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SEROLOGIC SURVEY FOR HANTAVIRUS INFECTIONS AMONG WILD ANIMALS IN RURAL AREAS OF SÃO PAULO STATE, BRAZIL

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SUMMARY

A serosurvey was conducted in wild animals captured close to two areas where hantavirus cardiopulmonary syndrome (HCPS) occurred in São Paulo State, Brazil. Serum samples from a total of 43 mammals were tested for antibodies reactive with Sin Nombre (SN) hantavirus using a strip immunoblot assay. RNAs from the blood clots of the positive samples were submitted to reverse transcriptase-polymerase chain reaction (RT-PCR). Two rodents of the genus *Oligoryzomys* were positive for hantavirus antibodies. These animals were captured in the Iguape region and represented 16.7% (2/12) of the sera from rodents and 100.0% (2/2) of the *Oligoryzomys* captured in that area. RT-PCR failed to amplify any viral cDNA. These results are in agreement with other data that suggest that members of this genus are important reservoirs of hantaviruses in Brazil.

KEYWORDS: Hantavirus pulmonary syndrome; Hantavirus cardiopulmonary syndrome(HCPS); Reservoirs; Strip immunoblot assay (SIA); Reverse transcriptase-polymerase chain reaction (RT-PCR); Araraquara; Iguape.

INTRODUCTION

In December 1993 an outbreak of hantavirus cardiopulmonary syndrome (HCPS) was recognized in Juquitiba, one of the districts of the São Paulo metropolitan area in southern Brazil. Three inhabitants of a rural area developed respiratory disease with fast evolution and two deaths. Since then, over forty cases have been reported in Brazil, with twenty-one of those in São Paulo State[13].

Members of the genus Hantavirus, family Bunyaviridae, cause HCPS or the related disease hemorrhagic fever with renal syndrome (HFRS). Hantaviruses are distributed worldwide, each in close association with a single species or genera of rodents. The best-known etiologic agent of HCPS is Sin Nombre (SN) virus. In nature, hantaviruses cause asymptomatic and persistent infection of rodents, which shed virus in their urine, feces and saliva. Humans contract most hantavirus infections by accidental inhalation of such excretions or secretions.

Only one hantavirus has been isolated in Brazil. A virus antigenically similar to a Seoul group virus was isolated from a *Rattus norvegicus* captured in Pará State, northern of Brazil, in the early 1980s[7,16]. Another hantavirus, Juquitiba (JUQ) virus, was detected in a human autopsy tissue sample from one of the HCPS patients from the 1993 cluster, using reverse transcriptase-polymerase chain reaction (RT-PCR)[10]. Recently, RNA from the viruses Araraquara (ARA) and Castelo dos Sonhos (CAS) was identified in the sera from HCPS patients by RT-PCR amplification[6].

Little is known about hantavirus reservoirs in Brazil. A serosurvey of wild rodents was conducted in the northwest region of São Paulo State, where 4 HCPS cases occurred between 1998 and 1999. From 365 animals examined, sixty-nine had hantavirus antibodies. All positives were *Bolomys lasiurus*, suggesting that this specie could be the virus reservoir in that area[11]. Another serosurvey was done in the Atlantic Forest, close to Juquitiba, were the first HCPS cases were diagnosed. In this region, some rodents of the species *Akodon cursor* and *Oligoryzomys nigrips* had hantavirus antibodies[12]. These findings suggest that different species of rodents are actuating as possible reservoirs of Hantavirus in Brazil.

The objective of this study was to conduct a serosurvey in animals captured close to some areas where HCPS have been occurred. Non-rodent species were included because few surveys have included non-rodents, which could lead to failure to detect unexpected reservoir species. Sera with hantavirus antibodies were subsequently tested with a RT-PCR in an attempt to identify the specific virus.

MATERIALS AND METHODS

Study area – Animals were collected from two areas of São Paulo State, southeastern Brazil: Araraquara and Iguape. Araraquara is located on the boarder of the Western Paulista Plateau. It is characterized by intense, widespread agricultural activity. The captures were done in an ecological reserve. One case of HCPS occurred in Araraquara in 1996.

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Iguape is located in Ribeira Valley, south of São Paulo State, near the Coast. The area is still covered by forest and has a very humid tropical climate. Is a place of considerable arbovirus activity. The captures were conducted every two months from March 1997 until March 1999.

Trapping of animals and collection of blood samples – Serum samples were obtained from 43 animals. Twelve animals were trapped in Iguape. They were rodents from the genera *Akodon* (N = 5), *Oryzomys* (N = 4), *Oligoryzomys* (N = 2) and one *Nectomys squamipes*. Thirty-one animals were captured in Araraquara. The twenty-five rodents were 16 *Akodon*, 6 *Nectomys*, *2 Oligoryzomys* and one animal was unidentified. Six animals were *Carnivora* from the specie *Nasua nasua*.

The identification of the species was made by specialists from The Zoology Museum, University of São Paulo, based on international references for taxonomy of mammals[15]. The voucher specimens are cataloged and archived at the Pau-Brasil Institute of Natural History.

Rodents were trapped in Sherman live traps baited with peanut butter. The *Carnivora* were trapped in modified Tomahawk traps baited with fresh fruits and corn. The traps were placed during the early evening at sites considered to be suitable habitats. The traps were picked up early the next morning. Traps were transported to a staging and processing area.

Blood processing – The rodents were bled through the retro-orbital sinus with a sterile thin Pasteur pipette or by cardiac puncture with a 10 mm long, 25 or 26 gauge needle and a 1 cm^3 tuberculin syringe. The carnivorous were bled by puncture of the tail lateral vein or the cardiac muscle with a 21G (25mm) needle and a 3 cm^3 syringe. The blood was ejected into 1.5 ml snap-cap vials (Eppendorf). Spent needles and syringes were disposed in a sharps container after being rinsed in a 10% bleach solution. The whole blood samples were centrifuged and sera were transferred into 1.5 ml disposable microcentrifuge tubes and stored in a freezer at – 20 °C. The specimens were transported to the laboratory on ice in less than four hours. The samples were then lyophilized and were subsequently reconstituted and tested at University of New Mexico School of Medicine (UNM in Albuquerque, NM, USA).

Antibody testing - Strip immunoblot assay – The sera were tested for detection of antibodies to SN virus using the technique described by BHARADWAJ *et al*.[2]. The recombinant viral nucleocapsid antigen was suctioned onto a nitrocellulose membrane under vacuum. Two micrograms of affinity purified recombinant nucleocapsid antigen of SNV was diluted into 2 ml of water and vacuumed onto a wetted 8 by 10 cm nitrocellulose membrane (model BA 85, Schleicher and Schuell, Keene, New Hampshire). While still wet, the membrane was shredded lengthwise with a hand-held paper shredder into 1.6 mm long strips, and the strips were stored in 5% powdered milk in phosphate-buffered saline (PBS). Each strip was placed into wells of a Western blot tray (Biorad Laboratories, Hercules, California) containing 1 ml of 5% powdered milk in PBS along with 5 µl of the sera of each animal. Membranes were rocked gently overnight at room temperature, allowing antibodies to bind to the immobilized nucleocapsid antigen. After 3 washes in a Tris buffer with 0.1% deoxycholic acid and 0.1% Triton X-100, a 1:1000 dilution of goat antibody to *P. leucopus* immunoglobulin G (IgG), labeled with alkaline phosphatase, was added and rocked gently for 1 hour for the rodent sera. To test the carnivore sera was used an anti cat IgG conjugate. After 3 more washes, membrane-bound alkaline phosphatase was detected with a chromogenic substrate (nitroblue tetrazolium and 5-bromo-4-chloro-3-indoyl-phosphate). Positive reactions were evident as darkly colored bands. A positive reaction reflects the binding of immunoglobulin G antibodies of *Peromyscus* to the immobilized nucleocapsid antigen of SN virus.

Reverse transcriptase-polymerase chain reaction (RT-PCR) – The protocol to the RNA extraction from the blood clots of the positive animals protocol is presented elsewhere[5]. The virus-specific primer pairs used to amplify SNV genomic cDNA from the blood clots of the seropositive animals were Bay S 1+/Bay S 626- (nest with Bay S 184+/ Bay S 626-). These primers showed to be effective in amplification of a wide variety of hantavirus S genomes from hantaviruses from the western hemisphere[1].

RESULTS

Seroprevalence – Two specimens of *Oligoryzomys* spp were positive for SN virus antibodies. These animals were captured in Iguape in October 1998 and represent 16.7% (2/12) of the sera from rodents and 100.0% (2/2) of the *Oligoryzomys* captured in that area. All other species of rodents were seronegative for antibodies reactive with SN virus N antigen.

Reverse transcriptase-polymerase chain reaction - The RT-PCR of blood clots from the only two seropositive animals failed to amplify any viral sequence, although an appropriate positive control amplification reaction was successful.

DISCUSSION

The fact that two *Oligoryzomys* had antibodies to hantavirus suggests that those agents were circulating in that area in agreement with other findings[12]. The tentative to implicate a hantavirus reservoir host from field studies require as a minimum some evidence that the virus causes a persistent infection in the putative reservoir specie. Since it was not possible to demonstrate the viral RNA in tissues of the antibody positive individuals, they cannot be implicated as reservoirs solely from the data presented herein. Also, many non-reservoir species have been found with antibody to hantaviruses, including cats and dogs that could be infected through contact with the true reservoir[9]. As other members of the genus have been implicated as reservoirs of hantaviruses in other countries[4,8,14], the involvement of *Oligoryzomys* spp. as reservoir of important human pathogenic hantaviruses makes this genus an important candidate as host of Juquitiba virus and the other known human pathogens in Brazil as well.

At least three genetically distinguishable virus lineages are associated with HCPS in Brazil but only Juquitiba (JUQ) is known to have caused HCPS in that region of São Paulo State.

Since hantaviruses are linked with specific species of rodents, identification of those reservoirs will be necessary to design appropriate control regimens.

In Brazil, all of the species with hantavirus antibodies detected thus far belong to the family *Muridae*, subfamily *Sigmodontinae* as is true in the other regions with hantaviruses that cause HCPS. Thus far, few sizable

surveys of other potential natural (non-rodent) reservoirs for the agents of HCPS have been carried out, but thus far domestic animals and carnivores have not appeared to be significant in the epidemiology[9], as noted here.

We were not able to clearly implicate *Oligoryzomys* spp as hosts of any of the pathogenic Brazilian hantaviruses herein. The failure to amplify the genome of the virus is most likely due to the lack of adequate levels of viremia in infected specimens. Experimental studies with hantaviruses have shown that viremia can be rather transient after infection and that viral RNA titers do not reach very high levels[3]. However, our results indicate that further attention should be paid to *Oligoryzomys* spp as potential reservoirs for hantaviruses in Brazil.

RESUMO

Inquérito sorológico para infecções por arbovírus em animais silvestres de áreas rurais do Estado de São Paulo, Brasil

Realizou-se inquérito sorológico em animais silvestres capturados em áreas próximas a duas regiões do Estado de São Paulo, Brasil, onde ocorreram casos de Síndrome Cardiopulmonar por Hantavírus (HCPS). Pesquisou-se a presença de anticorpos para o vírus Sin Nombre (SNV) em amostras de soro de 43 mamíferos, utilizando-se a técnica de Strip Immunoblot Assay (SIA). Empregou-se a técnica de reação em cadeia de polimerase – transcriptase reversa (RT-PCR) nos coágulos sangüíneos dos espécimes positivos. Dois roedores do gênero *Oligoryzomys* apresentaram anticorpos para hantavírus. Esses animais foram capturados na região de Iguape e representavam 16,7% (2/12) dos roedores e 100,0% dos *Oligoryzomys* capturados naquela área. O RT-PCR não detectou cDNA viral nas amostras. Os resultados estão de acordo com outros trabalhos que sugerem que membros desse gênero são importantes reservatórios de hantavírus no Brasil.

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