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A SMALL-SCALE SURVEY OF HANTAVIRUS IN MAMMALS FROM INDIANA

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ABSTRACT: In order to determine if hantaviruses were present in mice and other small mammals in Indiana (USA), small mammals were trapped in Brown, LaPorte, Tippecanoe and Whitley counties. Sixty-seven small mammals were trapped during August and September 1994. Sixty-three *Peromyscus leucopus*, one *Microtus pennsylvanicus*, one *Zapus hudsonius* and two *Blarina brevicauda* were captured and tested for hantaviruses. Six *P. leucopus* were found to have antibody to Sin Nombre virus (SN) by IgG ELISA, and a 139 bp fragment of SN-like hantavirus was amplified from five of them. All six of the positive *P. leucopus* were from LaPorte County. No other small mammals had evidence of infection with SN virus. This study presents the first report of Sin Nombre-like hantavirus in *P. leucopus* from Indiana.

Key words: Antibody, hantavirus, *Peromyscus leucopus*, Sin Nombre virus, survey.

INTRODUCTION

The rodent-borne hantaviruses are serologically-related, negative-strand RNA viruses which belong to the family Bunyaviridae (Elliott et al., 1991; Chu et al., 1994; Xiao et al., 1994). Hantavirus genomes consist of large (L), medium (M), and small (S) segments which encode the virus polymerase protein (L), envelope glycoproteins G1 and G2, and the nucleocapsid protein (N), respectively (Schmaljohn et al., 1987; Giebel et al., 1989; Arikawa et al., 1990; Gonzalez-Scarano and Nathanson, 1990; Parrington et al., 1991; Spiropoulou et al., 1994). The hantaviruses are associated with specific rodent reservoirs: Seoul (SEO) virus with the Norway rats (*Rattus norvegicus*), Hantaan (HTN) virus with the striped field mouse (*Apodemus agrarius*), Thailand (Thai) virus with a bandicoot (*Bandicota indica*), Tula virus with the European common vole (*Microtus arvalis*), Dobrava (DOB) virus with the yellow-necked field mouse (*Apodemus flavicollis*), Puumula (PUU) virus with the bank vole (*Clethrionomys glareolus*), Prospect Hill (PH) virus with the meadow vole (*Microtus pennsylvanicus*), Sin Nombre (SN) virus with the deer mouse (*Peromyscus maniculatus*), Black Creek Canal (BCC) virus and Muleshoe virus with two distinct subspecies of cotton rats (*Sigmodon hispidus*), and Bayou (BAY) virus with the rice rat (*Oryzomys palustris*) (McKee et al., 1991; Childs et al., 1994; Morzunov et al., 1995; Rawlings et al., 1996; Rollin et al., 1995; Torrez-Martinez and Hjelle, 1995).

In the past, hantaviruses were thought to be mainly associated with human diseases jointly called hemorrhagic fever with renal syndrome (HFRS) in Europe (PUU) and Asia (HTN, SEO) (McKee et al., 1991). Prospect Hill virus, the only hantavirus known to be indigenous to North America, was not known to cause disease in humans (LeDuc, 1987). However, the occurrence of a group of fatalities associated with acute respiratory distress in the Four Corners area of the southwestern United States led to the identification of SN virus as a human pathogen and *P. maniculatus* as its rodent reservoir (Nichol et al., 1993, Childs et al., 1994; Elliott et al., 1994). The symptoms associated with SN are referred to as hantavirus pulmonary syndrome (HPS) (Duchin et al., 1994). At the onset of illness, symptoms include fever, muscle pain and respiratory symptoms which are followed by rapid progression to

respiratory distress. Other symptoms include headache and gastrointestinal complaints. The early symptoms of HPS are similar to those for influenza. The time from the beginning of the symptoms until development of acute respiratory distress syndrome (ARDS) varies from 2 to 10 days.

In January of 1994, a fatal case of HPS occurred in Hendricks County (west of Indianapolis, Indiana, USA) (Slama and Zon, 1994). This case has been studied by personnel from the Centers for Disease Control and Prevention (CDC; Atlanta, Georgia, USA) and the Indiana State Department of Health (Indianapolis, Indiana, USA). These researchers trapped *P. maniculatus* from the home and surrounding area of the patient and sent sera and tissue samples to CDC for analysis to determine if a hantavirus was present. Several samples were antibody positive when tested with SN virus antigens, suggesting that the patient was infected via aerosolized rodent excreta (feces or urine) in his home. However, it was not known to what extent hantaviruses were present in Indiana. Therefore, the purpose of the current limited study was to determine if small mammals infected with hantaviruses could be found in other locations in Indiana.

MATERIALS AND METHODS

In August and September 1994, small mammals were trapped from sites in LaPorte (41°35′N, 86°53′W), Whitley (41°16′N, 85°28′W), Tippecanoe (40°24′N, 87°04′W) and Brown (39°12′N, 86°22′W) Counties (Indiana, USA). These sites were chosen to represent northwest, northeast, west-central, and southern parts of the state, respectively. Standard live-trapping protocols were followed (Mills et al., 1995). To minimize the chance for infection while handling potentially infected small mammals the following special precautions were taken. Processing was done out-of-doors away from any populated areas. Protective outfits worn by all personnel included: latex examination gloves (doubled), surgical gowns, booties and positive air pressure respirators with HEPA filters (Mills et al., 1995). Following capture, the small mammals were weighed and morphological characteristics were measured to aid species identification. They were then anesthetized with Metofane (Mallinckrodt, Mundelein, Illinois, USA), bled from the retro-orbital sinus, euthanized by overanesthetization with Metofane and the heart, lungs, liver, spleen, and kidneys were removed (Mills et al., 1995). The whole blood and tissue samples were placed in dry ice for shipment to the laboratory where they were stored at −70 C. Finally, the samples were shipped to CDC for hantavirus analysis. Initially, carcasses were stored in the laboratory at −70 C. Carcasses which were found positive for hantaviruses were sent to the CDC to verify species identification. Mitochondrial DNA (mtDNA) typing of *Peromyscus* sp. was done by sequencing a 384-nucleotide fragment of the replication control region of the rodent mitochondrial genome, using specially designed primers and previously obtained reference sequences (S. P. Morzunov, unpubl. data).

The IgG ELISA was performed as previously described employing an *Escherichia coli*-derived recombinant Immunosorbent Assay (ELISA), nucleocapsid antigen and an appropriate negative control antigen (Feldmann et al., 1993). Whole blood specimens were initially diluted 1:100, followed by four-fold dilutions through 1:6400, in 5% skim milk in phosphate-buffered saline (PBS)-Tween (SM-PBS-TW) and allowed to react with the antigen-coated wells. Bound IgG was detected with a mixture of goat anti-rat and goat anti-*Peromyscus* IgG conjugated to horseradish peroxidase (K&P, Gaithersburg, Maryland, USA). Optical densities at 410 nm (OD_{410}) were recorded on a microplate spectrophotometer (Dynatech Laboratories, Chantilly, Virginia, USA) and the OD_{410} of the uninfected, antigen-coated well was subtracted from its corresponding virus antigen-coated well to yield the adjusted OD_{410}. The sum of the adjusted OD_{410} for the four dilutions was calculated and sera which had sums of >0.95 were considered positive.

RESULTS

Sixty-seven small mammals were captured during the study and an additional two mice were taken from the Purdue University Biology *Peromyscus leucopus* Colony. The small mammals examined included 65 *Peromyscus leucopus* (white-footed mouse), one *Microtus pennsylvanicus* (meadow vole), one *Zapus hudsonius* (meadow jumping mouse) and two *Blarina brevicauda* (short-tailed shrew). In LaPorte County, 26 *P. leucopus* and one *M. pennsylvanicus* were captured. In Whitley

County 10 *P. leucopus* were captured. Including the two mice from the Purdue Biology Colony, 22 *P. leucopus*, one *B. brevicauda* and one *Z. hudsonius* were obtained from Tippecanoe County. Finally, Brown County yielded seven *P. leucopus* and one *B. brevicauda*. *Peromyscus leucopus* represented 94% of the captures.

Six blood samples were positive for SN virus antibodies. All six samples were obtained from *P. leucopus* captured in LaPorte County. No other small mammals were serologically positive for SN virus during the course of this study. Serologic findings in five of the six antibody positive animals were confirmed by reverse transcriptase - polymerase chain reaction (S. P. Morzunov, unpubl. data).

DISCUSSION

Previous investigations of SN virus have suggested that *P. maniculatus* serves as the primary reservoir (Nichol et al., 1993; Childs et al., 1994). In Indiana, *P. maniculatus* is found in prairie or grassland habitats and never occurs in woods (Mumford and Whitaker, 1982). The fatal case in Hendricks County, Indiana (USA) involved *P. maniculatus* which were captured in the grassland area where the patient lived, and three were found antibody positive for SN virus (Slama and Zon, 1994). However, in our study, all *Peromyscus* sp. captured were identified as *P. leucopus* based on morphological features. *Peromyscus leucopus* is found mainly in woodlands and in the ecotone between woodlands and grasslands (Mumford and Whitaker, 1982). All of the small mammals collected in the present study were captured in typical *P. leucopus* habitats. Thus, the morphological and ecological evidence supported the identification of *P. leucopus* in our study. However, *P. leucopus* and *P. maniculatus* exhibit a great deal of overlap in morphological features and in geographical distribution. Therefore, we utilized mtDNA analysis of the SN-positive carcasses to determine which species of *Peromyscus* harbored hantavirus at the LaPorte County site. The mtDNA analyses verified the identification of the hantavirus-positive rodents as *P. leucopus*. The *Peromyscus* sp. trapped in Brown, Tippecanoe and Whitley Counties also were morphologically identified as *P. leucopus*, but none of these were positive for SN virus antibody and their identities were not verified using mtDNA analysis.

The presence of SN-like virus in *P. leucopus*, as indicated by antibodies to SN virus, could be simplistically explained as "spillover" from *P. maniculatus* the primary reservoir (Childs et al., 1994). However, in our case it is an unlikely scenario as no *P. maniculatus* were captured in our study areas, and the LaPorte County and Hendricks County sites are over 150 km apart. Furthermore, PCR and nucleotide analysis studies have found significant sequence (up to 20% in the M genomic segment) (Nichol, pers. comm.) and phylogenetic differences between the virus in *P. leucopus* from LaPorte County (Blue River virus) and "classic" western SN virus detected in the Hendricks County human case (S. T. Nichol, pers. commun.; S. P. Morzunov, unpubl. data). Two other "Sin Nombre-like" hantaviruses were recently identified in *Peromyscus* sp. from the eastern United States. Hjelle et al. (1995) found a hantavirus, known as New York (NY) virus, in *P. leucopus* captured on Shelter Island (New York, USA). NY virus was implicated in two fatal cases of HPS, one in Rhode Island (Brackett et al., 1994) and the other in New York. Both cases are out of the geographic range of *P. maniculatus*. Another eastern SN-like hantavirus (known now as Monongahela virus) was sequenced from morphologically and phylogenetically distinct subspecies of *P. maniculatus* (*P. maniculatus nubiterrae*, cloudland deer mouse) in West Virginia (USA) (Song et al., 1996). The present study provides further evidence that *P. leucopus* can harbor its own hantavirus strains and may have a role in the epidemiology of zoonotic hantaviruses east of the Mississippi River.

An uneven rate of hantavirus infection

in local populations of *Peromyscus* sp. is not unusual and has been previously reported (Childs, et al., 1994; Rowe et al., 1995). Although *Peromyscus leucopus* positive for SN virus were trapped only at the LaPorte County site, it is probable that the virus is more widespread in Indiana. Considering the co-existence of two *Peromyscus* spp. in the region, and sequence differences between SN virus implicated in the Indiana human HPS case and SN-like hantavirus (BR virus) collected at our study site, it is apparent that at least two distinct SN-like hantaviruses are present in Indiana. The present study was limited in scope and a more comprehensive study with additional trap sites throughout more counties in Indiana would doubtless elucidate additional evidence of hantaviruses in small mammals from Indiana.

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