# HANTAVIRUSES AND THEIR RODENT RESERVOIRS IN THE UNITED STATES

**JAMES E. CHILDS, THOMAS G. KSIAZEK, PIERRE E. ROLLIN, JOHN W. KREBS, SHERIF ZAKI, STUART T. NICHOL, and C. J. PETERS,** Division of Viral and Rickettsial Diseases, Centers for Disease Control and Prevention, MS G-13, 1600 Clifton Road, Atlanta, Georgia 30333.

**GREGORY E. GLASS,** Department of Immunology and Infectious Diseases, Johns Hopkins School of Hygiene and Public Health, 615 North Wolfe Street, Baltimore, Maryland 21215.

ABSTRACT: Since 1993, three novel hantaviruses have been identified from the United States of which at least two can cause a severe respiratory disease termed hantavirus pulmonary syndrome (HPS). Rodent reservoirs have been identified for two viruses; *Peromyscus maniculatus* is the primary host of Muerto Canyon Virus (MCV) in the western United States and genetic analyses have implicated *Sigmodon hispidus* as the probable host of a hantavirus in Florida. Of 813 *P. maniculatus* tested in the southwestern United States 30.4% were infected; 12 of 90 (13.3%) *S. hispidus* from Florida were infected. The *S. hispidus*-associated virus has not been isolated in cell culture and its etiologic role in human disease is unproven. The rodent reservoir for the third virus, associated with a fatal case of HPS in Louisiana, is unknown.

These viruses are genetically distinct from their old world relatives, and cause a different spectrum of human disease. In the United States, respiratory disease is prominent while renal disease is most often reported from Eurasia. As yet the number of HPS cases occurring annually in the United States is unknown, but since the syndrome was identified in May, 1993, 50 cases have been reported from 15 states with an overall mortality ratio of 60%. The risk to groups occupationally exposed to rodents is being investigated.

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## INTRODUCTION

Hantaviruses are zoonotic agents maintained in rodent reservoirs and transmitted to humans via infectious virus shed in the urine, feces, or saliva of the host (Tsai et al. 1987). Transmission is believed to occur by inhalation of small droplet aerosols of the contaminated excreta, however transmission by other types of contact with excreta has not been ruled out. The viral genome of hantaviruses consists of single stranded, tri-segmented RNA (Schmaljohn et al. 1985). Across Eurasia hantaviruses cause approximately 200,000 cases of hemorrhagic fever with renal syndrome (HFRS), of which the major clinical presentation involves kidney dysfunction (Lee et al. 1989). Three major groups of viruses within the genus *Hantavirus* (Family: Bunyaviridae) are primarily responsible for HFRS; Hantaan and related viruses are maintained by rodents in the genus *Apodemus*; Puumala and related viruses are maintained by rodents of the genus *Clethrionomys*; and Seoul and related viruses are maintained by rodents in the genus *Rattus*.

Before 1993 there was limited data indicating that these important agents of human disease in Asia and Europe caused any significant human disease on other continents.

## PRE-1993 HISTORY

Prior to 1993, two hantaviruses were known to circulate among the rodent fauna of the United States. Prospect Hill virus (PHV) was the sole hantavirus associated with an indigenous species of North American rodent. Prospect Hill virus was isolated in 1985 from lung tissue obtained from a meadow vole, *Microtus pennsylvanicus*, captured in Frederick, Maryland (Lee et al. 1985). This virus is most closely related by RNA and protein sequence analysis to the old world hantaviruses of which the representative serotype is Puumala (Xiao et al. 1994). These agents are also maintained by Arvicolid rodents, but of a different genus (*Clethrionomys*), and cause a mild form of HFRS. Antibodies to PHV have been found in different human populations of North America (Yanagihara et al. 1984, Yanagihara et al. 1990), but no disease has been associated with the presence of antibody.

The second hantavirus characterized from rodents in the United States was Seoul virus (SEOV) which infects at least two species of the genus *Rattus*; *R. norvegicus* and *R. rattus* (Lee et al. 1982). Infected Norway rats have been identified from many cities in the United States and around the world (LeDuc et al. 1986). These Murid rodents are of Asian origin and have been inadvertently introduced throughout the world by human activities. Four strains of SEOV have been obtained from *R. norvegicus* captured from the cities of Philadelphia, Houston, New Orleans and Baltimore in the United States (LeDuc et al. 1984, Tsai et al. 1985, Childs et al. 1987).

In some Asian cities SEOV has been associated with urban cases of HFRS, including epidemics of this disease. Neither PHV or SEOV have been associated with outbreaks of hantavirus disease in the United States, although SEOV does produce a mild, acute disease in some infected persons (Glass et al. 1993). The previous demonstration of antibodies reactive to PHV in mammalogists and other populations suggests that infection with PHV occurs. However with the identification of the novel, highly pathogenic hantaviruses discussed below, these prior observations must be reconsidered. Antibodies to PHV are highly cross-reactive with antigens from the newly identified pathogenic hantaviruses so the possibility exists that the antibody previously described as associated with PHV

infection actually indicated prior infection with a different virus.

SEOV, although common in inner city rat populations where antibody prevalences can exceed 40%, apparently only rarely infects humans in the United States, and of those infected few develop acute disease (Glass et al. 1993). However, a consistent association between SEOV infection and chronic, hypertensive renal disease has been found in Baltimore patient populations (Glass et al. 1993). The implications of this observation are serious, if sequelae leading to chronic renal disease are a frequent outcome of SEOV infections (LeDuc et al. 1992).

Prior to May, 1993, hantaviruses in the United States were regarded as virological and medical curiosities, rather than a major public health concern. However, surveys attempting to link previously identified North American hantaviruses to human disease had for the most part looked for indications of renal involvement, as this is the dominant clinical presentation of HFRS as known from Asia and Europe. Thus, it is entirely possible that disease was not identified because the wrong patient populations were studied. It was not until 1993 that the major respiratory involvement in hantavirus disease was identified in patients in the southwestern United States. Up until that time respiratory involvement in HFRS had been noted (Linderholm et al. 1992), but was considered a relatively minor component of the disease spectrum.

## AN ABRUPT APPEARANCE

In May, 1993, many of our preconceived notions concerning hantaviruses changed. Serologic tests linked a hantavirus to a mysterious outbreak of adult respiratory distress syndrome (ARDS) in the Four Corner States of New Mexico, Colorado and Arizona (CDC 1993a). Immunohistochemical staining of fixed lung tissue obtained from fatal cases of ARDS revealed hantaviral antigens (CDC 1993b). The use of molecular genetic analyses performed with reverse transcriptase-polymerase chain reaction (RT-PCR) allowed not only additional confirmation that a hantavirus was causing these illnesses, but also provided the sequence data to show that this was a novel virus (Nichol et al. 1993). The sequence data indicated that this new hantavirus was most closely related to PHV, but differed enough to form a new group within the genus *Hantavirus*. This virus has been adapted to cell culture growth and given the name Muerto Canyon virus (MCV) (Elliott et al., manuscript submitted).

## THE RESERVOIR

Rodent collections from the Four Corners region indicated that the greatest prevalence of antibody reactive to hantavirus antigens was in *Peromyscus maniculatus* (Childs et al. 1994). Of 813 *P. maniculatus* tested 30.4% were seropositive, although indications of infection were also found in other rodents of the genus *Peromyscus* [*P. truei* (19.6% positive) and *P. boylii* (5.9%)], and in the genera *Tamias* [*T. quadrivitattus* (13.3%) and *T. dorsalis* (3.6%)], *Mus* [*M. musculus* (3.9%)], *Neotoma* [*N. albigula* (2.9%)] and *Reithrodontomys* [*R. megalotis* (22.2%)]. Two seropositive rabbits (16.3%; *Sylvilagus audubonii*) were also identified.

The use of RT-PCR revealed that the virus present in these rodents was identical to that being sequenced from human autopsy material (Nichol et al. 1993). In addition, viral nucleic acid could be amplified from >95% of the seropositive deer mice, suggesting that they are persistently infected and chronic carriers of virus, even in the presence of a vigorous antibody response. This pattern of infectious virus sheding from antibody positive rodents is a classic feature of the type of chronic infections hantaviruses establish in their primary rodent reservoir species. *P. maniculatus* was also the most common small mammal trapped in the area and frequently was obtained from within human dwellings. Based on this constellation of findings, *P. maniculatus* was implicated as the primary reservoir for this new hantavirus in the southwestern United States (Childs et al. 1994).

The distribution of *P. maniculatus* extends almost throughout the North American continent, with the exception of the far north, the eastern coastal region, and the southeastern United States (Carleton et al. 1989). Infected *P. maniculatus* have been identified from many areas within their geographic range and cases of hantavirus pulmonary syndrome (HPS) have been identified from 15 states. New HPS cases are still being discovered from states within the range of *P. maniculatus*, but it was a surprise when a case of HPS was diagnosed from Louisiana and a possible case occurred in Florida (CDC 1993c, CDC 1994). These states are outside the known geographic range of *P. maniculatus*.

## MORE NOVEL HANTAVIRUSES

Once again, RT-PCR provided insight to this apparent puzzle. The viral sequences obtained from the fatal case of HPS in Louisiana indicated it was not the same as MCV (CDC 1993c). It was approximately as different from MCV by sequence analysis as MCV is from PHV. Despite initial efforts, the rodent reservoir for the Louisiana virus has not yet been identified. Again, investigations surrounding the suspicious Florida case did turn up a surprising finding. Twelve of 90 cotton rats, *Sigmodon hispidus*, captured from around Miami, were serologically positive for a hantavirus (1994). RT-PCR studies indicated the virus from *S. hispidus* was yet a fourth North American hantavirus, different from MCV, PHV, and the virus associated with HPS in Louisiana (Table 1). The virulence of the *S. hispidus* virus for humans remains unproven, because the serologic data do not permit precise timing of the infection relative to the case-patient disease episode. However, there is a large and diverse group of North American hantaviruses and their reaction to human disease is actively being investigated.

It is apparent from the rapidity with which new findings are being reported on HPS and hantaviruses, that any conclusive statement would be premature. However, in a matter of six months a group of viruses that were previously regarded as biological and medical curiosities in the United States, have achieved a public health prominence that demands our attention.

Table 1. Hantaviruses, their implicated rodent reservoirs, and their association with human disease (HFRS= hemorrhagic fever with renal syndrome; HPS= hantavirus pulmonary syndrome) in the United States.

| Virus | Rodent Reservoir* | Human Disease |
|---|---|---|
| ISOLATED IN CELL CULTURE | | |
| Seoul | *Rattus norvegicus* | HFRS (mild type) |
| Prospect Hill | *Microtus pennsylvanicus* | unknown |
| Muerto Canyon | *Peromyscus maniculatus* | HPS |
| SEQUENCE DATA ONLY | | |
| "Louisiana"** | unknown | HPS |
| "Florida"** | *Sigmodon hispidus* | unknown |

* Other reservoirs may exist.
** Formal names do not exist as these viruses have yet to be adapted to cell culture growth.

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