SHORT COMMUNICATION

Study of Hantavirus Infection in Captive Breed Colonies of Wild Rodents

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Wild sigmondontine rodents are known to be the reservoir of several serotypes of New World hantaviruses. The mechanism of viral transmission is by aerosol inhalation of the excreta from infected rodents. Considering that the captive breed colonies of various wild mammals may present a potencial risk for hantaviral transmission, we examined 85 speciemens of Thrichomys spp. (Echimyidae) and 17 speciemens of Nectomys squamipes (Sigmodontinae) from our colony for the presence of hantavirus infections. Blood samples were assayed for the presence of antibodies to Andes nucleocapsid antigen using enzyme-linked immunosorbent assay (ELISA). Additionally, serum samples from workers previously exposed to wild rodents, in the laboratories where the study was conducted, were also tested by ELISA to investigate prevalence of anti-hantavirus IgG antibodies. All blood samples were negative for hantavirus antibodies. Although these results suggest that those rodent's colonies are hantavirus free, the work emphasizes the need for hantavirus serological monitoring in wild colonized rodents and secure handling potentially infected rodents as important biosafety measures.

Key words: hantaviruses - rodents - hantavirus cardiopulmonary syndrome (HPS)

Hantaviruses, members of the *Bunyaviridae* family, are spherical, lipid-enveloped, single-stranded, negative-sense RNA viruses, measuring 80-120 nm. Hantaviruses are enzootic viruses of wild rodents that cause persistent infections in their natural hosts in the presence of an apparent immune response. Many hantaviruses are pathogenic to humans, causing quite serious illness, including hemorrhagic fever with renal syndrome (HFRS), spread throughout Europe and Asia (Old World hantaviruses), and hantavirus cardiopulmonary syndrome (HPS), in the Americas (New World hantaviruses). Transmission to man occurs via inhalation of viral particles from aerolized excreta from infected rodents (Lee et al. 1982, Nichol et al. 1993).

Although the finding of hantavirus-infection among non-reservoir rodent species may be detected, the natural host and reservoir of hantavirus is murid rodents (order Rodentia, family Muridae). Many hantaviruses have been described among indigenous American sigmondontine rodents and some of them associated with HPS. There are currently 22 well described hantaviruses; but less than 5% of the estimated 2000 species of murid rodents have been tested for hantavirus infection (Henttonen et al. 1995).

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elemos@ioc.fiocruz.br Received 23 December 2003 Accepted 10 August 2004 Following the HPS outbreak in the Southwestern United States, clusters of HPS cases have been reported in Argentina, Bolivia, Brazil, Canada, Chile, Panama, Paraguay, and Uruguay (Padula et al. 2000a, PAHO 2004). In Brazil, more than 350 cases of HPS have been reported until now, but little is known about hantavirus reservoirs (Romano-Lieber et al. 2001, Ministry of Health 2004).

Laboratory transmission of hantaviruses from rodents to humans via aerosol route is well documented (Desmyter et al. 1983). Exposures to rodent excreta, fresh necropsy material, and animal bedding are presumed to be associated with risk. In animal holding areas, the period of exposure to infectious animal excreta required for transmission may be short (Lee et al. 1982, Kawamata et al. 1987). Other potential routes of laboratory infection include ingestion, contact of infectious materials with mucous membranes or broken skin, and, in particular, animal bites (CDC 1994). Previous study have suggest that Seoul virus, which has a worldwide distribution, is the most likely candidate for the numerous Hantaan-like virus outbreaks that have been identified in laboratory rats in Asia and Europe (LeDuc et al. 1986).

Although hantavirus illness as HPS or HRFS in animals exposure workers is uncommon, serologic analysis has indicated that mammalogists and laboratory technicians that trap or work with wild rodents are at high risk of exposure to hantaviruses (Lee & Johnson 1982, Childs et al. 1995).

We carried out a serosurvey of captive breed colonies of wild rodents maintained at the laboratories of Oswaldo Cruz Institute-Fiocruz and Department of Ecology of Federal University of Rio de Janeiro (UFRJ) and with the researchers and technicians that handle these field-trapped rodents. The study was performed according to the rules established by Institute Oswaldo Cruz Biosafety Comission (CiBio-IOC).

We examined 85 specimens of *Thrichomys* spp. (Echimyidae) and 17 specimens of *Nectomys squamipes* (Sigmodontinae) for the presence of hantavirus infections. The colonies were composed by different species captured in several states of Brazil, including *T. apereoides laurentius* from Bahia (6), Ceará (14), and Piauí (25), *T. pachyurus* from Pantanal (40), and *N. squamipes* in Rio de Janeiro (17).

Additionally blood samples were drawn from 12 apparently healthy mammalogists, and rodent trappers, in order to detect the presence of hantavirus infection.

Blood samples of all rodents maintained at the laboratories were analyzed for antibodies against hantavirus by IgG enzyme-linked immunosorbent assay (ELISA) using Andes recombinant nucleoprotein (N) as antigen (Padula et al. 2000b). Rodents and control sera were diluted 1:400 and 1:1600. Signal reaction was detected using 2,2'-azino-di (3-ethylbenzthiazolinesulphonate) (ABTS) as a substrate for peroxidase and absorbance at 405 nm was measured with an Organon Teknika 230S microplate reader. The values were expressed as the optical density (OD) obtained with Andes antigen substracted from the OD of the control antigen. The average of negative controls values (3) plus three times its standard deviation is considered the cut off of the test. All commercial reagents were from Kirkegaard and Perry.

All rodent and human samples were Andes IgG negative for the presence of hantavirus specific antibodies by ELISA. This result suggests that those rodent's colonies are hantavirus free. However this kind of survey appears to be essential to provide research personnel with adequate safety and to prevent the introduction of rodent's pathogens into established pathogen-free colonies in laboratories. New World hantaviruses are a newly emerging and recently recognized group of human pathogens with scarce data. So far, most studies about hantavirus seroprevalence in rodents have been limited to Muridae family. It seems important to include in the monitoring other species of captive breed rodents. This was a reason why we analyzed infection status of Echimyidae rodent *Thrichomys* spp.

Inapparent exposure to hantavirus infected rodents have led to human morbidity and to an occasional fatality in research labs, emphasizing the need for vigilance in screening laboratory rodents for hantavirus infections. Moreover, specifics measures for personnel who frequently handle or are exposed to wild rodents must be

recommended in order to reduce the exposure to hantavirus or other pathogenic agents transmitted by wild animals.

REFERENCES

- CDC 1994. Laboratory management of agents associated with hantavirus pulmonary syndrome: interim biosafety guidelines. *MMWR* 43: 1-7.
- Childs JE, Mills JN, Glass GE 1995. Rodent-borne hemorrhagic fever viruses: a special risk for mammalogists? *J Mammal* 76: 664-680.
- Desmyter J, LeDuc JW, Johnson KM, Brasseur F, Deckers C, van Ypersele de Strihou C 1983. Laboratory rat associated outbreak of haemorrhagic fever with renal syndrome due to Hantann-like virus in Belgium. *Lancet ii*: 1445-1448.
- Henttonen H, Vapalahti O, Vaheri A 1995. How many kinds of hantaviruses? *Trends Ecol Evol 11*: 7-8.
- Kawamata J, Yamanouchi T, Dohmae K, Miyamoto H, Takahaski M, Yamanishi K, Kurata T, Lee HW 1987. Control of laboratory acquired hemorrhagic fever with renal syndrome (HFRS) in Japan. *Lab Anim Sci* 37: 431-436.
- LeDuc JW, Smith GA, Childs JE, Pinheiro FP, Maiztegui JI, Niklasson B, Antoniades A, Robinson DM, Khin M, Shortridge KF, Wooster MT, Elwell MR, Ilbery T, Koech D, Rosa EST, Rosen L 1986. Global survey of antibody to hantaan-related viruses among peridomestic rodents. *Bull WHO 64*: 139-144.
- Lee HW, Johnson KM 1982. Laboratory-acquired infections with Hantaan virus, the etiologic agent of Korean hemorrhagic fever. *J Infect Dis* 146: 645-651.
- Lee HW, Baek LJ, Johnson KM 1982. Isolation of Hantaan virus, the etiologic agent of Korean hemorrhagic fever, from wild urban rats. *J Infect Dis* 146: 638-644.
- Ministry of Health, Brazil, 2004. Reported on Hantavirus Pulmonary Syndrome until July, 2004.
- Nichol ST, Spiropoulou CF, Morzunov S, Rollin PE, Ksiazek TG, Feldmann H, Sanchez A, Childs J, Zaki S, Peters CJ 1993. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science* 262: 914-917.
- Padula PJ, Colavecchia SB, Martínez VP, Gonzalez Della Valle MO, Edelstein A, Miguel SDL, Russi J, Mora Riquelme J, Colucci N, Almirón M, Rabinovich RD 2000a. Genetic diversity, distribution, and serological features of hantavirus infection in five countries in South America. *J Clin Microbiol* 38: 3029-3035.
- Padula P, Rossi C, Della Valle MO, Martinez PV, Colavecchia SB, Edelstein A, Miguel SD, Rabinovich RD, Segura EL 2000b. Development and evaluation of a solid-phase enzyme immunoassay based on Andes hantavirus recombinant nucleoprotein. *J Med Microbiol* 49: 149-155.
- PAHO, 2004. Reports to PAHO from the Ministries of Health of the American countries. Last Uptade: 26 April 2004.
- Romano-Lieber NS, Yee J, Hjelle B 2001. Serologic survey for hantavirus infections among wild animals in rural areas of São Paulo State, Brazil. *Rev Inst Med Trop São Paulo 43*: 325-327.