Seoul Virus in Patients and Rodents from Beijing, China

Shu-qing Zuo, Pan-he Zhang, Jia-fu Jiang, Lin Zhan, Xiao-ming Wu, Wen-juan Zhao, Ri-min Wang, Fang Tang, Zhe Dun, and Wu-chun Cao*

Beijing Institute of Microbiology and Epidemiology, State Key Laboratory of Pathogen and Biosecurity, Beijing, China; Center for Disease Control and Prevention of Dongcheng District, Beijing, China; Center for Disease Control and Prevention of Haidian District, Beijing, China

Abstract. Hemorrhagic fever with renal syndrome (HFRS) is a significant public health problem with an increasing incidence in Beijing, China (report of disease surveillance from the Center for Disease Control and Prevention of Beijing, China). Hantaviruses were detected using RT-PCR method in blood samples of HFRS patients and lung tissues of rodents captured in Beijing. Phylogenetic analyses of 724bp partial S segment of the hantavirus gene showed that the detected Seoul virus (SEOV) fell into three different lineages, two of which circulated in Beijing. A nucleotide sequence identity of 99.7% for one of the cases of HFRS—the human- and Rattus norvegicus-originated SEOV sequences—had only two silent substitutions, suggesting genetic analysis is an essential tool for “case-investigation.”

INTRODUCTION

Hantaviruses, in the family Bunyaviridae, are enveloped, single-stranded, negative-sense RNA viruses. The genome consists of 3 segments, designated as large (L), medium (M), and small (S), respectively encoding the RNA polymerase, the glycoprotein precursor (GPC) protein that is processed into 2 separate envelope glycoproteins (Gn and Gc), and the nucleocapsid (N) protein. Until now, more than 20 hantavirus species have been identified, each of which is predominately associated and co-evaluated with a few related rodent species. Consequently, hantaviruses form 3 large groups according to their rodent hosts: Murinae-, Arvicolinae-, and Sigmodontinae-associated hantaviruses. The Murinae-associated Hantaan virus (HTNV), Seoul virus (SEOV), Dobrava virus (DOBV), and the Arvicolinae-associated Puuma virus (PUUV) are causative agents of hemorrhagic fever with renal syndrome (HFRS). Sin Nombre virus (SNV), Andes virus (ANDV), Black Creek Canal virus (BCCV), Laguna Negra virus (LANV), and other related viruses cause hantavirus pulmonary syndrome (HPS).

Hemorrhagic fever with renal syndrome is a severe endemic in mainland China accounting for 90% of the total cases reported in the world. Although integrated intervention measures involving rodent control, environment management, and vaccination have been implemented, HFRS remains a significant public health problem with 20,000 to 50,000 human cases diagnosed annually. SEOV and SEOV carried by Apodemus agrarius and Norway rat (Rattus norvegicus), respectively, are known to be the causative agents of HFRS in mainland China. In recent years, the natural foci of the disease have extended from rural to urban areas, with a remarkable increase of incidence in the novel endemic areas.

In Beijing, the first HFRS case was reported in 1986 and was shown to be imported from another place. Thereafter, a few sporadic cases were diagnosed. Since 1996, the incidence of the disease has increased. It reached more than 200 cases annually in the early 2000s from less than 10 cases annually in the late 1990s (surveillance report from CDC of Beijing, China). In 1989, a strain related to HTNV was detected from a striped field mouse (A. agrarius) captured in the suburbs. Recently, epidemiological surveys found that the most common strains of hantaviruses in rodents were SEOV. In this study, we detected SEOV in HFRS patients and rodent hosts captured from the presumed infection sites of the cases to determine the infection sites of HFRS cases, investigate genetic diversity of the viruses circulating in Beijing, and learn more about mutations in the HV genome related to the host-switch.

MATERIALS AND METHODS

Samples collection. From 2003 to 2005, blood samples were collected from HFRS patients during their acute phase of illness. All of the clinical cases were diagnosed and confirmed by serologic tests (ELISA kit, Lanzhou Institute of Biological Products, China). After obtaining informed consent, blood samples were collected from each participant. At the same time, related information was obtained through a questionnaire.

In the meantime, rodents were captured using snap traps at the presumed infection sites (Figure 1). After identification of species and sex, lung tissues were removed from the captured rodents and kept in liquid nitrogen until tested. For unidentified species in the field, the craniums were brought to the laboratory for further identification.

Extraction of RNA, RT–PCR, and sequencing. RNA was extracted from blood clots or sera of HFRS patients and from lung tissues of rodents using TRIzol® Reagent (Invitrogen Inc., USA) according to the manufacturer’s instruction. Reverse transcription (RT) of hantaviral RNA was performed with SuperScript III (Invitrogen Inc., USA) and oligonucleotide primer P14 (5’-TAGTAGTAGACTCC-3’). Heminested polymerase chain reactions (PCR) were conducted to amplify the partial S segment of SEOV in the GeneAmp® PCR System 2700 (Applied Biosystems Inc., Singapore). The outer primer pair SF424 (5’-TCATTYGTGGTCC CRATCATCTT-3’, nt 424-445) and SR1148 (5’-TATATCCCATGATTG TG-3’, nt 1130-1148) was used first. Hemi-nested reactions were performed with primer pair SF424, SR1008 (5’-CCTAAYTCAGCCATCCTCCG-3’, nt 1008-1028) and primer pair SF812 (5’-CTGGGAATTCTGTRAATCGTG-3’, nt 812-832), SR1148, respectively. All the RT-PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide, and visualized using UV light. Specific DNA fragments were purified according to the manu-

* Address correspondence to Wu-chun Cao, 20 Dong-Da Street, Fengtai District, Beijing, 10071. E-mail: caowc2000@yahoo.com.cn
manufacturer's instructions using QIAquick Gel Extraction kit (Promega Inc., USA). Direct sequencing was performed with the ABI PRISM 377 or 3730 Genetic Analyzers (ABI Inc., USA). To avoid contamination, RNA extraction, reagent setup, amplification, and gel electrophoresis were performed in separate rooms.

**Phylogenetic analyses.** The 724bp sequences generated from overlapping fragments were analyzed using DNAStar software package (DNAStar, Inc.). Sequence was aligned using Clustalx1.8 software with default parameters and checked manually.18

Phylogenetic analysis was carried out with PHYLIP (version 3.65) software package,19 using the neighbor-joining (NJ) method, with an empirical transition/transversion bias of 2.0. The stability of the phylogenetic tree was evaluated by bootstrap analysis with 1,000 replications. Distance matrices for the aligned segments were calculated by F84 model. GenBank accession numbers of the previously published sequences of the hantaviruses used in this study are listed in the legend of Figure 2.

To better understand the phylogenetic relationship of the virus, Neighbor-net networks were constructed using SplitsTree4 V4.3 software with an uncorrected-P method.20

As the measure of support, the bootstrap values were also estimated by bootstrap analysis with 1,000 replications.

**RESULTS**

A total of 22 specimens were collected from laboratory-confirmed HFRS patients. Patient samples were obtained between 3 and 15 days after the onset of the illness. The age of the patients ranged from 19 to 70 years with a median of 37.2 years. Most patients were men (18 of 22). They were distributed in a large-scale area (Figure 1) and all were diagnosed during the winter and spring seasons from November to April. The clinical characteristics of the studied patients are summarized in Table 1.

At the same time, a total of 923 animals, including 649 R. norvegicus, 136 Mus musculus, 124 A. agrarius, and 14 other little animals were captured and subjected to RT-PCR detection. SEOV was detected in 7 of 22 (33.3%) blood samples collected from HFRS patients. None of the samples collected from HFRS patients 9 days after the onset of the symptoms was positive by RT-PCR detection. The 724bp fragments corresponding to position 424-1148 of the S segment gene of the

---

**FIGURE 1.** Location of hemorrhagic fever with renal syndrome (HFRS) cases and positive rodents captured in different areas in Beijing. Cross indicates location of probable site of exposure for every patient. Triangle indicates location of Norway rats whose sample was positive by RT-PCR detection. Rotundity indicated location of *Mus musculus* whose sample was positive by RT-PCR detection. Blue indicates strains fallen into the first lineage. Red indicates strains fallen into the second lineage. Purple indicates location of patients whose sample was negative by RT-PCR detection. Pink indicates HuBJ20, a strain probably circulating in Tianjin. This figure appears in color at www.ajtmh.org.
positive samples were designated as HuBJ7, HuBJ9, HuBJ15, HuBJ16, HuBJ19, HuBJ20, and HuBJ22 to represent patients 7, 9, 15, 16, 19, 20, and 22, respectively (Figure 1).

Seoul virus was also detected in 47 R. norvegicus and 1 Mus musculus lung tissue specimens. PCR products of 5 positive samples from R. norvegicus captured in different sites and 1 sample from M. musculus were sequenced. They were designated as Rn-M11, Rn-DC8, Rn-YUE12, Rn-CP7, Rn-SHY17, and Mm-SHY6, according to their rodent hosts and geographical origin (Figure 1).

The nucleotide sequences of the 724bp partial S segment of SEOV genes amplified from the HFRS patients and rodents had 96.3% to 100% homology with each other and 85.1% to 99.9% identity to those of other SEOV deposited in GenBank. The analysis of deduced amino acid sequence of the N protein (aa residues 136–371) revealed that the identity was 98.3% to 100%. The nucleotide sequences identity to HTNV, DOBV, and SAAV was 64.4% to 74.1%. Other species of hantaviruses had only 52.1% to 58.7% identity.

The phylogenetic tree constructed by PHYLIP 3.65 software package showed that all strains from Beijing formed a clade within the SEOV subtype #3 according to previous studies, but with low bootstrap values for many lineages (Figure 2), far below the widely accepted 70% threshold limit. (No sequences of subtype #2 were included in the study because no sequence long enough could be available.) This was distinct from previous studies in that SEOV sequences could be divided clearly into 6 subtypes.

To further illustrate the phylogenetic relationship between strains from Beijing and other strains of SEOV, the neighbor-net graph was constructed using SplitsTree4 V4.3 software. The overall fit index was 97.79% and most branches had bootstrap support values between 70% and 100% (data not shown) when was performed using 1,000 bootstrap samples (Figure 3).

The neighbor-net graph showed that strains from Beijing fell into 3 different lineages. Strain HuBJ9 clustered together with several rodent-originated (wild-type) SEOV strains circulating in Beijing and formed a unique lineage distinct from previously reported SEOV lineage. The HuBJ9 strain detected from patient 9 was most closely related to the rod-

---

### Table 1

Clinical and laboratory records from patients with hemorrhagic fever with renal syndrome

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>Frequency</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>22/22</td>
<td>100</td>
</tr>
<tr>
<td>Headache</td>
<td>18/22</td>
<td>81.8</td>
</tr>
<tr>
<td>Lumbago</td>
<td>17/22</td>
<td>77.3</td>
</tr>
<tr>
<td>Myalgia</td>
<td>5/22</td>
<td>22.7</td>
</tr>
<tr>
<td>Hypotension (SP &lt; 90 mm of Hg)</td>
<td>13/22</td>
<td>59.1</td>
</tr>
<tr>
<td>Belllyache</td>
<td>9/22</td>
<td>40.9</td>
</tr>
<tr>
<td>Vomiting</td>
<td>13/22</td>
<td>59.1</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>8/22</td>
<td>36.4</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>4/22</td>
<td>18.2</td>
</tr>
<tr>
<td>Petechia (on skin or oral mucosa)</td>
<td>14/22</td>
<td>63.6</td>
</tr>
<tr>
<td>Oliguria</td>
<td>14/22</td>
<td>63.6</td>
</tr>
<tr>
<td>Hematuria</td>
<td>10/22</td>
<td>45.5</td>
</tr>
<tr>
<td>Albuminuria</td>
<td>18/22</td>
<td>81.8</td>
</tr>
<tr>
<td>Thrombocytopenia (&lt; 150,000/mm³)</td>
<td>21/22</td>
<td>95.5</td>
</tr>
</tbody>
</table>

SP = systolic pressure.
dent-originated strain BjHD01, which had been previously isolated from a Norway rat (*R. norvegicus*) captured at the location about 200 meters from the residence of the patient 9. The nucleotide identity was 99.7% with only 2 silent substitutions. Five SEOV sequences from HFRS patients, HuBJ7, HuBJ15, HuBJ16, HuBJ19, HuBJ22, clustered together with rodent-originated sequences Rn-SHY17 with 4 to 8 silent substitutions, respectively, suggesting they might share a common evolutionary ancestor. Interestingly, apart from HuBJ15, the other 5 strains had a homologous substitution of asparagine to threonine at position 260 of the deduced amino acid sequences of the N protein, which was distinct from all other SEOV. According to the patients’ record, the possible exposed sites for case 7, 15, and 22 were close to one another in the northwest area of Beijing near the common boundary between Beijing and Hebei Province. The possible exposed sites for case 16 and 19 were close to the capture site of the rodent host of strain Rn-SHY17 (Figure 1). Sequence HuBJ20 clustered with strains from Heilongjiang Province, which is more than 1,000 km from Beijing; it formed another lineage distinct from the two lineages mentioned above. In the patient’s record there was a note that the patient had occupationally been exposed to rodents in Tianjin City just 9 days before onset of the disease. (The distance between the supposed contact site and Beijing is about 140 to 160 km [Figure 1]). This suggests the case was infected outside of Beijing.

**DISCUSSION**

S segments were chosen to detect hantavirus RNA in patients and rodent hosts due to more S segment sequences available in the GenBank hantavirus database than that of the M or L genome segment.

Altogether 7 of 22 patients had virus RNA. SEOV strains detected from HFRS patients could be divided into 3 lineages, 2 of them circulating in Beijing. Among them, the first lineage included variants distributed in an area from northwest to southwest, most of which were near the center of Beijing and distinct from previous reported SEOV strains. The second lineage was represented by the variants circulating in northeast and northwest areas comparatively far away from the center of Beijing. The strains in the 2 lineages were geographically intercrossed in northeast areas because the *M. musculus* carrying strain Mm-SHY6 within the first lineage was captured at the same area with the Norway rat carrying strain Rn-SHY17 within the second lineage (Figure 1 and Figure 3). Notably, in recent years, the highest prevalence in human cases was reported in this area (personal communication, unpublished data). Thus further studies are needed to clarify if the high prevalence is related to complex status of hantavirus or rodents in this area. In addition, most strains within the second lineage diverged from all other SEOV strains on amino acid level. Five of six sequences in this lineage had a single amino acid substitution at position 260 of the N protein. Further studies that include more sequences are required to clarify if the amino acid substitution is the marker of the local strains.

Another purpose of the study was to compare nucleotide sequences of SEOV strains from humans and animal hosts to learn about changes that might accompany the host-switch of the virus. In the present study, comparison of the sequences of SEOV train (BjHD01) obtained from a Norway rat and a
HFRS patient (case 9) in the same place showed an identity of up to 99.7% at the nucleotide level with only 2 silent substitutions. The finding suggests a stabilizing selection operating on the protein level of SEOV. To our knowledge, this was the first finding of virtually the most similar viral sequences in a HFRS patient and natural rodent host although Norway rats have been known as SEOV carriers since the early 1980s in China.25 It was reported that cell-adapted variants of PUUV were represented mostly by variants with mutated noncoding regions for S segment.26 Unfortunately, the SEOV sequences recovered from clinical specimens were still short in length, and do not allow for more intensive comparative studies. However, the findings in the study indicate that genetic analysis is an essential tool for “case-investigations” and mapping of natural microfoci of SEOV.

Received April 3, 2007. Accepted for publication January 20, 2008.

Financial support: This research was supported by Natural Science Foundation of Beijing (70201004) and Natural Science Foundation of China (30725032, 30590374, 30771855).

Authors’ addresses: Shu-qing Zuo, Pan-he Zhang, Lin Zhan, Jia-fu Jiang, Xiao-ming Wu, Wen-juan Zhao, Fang Tang, Wu-chun Cao, Beijing Institute of Microbiology and Epidemiology, State Key Laboratory of Pathogen and Biosafety, Beijing 100071, China; Tel/Fax: (+86)10-63896082, E-mail: caowc@nic.bmi.ac.cn. Rì-min Wang, Jiang, Xiao-ming Wu, Wen-juan Zhao, Fang Tang, Wu-chun Cao, Beijing Institute of Microbiology and Epidemiology, State Key Laboratory of Pathogen and Biosafety, Beijing 100071, China; Tel/Fax: (+86)10-63896082, E-mail: caowc@nic.bmi.ac.cn. Ri-min Wang, Center for Disease Control and Prevention of Dongcheng District, Beijing 100030, China. Zhe Dun, Center for Disease Control and Prevention of Haidian District, Beijing 100027, China.

REFERENCES