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Fatal Illness Associated With a New Hantavirus in Louisiana

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A fatal case of hantaviral illness occurred in Louisiana, outside of the range of *P. maniculatus*, the rodent reservoir for Sin Nombre virus. Hantavirus RNA and antigens were detected in patient autopsy tissues, and nucleotide sequence analysis of amplified polymerase chain reaction (PCR) products identified a newly recognized unique hantavirus, provisionally named Bayou virus. Prominent features of the clinical illness are compatible with hantavirus pulmonary syndrome (HPS), but several features such as renal insufficiency and intraalveolar hemorrhage are more compatible with hemorrhagic fever with renal syndrome (HFRS), a disease associated with Eurasian hantaviruses.

KEY WORDS: bunyaviridae, hantavirus pulmonary syndrome, hantaviruses, nephropathy, Sin Nombre virus, HFRS, Bayou virus

INTRODUCTION

Recently, hantavirus pulmonary syndrome (HPS) was described in the southwestern United States [Nichol et al., 1993; Duchin et al., 1994; Zaki et al., 1995]. This syndrome is characterized by a febrile prodrome and severe noncardiogenic pulmonary edema simulating adult respiratory distress syndrome. HPS is caused by a novel hantavirus (Sin Nombre virus) and like other known hantaviruses, is associated with a specific primary rodent vector [McKee et al., 1991], in this case *Peromyscus maniculatus*, the deer mouse [Nichol et al., 1993; Childs et al., 1994; Elliot et al., 1994; Hjelle et al., 1994]. Shortly after the description of this clinical entity, additional cases were reported throughout the United States. As expected, the vast majority of cases occurred within the known geographic range of *P. maniculatus* [CDC, 1994b]. We discuss the salient clinical features of a fatal case of this clinical syndrome in an individual residing outside the geographical distribution of *P. maniculatus* and report polymerase chain reaction (PCR) and immunohistochemical staining data confirming infection with a hantavirus. In addition, nucleotide sequence analysis indicates that this virus, provisionally named Bayou virus, represents a newly recognized unique hantavirus [Morzunov et al., 1995] distinct from Sin Nombre virus and the recently discovered Black Creek Canal virus, detected in cotton rats (*Sigmodon hispidus*) from Florida [CDC, 1994a; Rollin et al., 1995b].

CASE REPORT

A 58-year-old Louisiana highway worker was admitted to the regional public health hospital on June 16, 1993, for evaluation of dizziness, fever, vomiting, and weakness after approximately a two to three day history consisting of fever, chills, night sweats, headache, nausea, vomiting, and bilateral lower extremity weakness. On admission, his temperature was 101.6°F, respiratory rate of 18, heart rate of 92, and blood pressure of 170/100 mm/Hg. His physical exam and chest radiograph were unremarkable on admission; his urine analysis showed trace protein. On the third day of hospitalization, he was noted to have scant concentrated urine despite aggressive administration of fluids (at least 3.7 liters), and underwent computerized head tomography with intravenous contrast for evaluation of his ongoing headache and mental obtundation. He also had developed progressive shortness of breath and chest radiograph evidence of bilateral diffuse interstitial infiltrates necessitating intubation. Later that day, he became hypotensive and required aggressive pressor support. By the fourth day of hospitalization, he was noted to have acute oliguric renal insufficiency, hypoalbuminemia, and disseminated intravascular coagulation. On the fifth day of hospitalization, he developed irreversible asystole and expired despite aggressive medical management for his suspected adult respiratory distress syndrome (ARDS) and presumptive septic

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TABLE I. Selected Laboratory and Clinical Results*

	Normal values	16-Jun	18-Jun	19-Jun	20-Jun
WBC (10^3/cu mm)	7.8–11.8	5.3	28.1		29.0
-neutrophils (%)	50–70	58	56		78
-bands (%)	2–6	10	9		1
-lymphocytes (%)	20–44	27	28		16
-monocytes (%)	2–9	7	6		5
Hemoglobin (g/dl)	16.0–18.0	15.8	14.1		9
Hematocrit (%)	47–52	44.8	43.2		25.7
Platelets (10^3/cu mm)	130–400	194	28		23
Amylase (U/L)	34–122	66		638	625
Albumin (g/dl)	3.5–4.8	4.4		1.7	
LDH (U/L)	94–172	211			
BUN (mg/dl)	7–22	13	36	40	53
Creatinine (mg/dl)	0.5–1.7	1.1	0.6	5.6	7.4
Bicarbonate (mmol/l)	23–33	31	14	14	16
PT (sec)	11–16	13.5		29.8	29.2
PTT (sec)	25–38	32.8		>240	>240
Fibrinogen (mg/dl)	150–350			144	
Fibrin split products (ng/ml)				>4,000	

*WBC, white blood count; LDH, lactic dehydrogenase; BUN, blood urea nitrogen; PT, prothrombin time; PTT, partial thromboplastin time.

shock. There was no history of nephrotoxic analgesic or antibiotic administration either prior to or during hospitalization. Significant laboratory values are depicted in Table I. All pre- and postmortem bacterial, fungal, and mycobacterial stains and cultures of sputum and blood were sterile. A complete clinical description was rapidly presented to notify clinicians of the increasing geographic spread of HPS [Steier and Clay, 1993].

Pertinent gross autopsy findings included: bilateral atelectasis of both lungs, left greater than right, almost generalized; bilateral severe pleural effusions; moderate peritoneal effusions; mild pericardial effusions; severe cerebral edema; generalized and severe subcutaneous edema, and cardiomegaly with left ventricular hypertrophy. Histopathologic examination of the lung revealed an interstitial pneumonitis with congestion and inter- and intra-alveolar mononuclear and neutrophilic leukocytic infiltrates. Focal areas of atelectasis, hemorrhage, as well as type II pneumocyte hyperplasia were observed. Extensive amounts of edema fluid and fibrin as well as large numbers of hemosiderin-laden macrophages were also seen within the alveoli. Both kidneys showed changes consistent with early renal tubular necrosis; the pancreas was histologically normal. Fresh frozen and formalin-fixed tissues were sent to CDC for hantavirus diagnostic testing. No serum sample was available.

For the six week period prior to his illness onset (usual extreme of hantavirus incubation periods) he had no travel outside northeast Louisiana where he was employed as a bridge and road maintenance worker. His duties routinely exposed him to rodents and included: repairing and replacing culverts; cleaning drainage pipes; and cleaning drifts and beaver dams from under bridges. On the weekend he operated a large mechanical plower on two local farms where rodent sightings were common in the fields and barns. He resided in a brick house in the residential area of a semirural town which faced an open field but no rodents were seen within the house. The family owned a horse at a local farm but no other household pets. Serum samples from all thirty-three rodents captured in these environs during the initial case investigation were seronegative for hantaviral antibodies (T.G. Ksiazek, personal communication). No other family member was ill during this six week time period.

METHODS

RNA Extraction, Amplification and Nucleotide Sequence Analysis

Ribonucleic acid (RNA) was extracted from lung and brain autopsy tissues as described previously [Nichol et al., 1993]. Briefly, approximately 100 mg of tissue was homogenized manually in 500 μl of acid guanidine thiocyanate solution, followed by the phenol-chloroform extraction and purification with an RNA matrix (RNaid kit, Bio101, La Jolla, CA). The extracted RNA was then assayed for the presence of hantavirus RNA using a nested reverse-transcriptase polymerase chain amplification technique as described previously [Nichol et al., 1993; Spiropoulou et al., 1994]. This assay amplifies a 185 nucleotide region of the G2 encoding region of the virus M segment. This area was chosen for analysis as it has been extensively analyzed for a number of hantaviruses, and phylogenetic trees generated based on data from this region have been highly predictive of the overal phylogeny of hantaviruses [Nichol et al., 1993; Spiropoulou et al., 1994; Rollin et al., 1995a,b]. The nucleotide sequence of a 139 bp region of the resulting product DNA (185 bp in length) was analyzed directly using the dyedeoxy cycle sequencing technique (Applied Biosystems, Foster City, CA).

Phylogenetic Analysis

Phylogenetic analysis of nucleotide sequence differences between previously characterized hantaviruses and PCR fragments generated from the hantavirus detected in the Louisiana case lung and brain tissues were analyzed by the maximum parsimony method using PAUP software [Swofford, 1991]. Analysis was carried out by a branch and bound search using a weighting of transversions over transitions of 4:1. This weighting was based on prior MacClade software analysis [Maddison and Maddison, 1992] of trees generated from nucleotide sequence differences detected among numerous Sin Nombre virus variants [Spiropoulou et al., 1994]. Such weighting is predicted to improve the effectiveness of the maximum parsimony method for estimation of the correct phylogeny [Hillis et al., 1994]. Bootstrap confidence limits were calculated by 1,000 heuristic search repetitions of the analysis. Previously published hantavirus sequences used in the analysis include: Hantaan strains 76–118 [Genbank M14627, Schmaljohn et al., 1987; X61034, Yoo and Kang, 1987], Lee [Genbank D00377, Schmaljohn et al., 1988], and HV-114 [Genbank L08753; Xiao et al., 1993], Seoul strains 80–39 [Genbank S47716, Antic et al., 1992], SR-11 [Genbank M34882, Arikawa et al., 1990], and Thai [Genbank L08756, Xiao et al., 1994], Puumala strains Sotkamo [Genbank X61034, Vapalahti et al., 1992], Cg18-20 [Genbank M29979, Giebel et al., 1989], Berkel [Pilaski et al., 1994] and 9013 [Rollin et al., 1995a]; Prospect Hill strain PHV-1 [Genbank X55129, Parrington et al., 1991]; Sin Nombre viruses NM H10 and CC107 [Nichol et al., 1993; Spiropoulou et al., 1994; Dexin et al., 1995]; and Black Creek Canal virus [Rollin et al., 1995b].

Immunohistochemistry

Immunohistochemical assays for virus antigens were performed as described previously using hantavirus-specific monoclonal and polyclonal antibodies [Zaki et al., 1995].

RESULTS

PCR and Nucleotide Sequence Analysis

Specific nested PCR DNA products of the expected size (185 bp) were obtained using total RNA extracted from tissues from the lung and brain of the patient (data not shown). No bands were obtained using RNA from control autopsy materials or RNA controls. The amplified DNA from the tissues were extracted from the agarose gel, and analyzed with an automated thermocycle sequencing technique with the same primers used for PCR product synthesis. The DNA bands were found to contain hantavirus-like sequences but the nucleotide sequence differed from that of any of the known hantaviruses by at least 24%. Phylogenetic analysis of the nucleotide sequence differences by the maximum parsimony method indicated that the hantavirus associated with this fatal HPS case in Louisiana was novel, representing a distinct lineage, and was most closely related to Black Creek Canal and Sin Nombre viruses (76% and 71% identity, respectively), the only other hantaviruses recovered from rodent species indigenous to North America and associated with HPS (Fig. 1).

Immunohistochemical Analysis

Viral antigens were detected in several organs including the lungs, spleen, and kidneys. The strongest immunostaining was seen using a cross-reactive monoclonal antibody (GB04-BF07) raised against Puumala virus nucleocapsid protein. This antibody had previously been shown to cross-react with a number of hantaviruses including Sin Nombre virus [Ruo et al., 1991; Zaki et al., 1995]. Immunostaining was characteristically punctate and observed primarily within microvascular endothelial cells, in a pattern similar to that seen in previously reported cases of HPS [Zaki et al., 1995]. Lesser amounts of antigens were detected using a high-titered polyclonal sera from SN virus infected *P. maniculatus*.

DISCUSSION

The results of the genetic analysis of a fragment of the virus G2 coding region indicates that the virus detected in the tissues of this patient is a newly recognized highly pathogenic hantavirus most closely related to Sin Nombre (SN) and Black Creek Canal (BCC) viruses found in *P. maniculatus* and *S. hispidus*, respectively [Child et al., 1994; Rollin et al., 1995b]. Subsequent phylogenetic analysis of the nucleotide sequences of the complete genome S and M segments of this and other hantaviruses revealed a similar tree topology to that reported here and suggested naming of this distinct virus from Louisiana as Bayou virus [Morzunov et al., 1995]. As *P. maniculatus* is absent from the southeastern United States [Baker, 1968], rodent trapping and serological testing are currently underway to determine the identity of the rodent reservoir, and to determine the geographic distribution of Bayou virus. The strong immunostaining of this patient's tissues observed with a broadly reactive hantavirus-specific monoclonal antibody but not with high-titered polyclonal sera from SN virus-infected *P. maniculatus* is consistent with the genetic findings. It is interesting that phylogenetic analysis places the hantaviruses associated with predominantly HPS-like illness, i.e., SN, BCC, and Bayou virus, in a distinct genetic clade.

Many of the clinical illness and laboratory findings of this patient are similar to those reported for SN virus-induced HPS cases in the Southwest [Duchin et al., 1994; Shefer et al., 1994; Zaki et al., 1995]. These include the febrile prodrome, the rapid onset of severe pulmonary disease, hemoconcentration, leukocytosis with a left shift, severe progressive thrombocytopenia, hypoalbuminemia, metabolic acidosis, and elevated PT and PTT. The elevated amylase has not been previously reported, although abdominal pain is a not uncommon feature of the SN virus-induced HPS clinical prodrome. The acute and profound renal insufficiency is most atypical compared to other reported HPS cases [Duchin

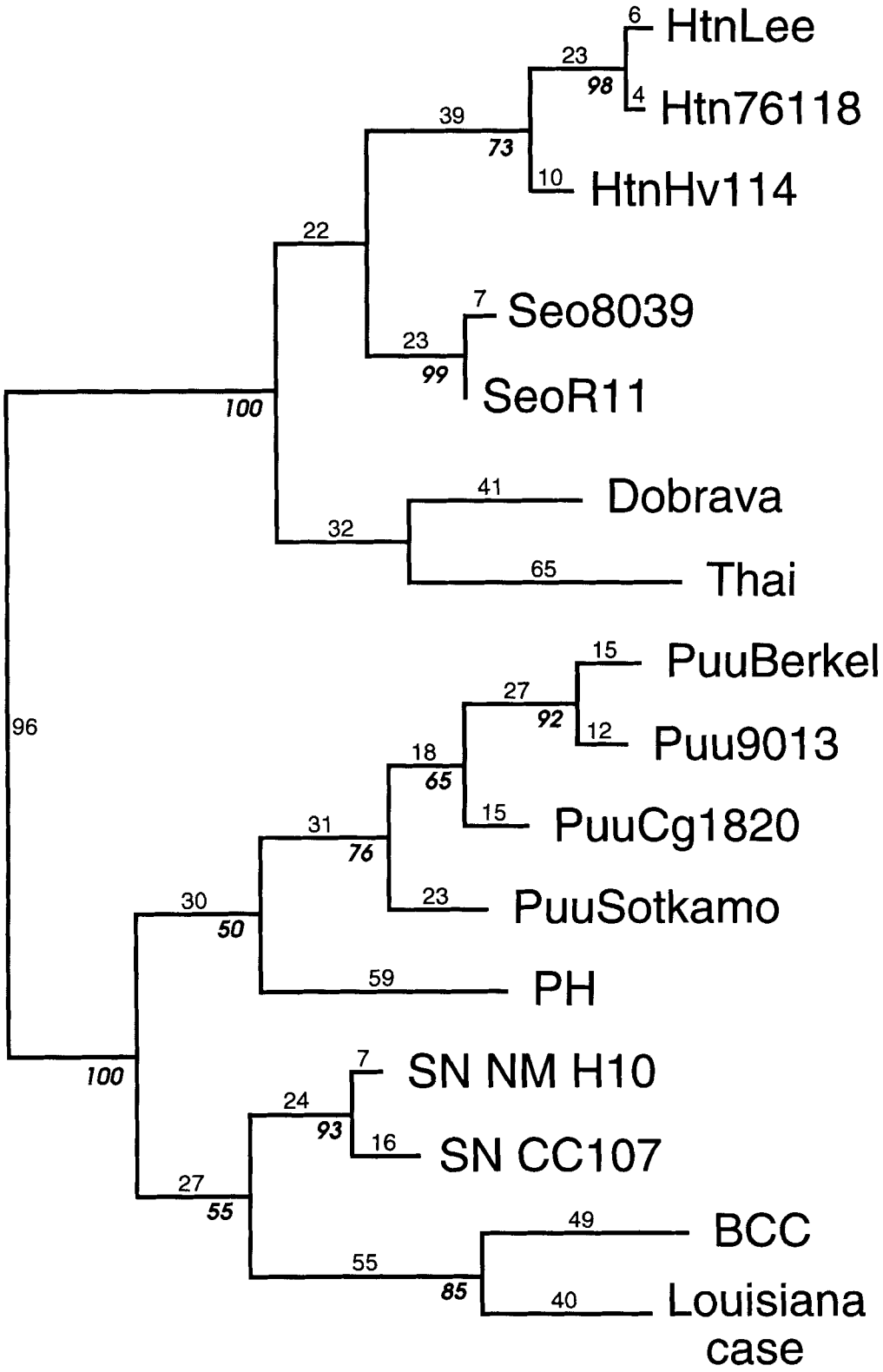

Fig. 1. Genetic relationship of virus detected in the Louisiana HPS case to previously characterized hantaviruses. Nucleotide sequence differences among the 139 bp of PCR fragements of the M segment of the virus detected in the Louisiana HPS case and previously characterized hantaviruses were analyzed by the weighted maximum parsimony method (see Materials and Methods). A single most-parsimonius tree was obtained. Horizontal lengths are proportional to nucleotide step differences (indicated above lines). Vertical distances are for graphic representation only. Bootstrap conficence limits wre calculated by 1,000 heuristic search repetitions, and limits in excess of 50% are indicated in bold italics at appropriate branch points.

et al., 1994; Zaki et al., 1995]. Although the renal disease in this individual may be attributable to the administration of intravenous contrast, a history of scant dark urine preceded the contrast study. Moreover, elements of the clinical prodrome, laboratory abnormalities, central nervous system manifestations, microscopic findings and the observed renal insufficiency are typical of hemorrhagic fever with renal syndrome (HFRS), the clinical entity caused by Eurasian hantaviruses [Sheedy et al., 1954; Cohen et al., 1983]. It is of note that renal insufficiency was also a prominent feature of the nonfatal case of HPS-like illness in Miami, Florida due to Black Creek Canal virus [CDC, 1994a; Rollin et al., 1995b]. The phylogenetic analysis places these two viruses from the southeastern U.S. together in another distinct genetic clade, suggesting that this genetic group of hantaviruses may be associated with clinical illness somewhat intermediate between HPS and HFRS, prominent respiratory illness associated with significant renal insufficiency. However, identification and analysis of a greater number of patients infected with these viruses will be needed to clarify the full spectrum of clinical disease.

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