

# Life-Threatening Sochi Virus Infections, Russia

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### Learning Objectives

#### Upon completion of this activity, participants will be able to:

- Analyze the demographics of patients infected with the Sochi virus in the current study
- Assess laboratory data available from patients infected with Sochi virus in the current study
- Distinguish the anatomic site of the highest concentration of Sochi virus among infected individuals
- Evaluate the prognosis of infection with Sochi virus.

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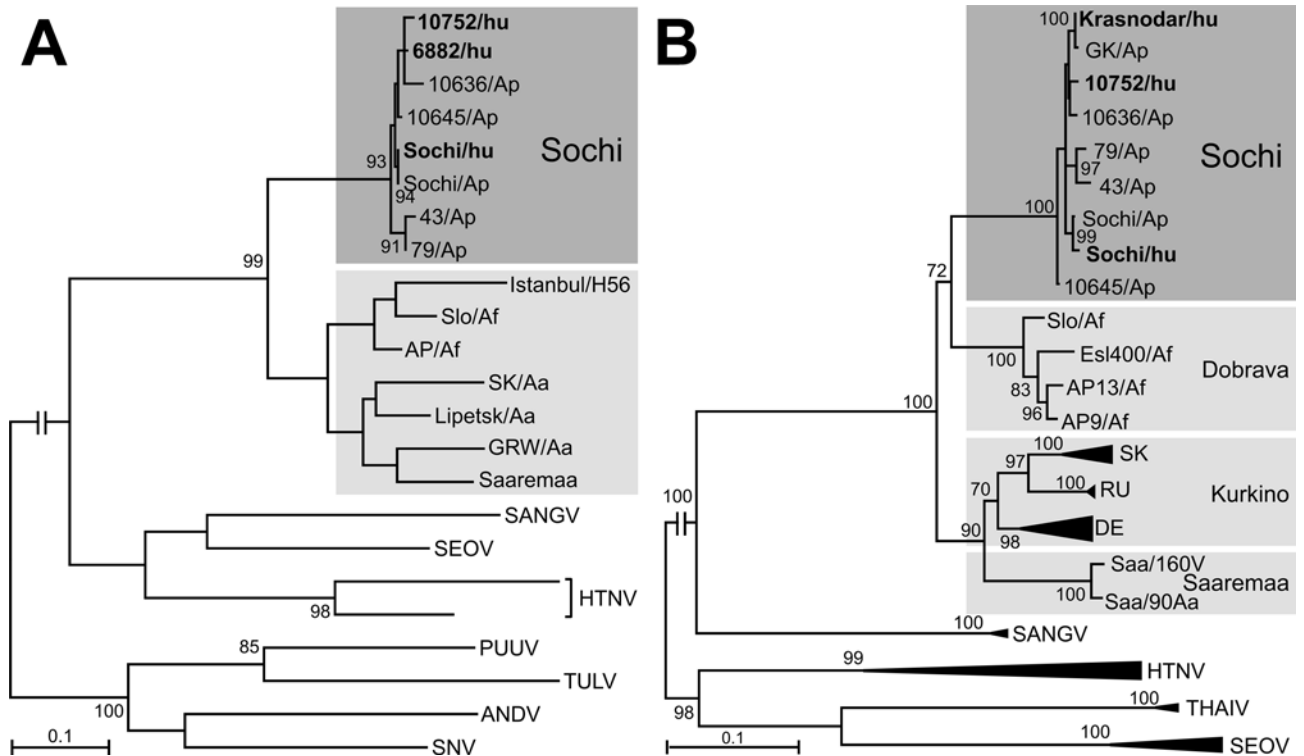
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Sochi virus was recently identified as a new hantavirus genotype carried by the Black Sea field mouse, *Apodemus ponticus*. We evaluated 62 patients in Russia with Sochi virus infection. Most clinical cases were severe, and the case-fatality rate was as high as 14.5%.

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**H**antaviruses are zoonotic pathogens transmitted from small animals to humans. Hantavirus disease in the Americas is called hantavirus pulmonary syndrome and in Asia and Europe is called hemorrhagic fever with renal syndrome (HFRS). Both syndromes can lead to cardio-pulmonary and renal failure (1). Recently we described a new hantavirus, Sochi virus, from the administrative region Krasnodar (including the city of Sochi), southern European Russia, which was isolated in cell culture from a Black Sea field mouse (*Apodemus ponticus*) and a



**Figure 1.** Phylogenetic analysis segment sequences of Sochi virus, Russia: A) 347-bp large (L) segment sequence; B) 1,197-bp small (S) segment sequence. Virus sequences derived from patients (shown in bold type) and *Apodemus ponticus* mice cluster within the Sochi genotype of DOBV. Evolutionary analysis was conducted in MEGA6 (6). The evolutionary history was inferred by using the maximum-likelihood method based on the Tamura 3-parameter model with a discrete gamma distribution and 5 rate categories (analysis in panel A) and on the general time reversible model with gamma rates and heterogeneous patterns (analysis in panel B), respectively, which were estimated to be the best-fit substitution model according to the Bayesian information criterion. Scale bars indicate an evolutionary distance of 0.1 substitutions per position in the sequence. Bootstrap values  $\geq 70\%$ , calculated from 500 replicates, are shown at the tree branches. GenBank accession numbers of all sequences used in the analysis are listed in online Technical Appendix Table 1 (<http://wwwnc.cdc.gov/EID/article/21/12/15-0891-Techapp1.pdf>). Dark gray shading indicates cluster of DOBV-Sochi strains; light gray shading indicates different clusters of strains from other DOBV genotypes. ANDV, Andes virus; DOBV, Dobrava-Belgrade virus; HTNV, Hantaan virus; PUUV, Puumala virus; SANGV, Sangassou virus; SEOV, Seoul virus; SNV, Sin Nombre virus; THAIV, Thailand virus; TULV, Tula virus.

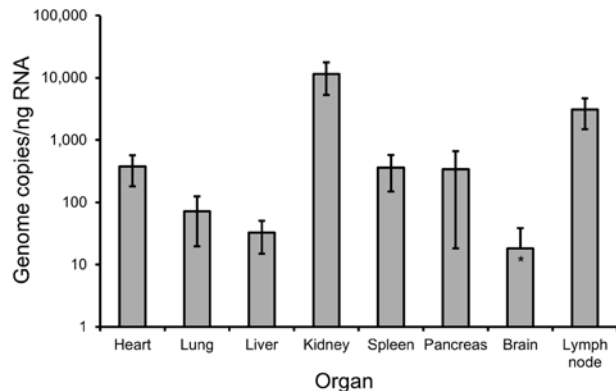
patient with fulminant hantavirus disease who died of shock and combined kidney and lung failure (2–4). Molecular taxonomical analyses identified Sochi virus as a new genotype within the Dobrava-Belgrade virus (DOBV) species (5). Here we show that HFRS caused by Sochi virus infection occurs in the geographic region where *A. ponticus* mice are prevalent. For 62 patients infected by this virus during 2000–2013, we evaluated clinical and epidemiologic data.

### The Study

Serum of patients with suspected acute hantavirus disease from the Krasnodar region were screened for hantavirus antibodies by indirect immunofluorescence assays and ELISA. Sixty-two patients showed clear DOBV IgG seropositivity. During the acute phase of illness, all

patients tested positive for DOBV IgM (data not shown). For 26 patients, sufficient volumes of follow-up serum were available for additional focus reduction neutralization assays to specify neutralizing antibodies. All serum samples exhibited substantially higher neutralizing titers toward DOBV than toward Puumala virus, Hantaan virus, and Seoul virus. When the neutralizing effect of DOBV-positive patients' serum were compared against the different human pathogenic genotypes of DOBV (Dobrava, Kurkino, and Sochi), all serum predominantly reacted with the Sochi genotype (online Technical Appendix Table 1, <http://wwwnc.cdc.gov/EID/article/21/12/15-0891-Techapp1.pdf>).

We successfully obtained virus genomic large (L) segment sequences from 2 patients (no. 51, specimen no. 6882; no. 59, specimen no. 10752). In the neighborhood



**Figure 2.** Quantification of hantavirus RNA in tissue biopsies from a 50-year-old Sochi virus-infected man (patient no. 59), Russia. Two independent approaches were performed to extract RNA from each organ. Quantitative reverse transcription PCR previously developed for DOBV (7) was used to measure virus load in the analyzed biopsy samples. Three quantitative reverse transcription PCR estimations were conducted for every RNA extraction, followed by calculation of mean values and SDs. Viral RNA levels are shown as genome copies per nanogram of total RNA isolated from the samples. Error bars indicate SD.

of the residence of patient no. 59, mice were trapped, and hantaviral L and small (S) segment regions from 2 *A. ponticus* animals (specimen nos. 10636, 10645) were amplified. The sequences obtained were deposited in GenBank under accession nos. KM192207–09 and KP878308–10 (L segment) and KP878311–13 (S segment) (online Technical Appendix Table 2). Samples from virus-positive mice were phylogenetically characterized by analysis of a 242-bp region of their *cytB* gene; all of them clustered with the previously identified *A. ponticus* animals (3) (data not shown). In addition, the *A. ponticus*-derived isolate Sochi/Ap (4), the patient-derived isolate Sochi/hu (5), an S segment sequence from a mouse (GK/Ap) trapped near the home of the previously described Krasnodar patient (4), and sequences originating from 2 *A. ponticus* mice sampled near the Black Sea coast, 43/Ap and 79/Ap, were included in the molecular analyses of the virus.

The patient-derived sequences 6882/hu, 10752/hu, and Sochi/hu clearly cluster with *A. ponticus*-derived sequences 43/Ap, 79/Ap, 10636/Ap, 10645/Ap, and Sochi/Ap

(Figure 1, panel A). In the analysis of the S segment, we obtained a very similar result; the patient-derived sequences 10752/hu, Krasnodar/hu, and Sochi/hu cluster with *A. ponticus*-associated sequences 43/Ap, 79/Ap, 10636/Ap, 10645/Ap, GK/Ap, and Sochi/Ap (Figure 1, panel B). In analysis of both L and S segments, the Sochi virus strains form a unique group, clearly distinguishable from all other DOBV genotypes.

Specimens from different organs of deceased patient no. 59 were analyzed for virus load. The highest concentration was detected in kidney (11,446 copies/ng RNA) and lymph node (3,086 copies/ng RNA), whereas the least virus RNA (10–100 copies/ng RNA) was detected in lung, brain, and liver (Figure 2).

The clinical disease severity of the 62 Sochi virus-infected patients investigated (Table 1) was subdivided into mild, moderate, or severe following the standard Russian criteria (i.e., length of febrile phase, minimal blood pressure in the hypotonic phase, extent of hemorrhagic symptoms, minimal urine production, serum creatinine level, and extent of proteinuria) (online Technical Appendix Table 3). The case-fatality rate (CFR) was as high as 14.5% (9/62 patients). Including fatalities, severe disease developed in nearly 60% of patients, whereas the remaining 40% of cases were moderate. The average age of all patients was 33 years. A significantly higher proportion of patients were males ( $p = 1.05 \times 10^{-9}$ ). Moreover, severe disease developed in most affected male patients (66.7%) but in only 35.7% of affected female patients ( $p = 0.037$ ). The fact that only 2 of 9 fatal cases occurred in female patients (Table 1) underscores this finding.

All 9 patients with fatal infections died of multiorgan failure and shock (Table 2). Postmortem examination showed multiple hemorrhages and edema in internal organs, including kidneys and lungs. The patients died within 8.2 days (range 3–16 days) after disease onset. An extraordinary fulminant course was observed for patient no. 47, who died 3 days after onset and before he could be hospitalized. This 19-year-old man was the son-in-law of patient no. 48, who also died after Sochi virus infection. Both men lived at the same rural address, and rodent contact during work in haystacks was reported.

**Table 1.** Comparisons in clinical outcome, age, and sex of 62 patients with Sochi virus infection, Russia\*

| Characteristic          | Total     |                       | Sex, no. (%)     |                  | Age, y, n/N (%)    |                     |
|-------------------------|-----------|-----------------------|------------------|------------------|--------------------|---------------------|
|                         | No. (%)   | Median age, y (range) | M, n = 48        | F, n = 14        | 7–15               | >15                 |
| No. patients            | 62 (100)  | 33.3 (7–57)           | <b>48 (77.4)</b> | <b>14 (22.6)</b> | <b>6/62 (9.7%)</b> | <b>56/62 (90.3)</b> |
| Outcome                 |           |                       |                  |                  |                    |                     |
| Died                    | 9 (14.5)  | 38.6 (19–53)          | 7 (14.6)         | 2 (14.3)         | 0/6                | 9/56 (16.1)         |
| Survived                | 53 (85.5) | 32.4 (7–57)           | 41 (85.4)        | 12 (85.7)        | 6/6 (100)          | 47/56 (83.9)        |
| Illness course          |           |                       |                  |                  |                    |                     |
| Severe, including fatal | 37 (59.7) | 33.1 (10–57)          | <b>32 (66.7)</b> | <b>5 (35.7)</b>  | 3/6 (50)           | 34/56 (60.7)        |
| Moderate, mild          | 25 (40.3) | 33.6 (7–57)           | <b>16 (33.3)</b> | <b>9 (64.3)</b>  | 3/6 (50)           | 22/56 (39.3)        |

\*Bold type indicates statistically significant differences between sex or age groups. Comparison of binomial population proportions analysis as implemented in Statlets (NWP Associates, Inc., <http://www.mrs.umn.edu/~sungurea/statlets/statlets.htm>) indicates rejection of the null hypothesis (claiming that the 2 proportions are equal) at significance level of  $p < 0.05$ .

**Table 2.** Characteristics of 9 deceased patients with Sochi virus infection, Russia\*

| Patient no. | Age, y/sex | Hospitalized, no. d after onset | GI symptoms | Max serum creatinine, $\mu\text{mol/L}\dagger$ | Min platelet count, $\times 10^9/\text{L}\ddagger$ | Died, no. d after onset | Clinical and postmortem findings   |
|-------------|------------|---------------------------------|-------------|--|--|-------------------------|--|
| 23          | 33/M       | 5                               | No          | 148  | 70   | 8                       | Pneumonia; renal, cardiovascular, multiorgan failure; multiple internal hemorrhages, edema                             |
| 29          | 29/M       | Same day                        | Yes         | 282  | 115  | 6                       | Renal, cardiovascular, multiorgan failure; multiple internal hemorrhages, edema  |
| 30          | 47/F       | 5                               | Yes         | 391  | 38   | 12                      | Renal, lung failure; shock; coagulation disturbance; hemorrhagic gastroenteritis; multiple internal hemorrhages, edema |
| 34          | 53/M       | 3                               | Yes         | 250  | 110  | 10                      | Multiorgan failure; coagulation disturbances; multiple internal hemorrhages  |
| 42          | 30/M       | 14                              | Yes         | 186  | 67   | 16                      | Uremic coma; multiorgan failure; multiple internal hemorrhages   |
| 47§         | 41/M       | Died before hospitalization     | Yes         | NR   | NR   | 3                       | Renal failure; multiple internal hemorrhages, edema  |
| 48§         | 19/M       | 4                               | Yes         | 192  | 54   | 6                       | Renal, cardiovascular failure; RDS, DIC syndrome; bleedings in pituitary, adrenal gland, intestine, etc.               |
| 56          | 35/F       | 4                               | Yes         | 410  | 49   | 6                       | Cardiovascular, renal, lung, liver failure; renal tubular necrosis; lung, brain edema                                  |
| 59          | 50/M       | 5                               | Yes         | 310  | 3  | 7                       | Renal, cardiovascular failure; RDS; multiple internal hemorrhages; pleurorrhea; lung, brain edema                      |

\*DIC, disseminated intravascular coagulation; GI, gastrointestinal; max, maximum; min, minimum; RDS, respiratory distress syndrome; NR, not reported.

†Reference range  $<96 \mu\text{mol/L}$  for female patients,  $<110 \mu\text{mol/L}$  for male patients.

‡Reference range  $150\text{--}400 \times 10^9/\text{L}$

§Patient no. 47 was the father-in-law of patient no. 48; both lived in the same rural residence.

## Conclusions

We have demonstrated the occurrence of human infections by Sochi virus and studied the clinical outcome for 62 patients. This virus is carried by the Black Sea field mouse (*A. ponticus*), which occurs naturally in the Transcaucasian region between the Black and Caspian Seas, including a part of southern European Russia. In anecdotal field studies in the coast region near Sochi, *A. ponticus* was the most abundant mouse species (71% of all trapped mice were identified as *A. ponticus*); moreover, 14% of trapped *A. ponticus* mice were serologically proven to be DOBV infected (8). This finding indicates that DOBV is the hantavirus indigenous in this geographic area and that *A. ponticus* mice are highly relevant as a hantavirus reservoir. All evidence from the natural virus reservoir, as well as serologic and molecular diagnostics of patients' serum, shows that the virus responsible for the infections is the DOBV genotype Sochi.

Most investigated patients found to be infected by Sochi virus exhibited a severe clinical course. With a calculated CFR of 14.5%, Sochi virus might be the most deadly hantavirus outside the Americas, where 35%–50% of hantavirus infections are fatal (1,9). Even Asian Hantaan virus is estimated to be less deadly; recent studies show CFRs of 1%–3% in China and South Korea, where Hantaan virus infections play an important role in HFRS morbidity (10,11). On the other hand, increased awareness in

diagnostics, treatment, and prevention by local physicians and public health authorities is expected to improve survival rates for Sochi virus infections.

Among the related viruses of the DOBV species, Sochi virus seems to have the highest level of virulence, similar to Dobrava virus (carried by *A. flavicollis* mice), which has a CFR of up to 10%–12% (12,13). As shown in larger studies, disease caused by infection with the related Kurkino genotype (carried by the western lineage of *A. agrarius* mice) is associated with a CFR of only 0.3%–0.9% (3,14). These phylogenetically related viruses exert a quite different pathogenicity in humans.

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Dr. Kruger is the head of the Institute of Medical Virology, Charité–University Medicine Berlin. His research focuses on the molecular epidemiology and clinical relevance of emerging virus infections.

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# Outbreak of a New Strain of Flu at a Fair



Dr. Karen Wong, an EIS officer with the Centers for Disease Control and Prevention, discusses her study about flu outbreaks at agricultural fairs.



<http://www2c.cdc.gov/podcasts/player.asp?f=8627464>

# Life-Threatening Sochi Virus Infections, Russia

## Technical Appendix

**Technical Appendix Table 1.** Details of serodiagnostic data (reciprocal antibody titers) of the 62 patients\*

| Patient no. | No. serum samples | Sampling day after disease onset | IFA  |       |       |      | ELISA |      |       |       | FRNT |       |         |         |      |      |
|-------------|-------------------|----------------------------------|------|-------|-------|------|-------|------|-------|-------|------|-------|---------|---------|------|------|
|             |                   |                                  | PUUV | DOBV  | HTNV  | SEOV | PUUV  |      | DOBV  |       | PUUV | DOBV  |         |         | HTNV | SEOV |
|             |                   |                                  |      |       |       |      | IgM   | IgG  | IgM   | IgG   |      | Sochi | Kurkino | Dobrava |      |      |
| 1           | 1,293             | 27                               | <16  | 1024  | 1024  | 512  | <128  | <128 | 16384 | 32768 | <40  | 1280  | 640     | 640     |      |      |
|             | 1312              | 104                              | <16  | 2048  | 512   | 512  | <128  | <128 | 2048  | 8192  | <40  | 1280  | 160     | 160     | 160  | <80  |
| 2           | 1308              | 22                               | <16  | 2048  | 1024  | 1024 | 256   | <128 | 4096  | 4096  | <40  | 640   | 80      |         | 160  | 80   |
|             | 1310              | 50                               | <16  | 8192  | 4096  | 4096 |       |      |       |       | <40  | 1280  | 640     | 640     | 160  | 160  |
|             | 4708†             | 5 y 9 m                          | 64   | 2048  | 2048  | 2048 |       |      |       |       | <40  | 2560  | 1280    | 2560    | 80   | 40   |
| 3           | 1307              | 15                               | <16  | 4096  | 2048  | 2048 | <128  | <128 | 8196  | 8196  | <40  | 640   | 320     | 320     | 160  | 160  |
| 4           | 1335              | 17                               | 64   | 8192  | 4096  | 4096 | 512   | 512  | 8192  | 16384 | <40  | 1280  | <80     | 80      |      |      |
|             | 1336              | 82                               | <32  | 4096  | 2048  | 1024 | 1024  | 512  | 4096  | 32768 | <40  | 640   | 320     | 640     | <80  | 160  |
|             | 4,709†            | 5 y 9 m                          | 64   | 4096  | 4096  | 2048 |       |      |       |       | <40  | 5120  | 1280    | 2560    | 160  | 80   |
| 5           | 1339              | 11                               | <32  | 4096  | 2048  | 2048 | 256   | <128 | 8192  | 8192  | <40  | 320   | 80      |         |      |      |
| 6           | 1642              | 3                                | <16  | 2048  | 1024  | 512  | <128  | <128 | 8196  | 2048  |      |       |         |         |      |      |
|             | 4716†             | 5 y 3 m                          | 128  | 4096  | 4096  | 4096 |       |      |       |       | <40  | 5120  | 1280    | 2560    | 160  | 160  |
| 7           | 1686              | 16                               | <16  | 2048  | 1024  | 512  | <128  | <128 | 2048  | 2048  | <40  | 640   | 80      | 80      |      |      |
| 8           | 4712              | 9                                | <16  | 512   | 256   | 256  | <128  | <128 | 2048  | 512   |      |       |         |         |      |      |
|             | 3507              | 15                               | 64   | 4096  | 2048  | 2048 | <128  | <128 | 8196  | 8196  | <40  | 640   | 160     | 320     |      |      |
| 9           | 4713†             | 1 y 5 m                          | 1024 | 8192  | 4095  | 4096 |       |      |       |       | <40  | 20480 | 5120    | 10240   | 320  | 160  |
|             | 3830              | 13                               | 128  | 8192  | 2048  | 2048 | 512   | 256  | 16384 | 8192  | <40  | 640   | 160     | 640     |      |      |
| 10          | 4714†             | 1 y 5 m                          | 512  | 16384 | 16384 | 8192 |       |      |       |       | <40  | 20480 | 5120    | 5120    | 80   | 40   |
|             | 3692              | 30                               | 64   | 4096  | 4096  | 2048 | 256   | 256  | 4096  | 16384 | <40  | 1280  | 160     | <160    |      |      |
| 12          | 3693              | 10                               | 32   | 2048  | 1024  | 1024 | <128  | <128 | 16384 | 8192  |      |       |         |         |      |      |
|             | 3694              | 18                               | 64   | 2048  | 2048  | 2048 |       |      |       |       | <40  | 640   | <80     | 80      |      |      |
| 13          | 3496              | 9                                | <16  | 4096  | 4096  | 2048 | <128  | <128 | 16384 | 16384 |      |       |         |         |      |      |
| 14          | 3802              | 11                               | 64   | 4096  | 2048  | 2048 | <128  | <128 | 8192  | 2048  | <40  | 1280  | 320     | 640     |      |      |
|             | 4711†             | 1 y 6 m                          | 512  | 8192  | 8192  | 4096 |       |      |       |       | <40  | 20480 | 20480   | 20480   | 160  | 80   |
| 15          | 3778              | 10                               | 64   | 8192  | 4096  | 4096 | <128  | <128 | 65536 | 8192  |      |       |         |         |      |      |
| 16          | 3824              | 11                               | 64   | 8192  | 8192  | 4096 | <128  | <128 | 16384 | 16384 |      |       |         |         |      |      |
| 17          | 3813              | 4                                | 64   | 8192  | 2048  | 2048 | <128  | <128 | 16384 | 16384 |      |       |         |         |      |      |
|             | 4710†             | 1 y 5 m                          | 64   | 2048  | 2048  | 2048 |       |      |       |       | <40  | 10240 | 5120    | 5120    |      |      |
| 18          | 3861              | 16                               | 256  | 8192  | 4096  | 4096 | <128  | <128 | 8192  | 2048  | <40  | 640   | 640     | 640     |      |      |

| Patient no. | No. serum samples | Sampling day after disease onset | IFA        |              |              |              | ELISA |      |       |       | FRNT |       |         |         |      |      |
|-------------|-------------------|----------------------------------|------------|--------------|--------------|--------------|-------|------|-------|-------|------|-------|---------|---------|------|------|
|             |                   |                                  | PUUV       | DOBV         | HTNV         | SEOV         | PUUV  |      | DOBV  |       | PUUV | DOBV  |         |         | HTNV | SEOV |
|             |                   |                                  |            |              |              |              | IgM   | IgG  | IgM   | IgG   |      | Sochi | Kurkino | Dobrava |      |      |
| 19          | 4715†<br>3855     | 1 y 4 m<br>9                     | 128<br><16 | 8192<br>4096 | 8192<br>2048 | 4096<br>2048 | <128  | <128 | 16384 | 2048  | <40  | 5120  | 2560    | 5120    | 80   | 40   |
| 20          | 4387              | 8                                | <16        | 512          | 256          | 256          | <128  | <128 | 1024  | 1024  |      |       |         |         |      |      |
| 21          | 4471              | 2                                | <16        | 512          | 512          | 256          | <128  | <128 | 4096  | 1024  |      |       |         |         |      |      |
| 22          | 4705              | ?                                | 128        | 2048         | 2048         | 2048         | 128   | <128 | 4096  | 4096  | <40  | 640   | 640     | 640     |      |      |
| 23          | 5049              | 7                                | <16        | 1024         | 512          | 512          | <128  | <128 | 4096  | 1024  |      |       |         |         |      |      |
| 24          | 5065              | 8                                | <16        | 2048         | 2048         | 2048         | <128  | <128 | 8192  | 8192  |      |       |         |         |      |      |
| 25          | 5066              | 8                                | <16        | 2048         | 1024         | 512          | <128  | <128 | 4096  | 8192  |      |       |         |         |      |      |
| 26          | 5067              | 8                                | <16        | 1024         | 512          | 512          | <128  | <128 | 4096  | 1024  |      |       |         |         |      |      |
| 27          | 5293              | 12                               | <16        | 4096         | 2048         | 2048         | <128  | <128 | 8192  | 8192  |      |       |         |         |      |      |
| 28          | 5312              | 5                                | <16        | 1024         | 1024         | 1024         | <128  | <128 | 4096  | 1024  |      |       |         |         |      |      |
| 29          | 6624              | 4                                | <16        | 4096         | 2048         | 2048         | <128  | <128 | 16384 | 4096  |      |       |         |         |      |      |
| 30          | 6625              | 6                                | 64         | 2048         | 2048         | 2048         | <128  | <128 | 4096  | 1024  |      |       |         |         |      |      |
| 31          | 6627              | 7                                | 64         | 4096         | 1024         | 1024         | <128  | <128 | 8192  | 8192  |      |       |         |         |      |      |
|             | 6627-2            | 73                               | 64         | 4096         | 512          | 512          |       |      |       |       | <40  | 2560  | 320     | 640     | 160  | 80   |
| 32          | 6623              | 13                               | 32         | 4096         | 4096         | 2048         | 128   | 128  | 16384 | 8192  |      |       |         |         |      |      |
| 33          | 6626-2            | 108                              | 256        | 8192         | 2048         | 2048         |       |      |       |       | <40  | 10240 | 1280    | 2560    | 160  | 160  |
| 34          | 6930              | 5                                | 256        | 4096         | 2048         | 2048         | <128  | <128 | 8192  | 8192  |      |       |         |         |      |      |
| 35          | 6845              | 4                                | <16        | 1024         | 512          | 256          | <128  | <128 | 8192  | 4096  |      |       |         |         |      |      |
|             | 6845-a            | 29                               | <32        | 1024         | 512          | 512          |       |      |       |       | <40  | 1280  | 160     | 320     | <40  | <40  |
|             | 6855-2            | 11 m                             | 512        | 4096         | 1024         | 1024         |       |      |       |       | <40  | 2560  | 640     | 640     | 80   | 80   |
| 36          | 6931              | 7                                | 64         | 2048         | 512          | 512          | <128  | <128 | 4096  | 8192  |      |       |         |         |      |      |
| 37          | 6918              | 3                                | <32        | 2048         | 1024         | 512          | <128  | <128 | 8192  | 16384 |      |       |         |         |      |      |
|             | 6918a             | 27                               | <32        | 2048         | 1024         | 512          |       |      |       |       | <40  | 2560  | 320     | 1280    | 40   | <40  |
|             | 6931-2            | 1y                               | 512        | 4096         | 1024         | 1024         |       |      |       |       | <40  | 5120  | 1280    | 2560    | 160  | 160  |
| 38          | 4481              | 2 m                              | 256        | 8192         | 8192         | 4096         | <128  | <128 | 2048  | 16384 |      | 2560  | 640     | 640     | 160  | 160  |
| 40          | 4482              | 9                                | 512        | 8192         | 4096         | 2048         | <128  | <128 | 16384 | 16384 | <40  | 2560  | 2560    | 2560    | <40  | <40  |
| 41          | 4483              | 9                                | 1024       | 16384        | 8192         | 8192         | 512   | 256  | 16384 | 16384 |      |       |         |         |      |      |
| 42          | 4484              | 15                               | 64         | 8192         | 2048         | 2048         | <128  | <128 | 16384 | 32768 | <40  | 320   | 80      | 80      | <40  | <40  |
| 43          | 4485              | 9                                | 128        | 8192         | 4096         | 4096         | 256   | 256  | 32768 | 32768 |      |       |         |         |      |      |
| 44          |                   | 12                               | 512        | 8192         | 4096         | 4096         | 512   | 512  | 16384 | 16384 |      |       |         |         |      |      |
| 45          | 6876              | 6                                | <16        | 4096         | 4096         | 4096         | <128  | <128 | 16384 | 8192  |      |       |         |         |      |      |
| 46          | 6878              | 11                               | 1024       | 32000        | 4096         | 4096         | 512   | 256  | 32768 | 32768 |      |       |         |         |      |      |
|             | 6878-a            | 33                               | 64         | 8192         | 4096         | 1024         |       |      |       |       | <40  | 5120  | 640     | 2560    | 160  | 80   |
| 47          | 6880              | 3                                | <16        | 4096         | 2048         | 2048         | <128  | <128 | 4096  | 1024  |      |       |         |         |      |      |
| 48          | 6929              | 4                                | <16        | 4096         | 4096         | 4096         | <128  | <128 | 16384 | 1024  |      |       |         |         |      |      |
| 49          | 6628              | 19                               | <32        | 2048         | 1024         | 1024         | <128  | <128 | 4096  | 8192  |      |       |         |         |      |      |
|             | 6875              | 26                               | <32        | 1024         | 512          | 512          |       |      |       |       | <40  | 2560  | 640     | 640     | 160  | 160  |
| 51          | 6882              | 17                               | 64         | 4096         | 2048         | 2048         | 256   | <128 | 8192  | 8192  |      |       |         |         |      |      |

| Patient no. | No. serum samples | Sampling day after disease onset | IFA  |      |      |      | ELISA |      |       |       | FRNT |       |         |         |      |      |     |
|-------------|-------------------|----------------------------------|------|------|------|------|-------|------|-------|-------|------|-------|---------|---------|------|------|-----|
|             |                   |                                  | PUUV | DOBV | HTNV | SEOV | PUUV  |      | DOBV  |       | PUUV | DOBV  |         |         | HTNV | SEOV |     |
|             |                   |                                  |      |      |      |      | IgM   | IgG  | IgM   | IgG   |      | Sochi | Kurkino | Dobrava |      |      |     |
|             | 6882-a            | 34                               | 64   | 4096 | 2048 | 1024 |       |      |       |       |      | <40   | 1280    | 320     | 640  | 40   | <40 |
| 52          | 6883              | 13                               | 256  | 2048 | 1024 | 1024 | <128  | <128 | 8192  | 8192  |      |       |         |         |      |      |     |
|             | 6883-2            | 10 m                             | 256  | 4096 | 2048 | 512  |       |      |       |       | <40  | 2560  | 80      | 160     | 40   | 40   |     |
| 53          | 7105              | 10                               | 256  | 4096 | 4096 | 2048 | 256   | 128  | 32768 | 32768 |      |       |         |         |      |      |     |
| 54          | 7110              | 9                                | 64   | 1024 | 1024 | 1024 | <128  | <128 | 2048  | 4096  |      |       |         |         |      |      |     |
| 55          | 7111              | 5                                | 64   | 1024 | 1024 | 512  | <128  | <128 | 8192  | 4096  |      |       |         |         |      |      |     |
| 56          | 8385              | 4                                | <16  | 1024 | 512  | 512  | <128  | <128 | 8192  | 2048  |      |       |         |         |      |      |     |
| 57          | 8386              | 8                                | 64   | 1024 | 1024 | 512  | 256   | 128  | 4096  | 8192  |      |       |         |         |      |      |     |
| 58          | 8380              | 6                                | 32   | 2048 | 1024 | 1024 | <128  | <128 | 8192  | 8192  |      |       |         |         |      |      |     |
| 59          | 8381              | 5                                | <16  | 128  | 64   | 64   | <128  | <128 | 1024  | 128   |      |       |         |         |      |      |     |
| 60          | 8382              | 9                                | 32   | 4096 | 4096 | 1024 | <128  | <128 | 32768 | 16384 |      |       |         |         |      |      |     |
| 61          | 8383              | 16                               | <16  | 4096 | 4096 | 2048 | <128  | <128 | 16384 | 16384 |      |       |         |         |      |      |     |
| 62          | 8384              | 24                               | 64   | 4096 | 4096 | 2048 | 256   | 256  | 8192  | 32768 |      |       |         |         |      |      |     |
| 63          | 8388              | 5                                | <16  | 4096 | 2048 | 2048 | <128  | <128 | 8192  | 8192  |      |       |         |         |      |      |     |

\*Patient serum samples were screened for hantavirus antibodies by indirect IFAs by using spot-slides containing a mixture of Vero-E6 cells infected by PUUV, DOBV, HTNV, and SEOV, as previously described (Dzagurova et al., Zh Mikrobiol Epidemiol Immunobiol, 2008; No.1: 12-16). Serum found to be positive was confirmed and further typed on IFA with "monovalent" spot-slides containing cells infected by only 1 of the mentioned viruses. Thereafter, IgG and IgM were determined by ELISAs on the basis of PUUV and DOBV antigens by established methods (Meisel et al., Clin Vaccine Immunol, 2006;13: 1349-57). For further serotyping, the neutralizing activity of serum was investigated by FRNT (Dzagurova et al., Zh Mikrobiol Epidemiol Immunobiol, 2008; No.1: 12-16), using Vero-E6 cells to propagate the following virus stocks; PUUV strain K-27/Ufa-85, DOBV (genotype Sochi) strain Ap1584/Sochi-01 (named Sochi/Ap), DOBV (genotype Kurkino) strain Aa1854/Lipetsk-02, DOBV (genotype Dobrava) strain Bel-1, HTNV strain P-88/ Khabarovsk-89, SEOV strain SR-11. DOBV, Dobrava-Belgrade virus; FRNT, focus-reduction neutralization assay; HTNV, Hantaan virus; IFA, immunofluorescence assay; PUUV, Puumala virus; SEOV, Seoul virus. Blank cells indicate testing not done.

†FRNT was performed with addition of 10% guinea pig blood serum (as complement).



**Technical Appendix Table 2.** Virus sequences and corresponding GenBank accession numbers used in the phylogenetic analyses

| Virus species          | Abbreviation | Strain name    | GenBank accession no. |                 |
|------------------------|--------------|----------------|-----------------------|-----------------|
|                        |              |                | S segment             | L segment       |
| Dobrava-Belgrade virus | DOBV         | 10636/Ap       | <b>KP878311</b>       | <b>KP878308</b> |
|                        |              | 10645/Ap       | <b>KP878312</b>       | <b>KP878309</b> |
|                        |              | 10752/hu       | <b>KP878313</b>       | <b>KP878310</b> |
|                        |              | 6882/hu        | –                     | <b>KM192207</b> |
|                        |              | 43/Ap          | JF920151              | <b>KM192209</b> |
|                        |              | 79/Ap          | JF920152              | <b>KM192208</b> |
|                        |              | Sochi/hu       | JF920150              | JF920148        |
|                        |              | Sochi/Ap       | EU188449              | EU188451        |
|                        |              | GK/Ap          | AF442622              | –               |
|                        |              | Krasnodar/hu   | AF442623              | –               |
|                        |              | SK/Aa          | AY961615              | GU904039        |
|                        |              | Esl29/Aa       | AY533118              | –               |
|                        |              | Esl81/Aa       | AY533120              | –               |
|                        |              | Kurk44/Aa      | AJ131672              | –               |
|                        |              | Kurk53/Aa      | AJ131673              | –               |
|                        |              | Esl856/Aa      | AJ269549              | –               |
|                        |              | Esl862/Aa      | AJ269550              | –               |
|                        |              | Lipetsk/Aa     | EU188452              | EU188454        |
|                        |              | GER/08/118/Aa  | GQ205407              | –               |
|                        |              | GER/08/131/Af  | GQ205408              | –               |
|                        |              | GER/07/293/Aa  | GQ205401              | –               |
|                        |              | GER/07/607/Af  | GQ205402              | –               |
|                        |              | GER/07/1064/Aa | GQ205404              | –               |
|                        |              | GER/05/239/Aa  | GQ205405              | –               |
|                        |              | GER/05/477/Af  | GQ205406              | –               |
|                        |              | Esl400/Af      | AY168576              | –               |
|                        |              | AP9/Af         | AJ410615              | AJ410617        |
|                        |              | AP13/Af        | AJ410619              | –               |
| Slo/Af                 | L41916       | AJ009779       |                       |                 |
| Istanbul/H56           | –            | KF039740       |                       |                 |
| Saa/160V               | AJ009773     | AJ410618       |                       |                 |
| Saa/90Aa               | AJ009775     | –              |                       |                 |
| Sangassou virus        | SANGV        | SA14           | JQ082300              | JQ082302        |
|                        |              | SA22           | JQ082303              | –               |
| Hantaan virus          | HTNV         | 76–118         | M14626                | NC_005222       |
|                        |              | SC-2           | –                     | AY675354        |
|                        |              | Z10            | AF184987              | –               |
|                        |              | LR1            | AF288294              | –               |
|                        |              | AH09           | AF285264              | –               |
|                        |              | 84FLi          | AY017064              | –               |
| Thailand virus         | THAIV        | 741            | AB186420              | –               |
|                        |              | NR/Bi0017      | AM397664              | –               |
| Seoul virus            | SEOV         | 80–39          | NC_005236             | NC_005238       |
|                        |              | Gou3           | AF184988              | –               |
|                        |              | Z37            | AF187082              | –               |
|                        |              | L99            | AF488708              | –               |
| Puumala virus          | PUUV         | CG1820         | –                     | M63194          |
| Tula virus             | TULV         | Mo5302         | –                     | NC_005226       |
| Andes virus            | ANDV         | Chile-9717869  | –                     | NC_003468       |
| Sin Nombre virus       | SNV          | NM H10         | –                     | NC_005217       |

\*The nucleotide sequences generated in this study were based on PCRs amplifying a 390-nt conserved region within the L segment (Klempa et al. Emerg Infect Dis. 2006;12:838–40) or a 599-nt fragment of the S segment (Klempa et al., J Clin Microbiol, 2005;43:2756–63). Bold type indicates new sequences. Dashes indicate that no sequence information was available.

**Technical Appendix Table 3.** Classification criteria of clinical severity of hemorrhagic fever with renal syndrome\*

| Symptoms                          | Clinical course                   |                                 |                             |
|-----------------------------------|-----------------------------------|---------------------------------|-----------------------------|
|                                   | Mild                              | Moderate                        | Severe                      |
| Duration of fever, d              | 3–4                               | 5–6                             | >6                          |
| Systolic blood pressure, mm Hg    | 100                               | <100                            | <80                         |
| Hemorrhagic syndrome              | Scleral, subcutaneous hemorrhages | Bleedings, not life threatening | Bleedings, life threatening |
| Duration of oliguria <500 mL/24 h | 24–48 h                           | 49–96 h                         | >96 h                       |
| Anuria >50 mL/24 h                | –                                 | –                               | Positive                    |
| Serum creatinine, μmol/L          | Normal (<96/F, <110/M)            | 120–250                         | >250                        |
| Proteinuria, g/L                  | <1                                | <5                              | >5                          |
| Pulmonary edema                   | –                                 | –                               | Positive                    |
| Cerebral edema                    | –                                 | –                               | Positive                    |
| Kidney rupture                    | –                                 | –                               | Positive                    |

\*Classification according to Russian standard criteria (Leshchinskaia et al., Vopr Virusol. 1990;35:42–5; Klempa et al., Emerg Infect Dis.2008;14:617–25). Dashes indicate that these symptoms were not observed.