INTRODUCTION

Hantavirus pulmonary syndrome (HPS) is a rodent-borne viral disease first identified in the Four Corners region of the southwestern United States in 1993. HPS often results from infection with one of multiple pathogenic viruses (i.e., Sin Nombre virus [SNV], Bayou virus, and Black Creek Canal virus in the United States), all members of the genus Hantavirus. After identification of SNV as the viral agent responsible for the 1993 HPS outbreak, -30 new Hantavirus genotypes have been identified throughout North, Central, and South America. Although the majority of US cases of HPS are identified in the Western states, rodent reservoirs of hantaviruses are present, and HPS cases occur throughout the United States. Before the incidents presented in this paper, only one hantavirus infection had been reported from West Virginia. That infection was retrospectively diagnosed in a wildlife biologist working in Randolph and Tucker Counties, who became ill in 1981. The deer mouse (Peromyscus maniculatus) and the white-footed mouse (P. leucopus), both known to carry SNV (including Monongahela and New York variants), inhabit West Virginia. Persons coming into contact with rodents or rodent excreta through work-related, recreational, or peridomestic activities are at risk for infection. In this report, we present the results of an investigation to identify the virus, the associated rodent reservoir, and the circumstances surrounding two HPS cases occurring within 1 week and 12 miles of each other in Randolph County, WV, in July 2004.

CASE REPORTS

Patient 1. A man, 32 years of age, who was a wildlife science graduate student working locally and a Randolph County resident. We interviewed family members and colleagues, reviewed medical records, and conducted environmental studies at likely exposure sites. Small mammals were trapped, and blood, urine, and tissue samples were submitted to the Centers for Disease Control and Prevention for laboratory analyses. These analyses confirmed that both patients were infected with Monongahela virus, a Sin Nombre hantavirus variant hosted by the Cloudland deer mouse, Peromyscus maniculatus nubiterrae. Other than one retrospectively diagnosed case in 1981, these are the first HPS cases reported in West Virginia. These cases emphasize the need to educate the public throughout the United States regarding risks and prevention measures for hantavirus infection.

Patient 2. A man, 41 years of age, residing in Randolph County, WV. The graduate student was reported to have not used personal protective equipment (i.e., respirator, gloves, gown, or coveralls) while handling rodents and to have eaten food without washing his hands after handling animals. On July 5, 2004, the graduate student (Patient 1) visited a Blacksburg, VA, emergency department (ED) complaining of fever, cough, and weakness. His medical history included chest pain, presumably from sneezing and coughing intermittently for ~1 month. Vital signs indicated a fever (102.7°F/39.2°C) and tachycardia (pulse, 117 bpm). A chest radiograph showed a faint infiltrate in the right lung. Initial blood work revealed a normal white blood cell count (5,600/mm³), with a slight left shift (87% neutrophils) and no bands and a lymphopenia (400/mm³). The platelet count (195,000/mm³) and hematocrit (48.9%) were both normal. Physical exam findings were consistent with pneumonia. Patient 1 received intravenous fluids, nonsteroidal anti-inflammatory drugs, and oral quinolone antibiotic therapy in the ED. Approximately 4 hours later, Patient 1 was released at his own request. While attempting to leave the ED parking lot, Patient 1 became nauseated and vomited and was readmitted to the ED; 12 hours later, he had acute respiratory failure requiring intubation and mechanical ventilation. He remained febrile with worsening hypotension. Chest radiographs on June 6 showed severe bilateral pulmonary edema. Blood work on June 6 revealed a mild thrombocytopenia (115,000/mm³), a normal white blood cell count, with left shift consistent with blood work on admission, but with bands (28%) now present, a normal hematocrit (50.5%), and a slightly increased prothrombin time (12.8 seconds). Multiple scratch wounds were noted on Patient 1’s arms, and his graduate advisor suggested possible hantavirus infection to his physicians. Possible diagnoses considered included pneumococcal pneumonia, pulmonary tularemia, and HPS. Despite aggressive therapy and empirical antimicrobial treatment, Patient 1 died on July 8.

Patient 2. A man, 41 years of age, residing in Randolph County, spent the first weekend of July 2004 at his family’s log cabin. He and other family members had been renovating the cabin over the past year. The Randolph County man (Patient 2) had multiple exposures to rodents while working at the cabin during the 2 months preceding this weekend stay. As an example of such exposures, on the evening of July 2, Patient
2, his wife, and their three children arrived at the cabin and found the interior reeking of urine. While airing out the cabin, his wife found two live mice, which Patient 2 immediately killed, and the remains of a dead mouse in a trash can. Later that evening, without using any personal protective equipment (PPE), he cleaned the trash can and saved the mouse carcasses for the farm cats. The family slept in the cabin that weekend, and other family members visited them at the cabin. Six more mice were trapped in the cabin during that weekend and were disposed of in a similar fashion.

On July 8, Patient 2 visited his primary-care provider (PCP) with a 2-day history of fever, fatigue, and a mild headache. His vital signs were normal, but laboratory results indicated hematuria, and he was treated as an outpatient for a presumptive urinary tract infection. The morning of July 9, he called his PCP to report a severe headache of ~12-hour duration; his PCP immediately referred him to the ED, where he was evaluated for headache and acute shortness of breath. He was hypotensive, with a relative bradycardia and an oxygen saturation of 88% on room air. Chest radiographs showed bilateral pulmonary vascular congestion with moderate-sized right pleural effusion and small left pleural effusion and bibasilar atelectasis. Bloodwork revealed a normal white blood cell count (7,200/mm$^3$) and a normal hematocrit (49.5%) but severe thrombocytopenia (3,300/mm$^3$). With ongoing hypoxia, hypotension, and bradycardia, Patient 2 was transferred that afternoon to a referral hospital, with a diagnosis of acute congestive heart failure and pneumonia.

At the referral hospital, Patient 2 was treated with broad-spectrum antibiotics and cardiovascular support. On July 10, he was transferred to the intensive care unit, where he was intubated and mechanically ventilated. HPS was considered as a possible diagnosis after the health alert had been distributed following the death of the graduate student (Patient 1). Bloodwork on June 10 indicated an on-going thrombocytopenia (6,600/mm$^3$), slightly improved, a normal white blood cell count with bands (9%), and a normal hematocrit (55.6%). Over the next few days, Patient 2’s condition deteriorated, with evidence of gastrointestinal bleeding and laboratory values that indicated thrombocytopenia, hypoalbuminemia, renal insufficiency, and disseminated intravascular coagulation.

However, by July 15, Patient 2’s health began to show limited progress. His condition improved slowly during the next month, and ventilatory support was removed on August 8. He remained in the hospital for >1 month and returned to work in November 2005, 4 months after his initial illness onset.

**Ecologic/small mammal investigation.** During August 3–6, 2004, a field investigation team consisting of the Centers for Disease Control and Prevention (CDC) and West Virginia State employees, assisted by staff members of the forest research station and colleagues of the graduate student (Patient 1), trapped rodents at three rural sites in Randolph County, WV. The collection sites were located near the towns of Helvetia and Cassity (Figure 1). Field notes kept by Patient 1 indicate high trap success rates (~40%) during June 2004 on his collection grids located ~1 mi from the research workers’ living quarters. The first site (Figure 1, site 1) included the research workers’ living quarters, a modern brick structure where Patient 1 had eaten meals. He reportedly had lifted ceiling tiles in the facility to place rat traps and had collected and killed at least one rat, *Rattus sp.*, which is not known to carry a hantavirus linked to human HPS. This building had multiple openings (i.e., crawl spaces, windows, and doors often left open), which could have allowed rodent entry, but no signs of rodent infestation were found during our survey. Only one deer mouse was collected near the building. Trapping was also conducted in two outbuildings and at the edges of the surrounding hardwood forest near the research workers’ living quarters; Patient 1 slept in a small tent located in this area. The tent, which had a floor and zip closure, was closed, undisturbed, and had no evidence of rodent infestation. The second trapping site (Figure 1, site 2) was near a trapping grid located in a selectively logged, old secondary growth deciduous forest, where Patient 1 had worked the week before his illness onset. The third site (Figure 1, site 3) was in and around Patient 2’s family cabin, located within a closed canopy deciduous forest, with a sparse understory consisting primarily of ferns. The interior of the cabin was clean, but multiple openings in the walls and the eves allowed easy rodent entry. (The researchers found immediately inside the cabin’s front door a container with several partially decomposed mice that appeared to have fallen into the container and become trapped since the family had locked the cabin.)

**RESULTS**

**Patient 1.** Serum specimens submitted to a private laboratory and the CDC, Special Pathogens Branch laboratory, were positive for both IgG and IgM antibodies reactive with SNV. Immunohistochemical staining of a splenic biopsy specimen acquired post-mortem also identified hantavirus antigen. In addition, hantavirus S segment RNA was detected by reverse transcriptase-polymerase chain reaction (RT-PCR), and nested PCR was performed on serum obtained July 8. Sequence analysis of the 394-nucleotide (nt) diagnostic nucleocapsid protein fragment revealed that the closest relationship was to the SNV Monongahela–l variant, with 97.5%
sequence identity at the nt level and 100% amino acid (aa) identity.\textsuperscript{13} Phylogenetic analysis further confirmed the identity of the sequence from Patient 1 as Monongahela virus (Figure 2).

**Patient 2.** Serum samples were sent to a private laboratory and were later confirmed by CDC laboratories. These were positive for specific IgG and IgM antibodies reactive with SNV antigen. Serum samples from July 10 were positive by RT-PCR and nested PCR for hantavirus RNA. Comparison of the nucleotide sequence to that from Patient 1 revealed 99.7% nt identity and 100% aa identity, indicating the SNV Monongahela virus variant as the causative agent of disease in this case as well. Phylogenetic analysis further supports this conclusion (Figure 2).

**Ecological/small mammal study.** Twenty small mammals were trapped at the three sites, including 15 deer mice (\textit{P. maniculatus}), 3 Northern short-tailed shrews (\textit{Blarina brevicauda}), 1 long-tailed shrew (\textit{Sorex sp.}), and 1 eastern chipmunk (\textit{Tamias striatus}). Trap success for the three sites ranged from 6% to 10%. One deer mouse trapped outside the family cabin of Patient 2 was SNV antibody positive. Lung tissue from this rodent yielded hantavirus RNA that was 100% identical to RNA encoding nucleocapsid protein from Patient 2 at the nt and aa levels. Phylogenetic analysis graphically shows the association of the human and rodent sequences to each other, to the SNV Monongahela-1 sequence, and to the sequences from other North American hantaviruses (Figure 2).\textsuperscript{13} The West Virginia human and rodent sequences clearly fall within the SNV Monongahela variant lineage. Phylogenetic analyses (parsimony and neighbor-joining), using PAUP* of 400 bp of the mitochondrial cytochrome \textit{b} gene of the rodent host of this virus, were consistent with the identification of this rodent as a Cloudland deer mouse (\textit{Peromyscus maniculatus nubiterrae}).\textsuperscript{14}

**DISCUSSION**

Sequencing of amplified nucleic acid from the two patients identified SNV Monongahela variant, a hantavirus unique to the eastern United States.\textsuperscript{13,15} Comparison of genetic sequences between rodent and human samples has been used on numerous occasions to help identify the host species,\textsuperscript{16,17} the likely geographic site of exposure,\textsuperscript{6,18} or even the mechanism of infection.\textsuperscript{19} Analysis of viral sequence derived from a Cloudland deer mouse captured near the family cabin of Patient 2 revealed identical sequence to that from Patient 2, directly linking the human infection with a specific rodent host. The similarity of the viral sequences from both Patient 2 and the Cloudland deer mouse provides a clear epidemiologic link between the rodents and the human patients in this area. The identification of the Cloudland deer mouse as the reservoir source of the human hantavirus cases described herein is consistent with the initial description of the SNV Monongahela variant, which was first identified in archival tissues from Cloudland deer mice captured in West Virginia.\textsuperscript{13} However, the first documented human disease caused by this virus was a fatal case in Pennsylvania and was reportedly associated with \textit{P. leucopus}.\textsuperscript{15} A recent study by Dragoo and others\textsuperscript{20} indicated that confusion exists regarding the classification within the rodent species traditionally described as \textit{P. maniculatus}. The complete understanding of the host-virus relationships will require continued and expanded studies to refine the taxonomy of the rodent hosts of hantaviruses in this and other regions where HPS is endemic.

Initial beliefs were that both patients had become infected through the inhalation of virus-contaminated dust (the graduate student while placing rodent traps in the suspended ceiling of the workers’ living quarters and the Randolph County man while cleaning in the family cabin or working elsewhere). However, we identified no evidence of rodent infestation in the students’ living quarters and the Randolph County man. Indeed, an open box of breakfast cereal that had been on the floor of the facility since the illness of Patient 1 showed no sign of disturbance by rodents. No holes for rodent entry or signs of rodent infestation were found in the tent in which the student slept. Additionally, interviews with colleagues of Patient 1 revealed that he had direct contact, including bites and scratches, with deer mice. Patient 2 had a desk job at a lumber yard and was known to
assist friends with home projects, which could have been possible sources of exposure; however, family members of Patient 2 reported that he had handled multiple deer mice that had been found inside the family cabin over the summer providing several opportunities for exposure. The likely specific mechanism of infection for Patient 1 was through a bite while handling rodents without gloves. Patient 2 may have been infected through inhalation of infectious aerosol within the family cabin or through contamination of broken skin or mucous membranes with virus while handling infected rodents or cleaning rodent-infested areas.

Although the events of July 2 as reported by Patient 2’s family members provided the most immediate, and therefore obvious, opportunity for exposure to infected hosts, in actuality, it is more likely that Patient 2 became infected on any one of several visits to his cabin in the previous weeks. Indeed, if infection occurred on July 2, the incubation period would have been only 4 days until the patient was symptomatic on July 6. An analysis of HPS case registry data suggested an incubation period for HPS of 9–33 days, with a median of 14–17 days. Therefore, both patients most likely became infected in June, several weeks before their illness.

These cases show the importance of appropriate PPE and prompt hand washing whenever handling rodents or contaminated materials. After the diagnosis of HPS in Patient 1, immediate notification of health care providers in West Virginia played a key role in the diagnosing of the Randolph County man’s (Patient 2) illness. Physicians throughout the United States should consider HPS as a possible diagnosis among patients with a history of rodent contact or respiratory distress, because symptoms may be non-specific initially.

Previous studies have identified a low prevalence of hantavirus antibodies among forest and park workers. Further studies are needed to assess the prevalence of hantavirus infection within rodent populations, particularly in the Appalachian region, and the environmental influences on infection prevalence. Occupational risk factors for exposure to hantaviruses should be clearly identified, and information must reach populations at risk. Persons exposed to rodents and their excreta, including workers in high-risk occupations and the general public, need to be educated about the risk of HPS and the methods available to reduce exposure to hantaviruses and other zoonotic agents.

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