz, Av. Brasil 4365, Pavilhão Rocha Lima, 5º andar, Manguinhos, Rio de Janeiro, RJ, 21045-900, Brazil. E-mail: elemos@ioc.fiocruz.br

Hantaviruses are the etiological agents of the Hantavirus Cardio-Pulmonary Syndrome, a serious rodent-borne disease in Brazil. In order to investigate the occurrence of hantavirus infection in wild rodents, a survey was conducted in three different suburban areas of the municipality of Pedreira, State of São Paulo, Brazil. Of the 145 wild animals captured belonging to 12 different species identified by morphology and karyological analysis, 107 were rodents of the following species: *Akodon montensis*, *Bolomys lasiurus*, *Calomys tener*, *Oligoryzomys nigripes*, *Oligoryzomys flavescens*, and *Myocastor coypus*. Blood samples from these rodents were assayed for the presence of antibodies against hantavirus by IgG ELISA using Andes recombinant nucleocapsid antigen. Antibody reactive to Andes virus was found in two different species, *O. nigripes* and *O. flavescens*. These results indicate a potential risk for hantavirus transmission to humans in this area, where reservoir rodents are present in peridomestic settings.

INDEX TERMS: Hantavirus, rodent, survey, Pedreira, São Paulo, Brazil.

RESUMO.- [Evidência de infecção por hantavírus em roedores silvestres capturados em uma área rural do estado de São Paulo, Brasil.] Hantavírus é o agente etiológico da síndrome cardiopulmonar por hantavírus (SCPH), uma importante doença transmitida por roedores no Brasil. Com o objetivo de se conhecer a ocorrência de infecção por hantavírus em pequenos roedores silvestres, uma análise sorológica foi conduzida em três diferentes áreas do subúrbio da cidade de Pedreira, São Paulo. Dos 145 animais silvestres capturados, pertencentes a 12 diferentes espécies identificadas por morfologia e por análise cariológica, 107 eram roedores das seguintes espécies: *Akodon montensis*,

Bolomys lasiurus, *Calomys tener*, *Oligoryzomys nigripes*, *Oligoryzomys flavescens*, e *Myocastor coypus*. Amostras de sangue desses roedores foram testadas para a presença de anticorpos IgG contra o antígeno do nucleocapsídeo do vírus Andes através do teste ELISA. Reatividade sorológica ao vírus Andes foi observada em duas espécies diferentes, *O. nigripes* and *O. flavescens*. Estes resultados indicam o potencial risco de transmissão de hantavírus nesta região, onde roedores reservatórios frequentemente são vistos em peridomicílio.

TERMOS DE INDEXAÇÃO: Hantavírus, roedores, prevalência, Pedreira, São Paulo, Brasil.

INTRODUCTION

Hantavirus cardiopulmonary syndrome (HCPS), a newly recognized viral disease that has been described in the American continent since 1993, is caused by several strains of the genus *Hantavirus*, family Bunyaviridae (Nichol et al. 1993, Elliott et al. 1994, Spiropoulou et al. 1994). A number of hantavirus serotypes (Sin Nombre, Black Creek Canal, Bayou, Andes, Laguna Negra, Rio Mamore, Juquitiba, Araraquara, Franca and Castelo dos Sonhos), among other hantaviruses, has been identified in different American countries (Lee et al. 1978, Rollin et al. 1995, Gonzalez-

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² Depto Virologia, Instituto Oswaldo Cruz, FIOCRUZ, Avenida Brasil 4365, Manguinhos, Rio de Janeiro, RJ 21045-900. E-mail: elemos@ioc.fiocruz.br

³ Depto Medicina Tropical Instituto Oswaldo Cruz, FIOCRUZ.

⁴ Depto Parasitologia Animal, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ 23890-000.

⁵ Instituto Nacional de Laboratórios e Instituto de Saúde Dr. Carlos Málbran, Buenos Aires, Argentina.

⁶ Secretaria de Saúde e de Meio Ambiente do Município de Pedreira, São Paulo, SP 13920-000.

Scarano & Nathanson 1996, Lopez et al. 1996, Bharadwaj et al. 1997, Johnson et al. 1997, Schamaljohn & Hjelle 1997, Silva et al. 1997, Vasconcelos et al. 1997, Levis et al. 1998, Johnson et al. 1999).

The natural reservoirs for hantaviruses in the Americas are rodents of the family Muridae, subfamily Sigmodontinae (Butler & Peters 1994, Morzunov et al. 1995, Rowe et al. 1995, Schamaljohn & Hjelle 1997, Mills et al. 1998, 1999, Young et al. 1998, Nichol 2001). In Brazil, rodents harbouring infection by hantaviruses have been detected by serological methods in different regions, where certain rodent species – mainly *Bolomys lasiurus*, *Akodon cursor* and *Oligoryzomys nigripes* – have been considered as important natural reservoirs for the virus (Ferreira et al. 1998, Johnson et al., 1999, Figueiredo et al. 2001, Romano-Liber et al. 2001).

Considering the scarce information about hantaviruses in Brazil, this study was conducted in reach for evidences of hantavirus infection in wild rodents in the region of Pedreira, State of São Paulo, Brazil, where HCPS had to date not been reported.

MATERIALS AND METHODS

This study was carried out in the County of Pedreira, located in the southeast of the State of São Paulo, Brazil, an area within the Brazilian Atlantic Forest domain, near the limits with the Cerrado Biome, at 22°44'21" latitude South and 46° 54'27" longitude West, 584m above sea level. The vegetation of this area has been altered mainly due to farming activities. Eucalyptus, high-growing grass, swamp and secondary forest were common.

Three localities were sampled: the Workers' Colony of Nadir Figueiredo Industry (NF), Fortaleza Farm (FF), and Jaguari Farm (JF), located along Jaguari River, 3 km away from the nearest urban center.

Field surveys were done in January 1996 and April/May and October 1997, totalling a capture effort of 1,830 trap-nights. Sherman and Tomahawk live traps were spaced at approximately every 10 meters in linear ground transects. The bait was a mixture of bacon, peanut butter, oatmeal and banana. Mammal specimens were karyotyped to confirm morphological identification. Chromosome preparations were obtained from short term bone marrow cultures with 80% RPMI 1640, 20% fetal calf serum, ethidium bromide (5 mg/ml) and colchicine 10^{-6} M (Bonvicino et al. 1996).

All species of rodents were analysed for antibodies against hantavirus by IgG ELISA using ANDES antigen (Padula et al. 2000). The recombinant nucleocapsid protein was applied to the solid phase of a microtiter plate. After washing, serum samples were added. After 1hour incubation at 37°C plates were washed again and a mixture of two conjugates (anti-*Peromyscus leucopus* IgG and anti-*Rattus norvegicus* IgG, Kirkegaard and Perry Laboratories) was used to detect immunoglobulins from various rodent phyla. The chromogenic substrate used was 2,2'-azino-di(3-ethyl-benzthiazoline sulfonate) (Kirkegaard and Perry Laboratories). Optical densities were read at 405 and 450 nm. A titer $>1=1:400$ was considered positive (Padula et al. 2000). Subsequently, lung tissue and blood samples from seropositive rodents were analysed for virus RNA by reverse transcriptase-polymerase chain reaction (RT-PCR; Lopez et al. 1996).

RESULTS

One hundred and forty-five wild animals belonging to 12 different species were identified by morphology and

karyological analysis and were subsequently deposited in the National Museum, Rio de Janeiro, Brazil. The following species were captured: the marsupials *Didelphis aurita* (18), *Didelphis marsupialis* (6), *Gracilinanus microtarsus* (1), *Lutreolina crassicaudata* (15), *Monodelphis sorex* (1), the rodents *Bolomys lasiurus* (24), *Akodon montensis* (35), *Nectomys squamipes* (21), *Oligoryzomys nigripes* (16), *Oligoryzomys flavescens* (4), *Calomys tener* (2) and *Myocastor coypus* (2). The overall trapping success for all species was 7.4%. Only rodents were assayed for the presence of antibodies to Andes recombinant antigen (ELISA). From 107 rodents submitted to serological testing, two individuals, one *O. nigripes* and one *O. flavescens*, captured in the Nadir Figueiredo area, had evidence of hantavirus infection with an antibody titer equal to or greater than 1:1600. These seropositive animals represented 10.0% (2/20) of genus *Oligoryzomys* captured in that county. Lung tissues and blood samples from these rodents did not reveal the presence of hantavirus genome by RT-PCR.

DISCUSSION

Although human cases of HCPS have not been reported in the county where this study was undertaken, several facts highlight the need to analyse the status of hantavirus infections in this region: 1) the usual contact of the human population with wild rodents in rural areas, 2) rural touristic activities, and 3) large farming areas. In fact, all rodents should be considered as potentially infected, since some reports have demonstrated hantavirus infections in small mammal populations without confirmed HPS human cases (Lee et al. 1982, LeDuck et al. 1985, Mills et al. 1998). Thus, the risk for hantavirus infection should be considered whenever individuals are frequently exposed to wild rodents.

The result of the present study suggest that seropositive *Oligoryzomys* rodent may be in a late stage of infection, when the immune response decreases the amount of viral antigen (Netski et al. 1999), although the lack of RNA detection through PCR may have been due to the fact that samples had been frozen and thawed several times. Even though the identity of the virus detected by serological tests could not be determined, the results obtained in this preliminary report suggest that rodents captured in peridomestic environments of Pedreira can be reservoirs of pathogenic hantaviruses. Further investigations should be carried out in different regions in Brazil for a better understanding of the distribution of such infection in the country.

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