INFECTIOUS DISEASES

FATAL CASE OF HEMORRHAGIC FEVER WITH RENAL SYNDROME ASSOCIATED WITH HANTAVIRUS SEOUL

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RESUME

Seoul orthohantavirus is one of the causative agents of hemorrhagic fever with renal syndrome (HFRS) in the Russian Far East. Foci of SEOV in Norway rats have been identified in the cities of Vladivostok and Khabarovsk.

The aim. *Study of a clinical case of HFRS with a fatal outcome, from Komsomolsk-on-Amur in 2025.*

Materials and methods. *Samples of blood serum and autopsy material (blood, lung, spleen, liver, kidney tissues) from a resident of Komsomolsk-on-Amur with a clinical diagnosis of HFRS were examined using enzyme-linked immunosorbent assay (ELISA) and reverse transcription-polymerase chain reaction (RT-PCR) followed by sequencing.*

Results. *The laboratory studies confirmed the clinical diagnosis. Antibodies to hantaviruses in a titer of 1:3200 (IgM) and 1:1600 (IgG) were detected by the ELISA in the blood serum. SEOV RNA was detected in blood serum and autopsy material (blood, lung, spleen, liver, kidney tissues) studying by the RT-PCR and using the OM-Screen-GLPS-RV test system. Analysis of genome fragments showed high homology of SEOV from Komsomolsk-on-Amur with virus strains previously identified in Khabarovsk, as well as those widespread in China. Genetic evidence of human infection, associated with the Seoul virus circulating in the city of Komsomolsk-on-Amur has been obtained, and two hypotheses have been put forward for the formation of the focus: importation with infected rats from China or Khabarovsk, and the possible existence of a mainland variant of the virus in a vast territory, including the eastern regions of China and Khabarovsk Krai.*

Conclusion. *Third urban focus of Seoul hantavirus was detected in Russia. The fatal outcome of HFRS could be caused by a comorbidity: chronic alcohol intoxication with multiple organ manifestations.*

Keywords: *hantavirus, Seoul virus, HFRS, Russia*

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ЛЕТАЛЬНЫЙ СЛУЧАЙ ГЕМОРРАГИЧЕСКОЙ ЛИХОРАДКИ С ПОЧЕЧНЫМ СИНДРОМОМ, ОБУСЛОВЛЕННЫЙ ЗАРАЖЕНИЕМ ХАНТАВИРУСОМ СЕУЛ

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РЕЗЮМЕ

Ортохантавирус Сеул (SEOV) является одним из возбудителей геморрагической лихорадки с почечным синдромом (ГЛПС) на Дальнем Востоке России. Очаги циркуляции SEOV в серых крысах выявлены в городах Владивосток и Хабаровск.

Цель работы. *Исследование клинического случая ГЛПС с летальным исходом, выявленного в г. Комсомольске-на-Амуре в 2025 году.*

Материалы и методы. *Образцы сыворотки крови и аутопсийного материала (кровь, ткани легких, селезенки, печени, почек) от жительницы г. Комсомольска-на-Амуре с предварительным диагнозом «ГЛПС, завершившаяся летальным исходом», были исследованы методами иммуноферментного анализа (ИФА) и обратной транскрипции – полимеразной цепной реакции (ОТ-ПЦР) с последующим секвенированием.*

Результаты. *Проведенные исследования подтвердили клинический диагноз. Методом ИФА в сыворотке крови обнаружены антитела к хантавирусам в титре 1:3200 (IgM) и 1:1600 (IgG). При исследовании методом ОТ-ПЦР с использованием тест-системы «ОМ-Скрин-ГЛПС-РВ» в сыворотке крови и аутопсийном материале (крови, тканях легких, селезенки, печени, почек) выявлена РНК SEOV. Анализ фрагментов генома показал высокую гомологию SEOV из Комсомольска-на-Амуре с вариантами вируса, выявленными ранее в Хабаровске, а также широко распространенными в Китае. Впервые получено генетическое доказательство инфицирования человека вирусом Сеул, циркулирующим в г. Комсомольске-на-Амуре, и выдвинуты две гипотезы формирования очага: завоз с инфицированными крысами из Китая либо Хабаровска и возможное существование материкового варианта вируса на обширной территории, включающей восточные районы Китая и Хабаровский край.*

Заключение. *В РФ выявлен третий городской очаг хантавируса Сеул. Летальный исход ГЛПС мог быть обусловлен фоновым заболеванием: хронической алкогольной интоксикацией с полиорганными проявлениями.*

Ключевые слова: *хантавирус, вирус Сеул, ГЛПС, Россия*

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INTRODUCTION

Hemorrhagic fever with renal syndrome (HFRS) is a potentially life-threatening infectious disease of humans that has been reported in various regions of Russia [1]. In the Far Eastern region, HFRS is caused by the Hantaan (HTNV), Amur (AMRV), and Seoul (SEOV) orthohantaviruses, which circulate among the stripped field mouse (*Apodemus agrarius*), Korean field mouse (*Apodemus peninsulae*), and Norway rat (*Rattus norvegicus*), respectively [2, 3]. Infection with HTNV and AMRV viruses can occur when humans visit natural foci, while SEOV forms urban foci of infection [4]. Global trade and human migration have contributed to the spread of the SEOV worldwide, including the emergence of two foci in the Far East [4-6]. Based on the genetic similarity between the SEOV variants from patients and Norway rats in these outbreaks and previously published data, it can be assumed that the virus was introduced to the port city of Vladivostok through infected rats on ships originating from Southeast Asian countries (Cambodia, Vietnam and Singapore). The outbreak in the city of Khabarovsk and the neighboring village of Priamursky, is associated with another variant of the SEOV, which is linked to cargo transportation from China [4, 6].

HFRS is characterized by a variety of clinical manifestations, ranging from mild febrile forms to more severe forms with systemic damage of small blood vessels, hemorrhagic diathesis, hemodynamic abnormalities, renal failure, and in severe cases, multi-organ failure [7]. HFRS variants associated with SEOV tend to be mild to moderate in severity, although severe clinical forms of the disease have been identified in 5.7 % of cases in Vladivostok [6, 8, 9].

The aim of this study was to analyze a fatal case of HFSR that occurred in Komsomolsk-on-Amur.

MATERIALS AND METHODS

The clinical diagnosis of HFRS was confirmed based on data from a laboratory study of the patient's blood serum using enzyme-linked immunosorbent assay (ELISA) with the VectoHanta-IgG and VectoHanta-IgM reagent kits (Vector-Best, Russia) and reverse transcription polymerase chain reaction (RT-PCR) with the OM-Screen-HFRS-RV reagent kit (Synthol, Russia). In addition, autopsy samples (blood, lung tissue, spleen, liver, and kidneys) were analyzed using RT-PCR with the same kit to further confirm the diagnosis.

RNA was isolated from blood samples using the RIBOprep kit (Central Research Institute of Epidemiology, Russia). Viral cDNA was synthesized using the M-MuLV-RH reverse transcriptase (Dia-M, Russia). Two-round amplification products were generated according to a standard protocol using a series of previously described primers for the L- and S-segments of the genome [4, 10], and Taq DNA polymerase Hot Start (Dia-M, Russia). The resulting amplicons were separated by electrophoresis on a 1.2 % agarose gel and then purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research, USA). Sanger DNA sequencing was conducted using an ABI Prism 310 genetic analyzer (Applied Biosystems, USA).

Nucleotide sequence alignment was conducted using the ClustalW algorithm in the Mega5 software. Neighbor-joining (NJ) method was employed to generate phylogenetic trees. The calculations were performed for 1,000 iterations.

Approval for the study was obtained from the Ethics Committee of the State Research Center of Virology and Biotechnology «Vector» of Rospotrebnadzor on February 10, 2025 (protocol no. 12).

A CLINICAL CASE

Patient H., a 41-year-old resident of Komsomolsk-on-Amur, was admitted to the Infectious Diseases Department No. 1 of the M.I. Shevchuk City Hospital on January 31, 2025. The patient complained of fever, generalized weakness, and back pain.

The patient's medical history reveals that, as a homeless person, she resided in a basement apartment between December 2024 and January 2025. During this time, she was exposed to a significant number of mice and rats, and her living conditions were substandard. The patient did not adhere to personal hygiene practices and relied on meltwater for drinking. On January 28th, 2025, she became acutely ill, presenting with fever (38.9–40°C), chills, severe general weakness, and a feeling of heaviness in her lower back. Additionally, she experienced a decrease in urine output. Upon admission, a physical examination revealed several findings. The patient exhibited swelling and hyperemia (excessive blood flow) of the face, neck, and collar zone, as well as scleral injection (blood vessels in the white of the eyes) and conjunctival hyperemia. Furthermore, a positive Pasternatsky sign was observed on both sides, indicating possible kidney involvement. A complete blood count showed moderate thrombocytopenia (97 $\times$ 10^9/L). The patient was admitted to the hospital with a preliminary diagnosis of hemorrhagic fever with renal syndrome (HFRS). Upon admission to the hospital, the patient gave informed consent in accordance with Article 20 of Federal Law No. 323-FZ dated November 21, 2011 «On the Basics of Public Health Care in the Russian Federation».

During the first day of admission, there was a significant decline in the patient's condition. The platelet count decreased to 57 x 10^9/L, lower back pain increased, anuria (no urine output through the catheter after saline infusion) developed, there were a single episode of vomiting containing blood, muscle pain, hypotension, and dyspnea. The patient was immediately transferred to the intensive care unit for further monitoring and treatment.

Within the next 24 hours, despite continued pathogenetic and symptomatic treatment, multiple organ dysfunction (renal, hepatic, respiratory, and cardiovascular)

deteriorated, leading to somnolence and coma. On February 2, 2025, death was recorded.

The sanitary and epidemiological investigation into the given case of a severe and fatal course of HFRS was conducted by the Epidemiology Department of the Center for Hygiene and Epidemiology in the Khabarovsk region (Komsomolsk-on-Amur).

Laboratory analysis results

Serum samples and autopsy materials were used to confirm the clinical diagnosis through laboratory testing. A vital serum sample collected on February 1, 2025, revealed specific antibodies to hantaviruses at titer levels of 1:3200 (IgM) and 1:1600 (IgG) using ELISA. In the autopsy material, the titers of specific antibodies to hantaviruses in the blood were 1:1600 for IgM and 1:1400 for IgG. The SEOV RNA was detected in the serum samples as well as in the autopsy materials (blood, lung, spleen, liver and kidney tissue) using RT-PCR.

Based on clinical, laboratory and pathological findings, the final diagnosis was hemorrhagic fever with renal syndrome in the anuric stage, which was severe, as confirmed by laboratory tests.

The underlying condition was chronic alcohol-induced intoxication, which had led to multiple organ complications, including alcoholic cardiomyopathy, chronic pancreatitis, and alcoholic hepatitis.

Complications of the underlying condition: grade 3 infection-related toxic shock. Renal nephritis, acute kidney failure. Hemorrhagic syndrome: mucosal and parenchymal organ hemorrhages. Hemorrhagic gastritis and enterocolitis. Pulmonary edema. Meningeal and brain tissue edema with brainstem displacement. Dystrophic and necrobiotic changes in parenchymal organs.

Phylogenetic analysis results

RT-PCR analysis using the OM-Screen-HFRS-RV reagent kit enabled us to identify the viral species responsible for HFRS. However, it did not permit us to compare the genome sequence of the virus with previously identified strains from various parts of the world to determine the genetic variant of SEOV and its origin. To this end, blood samples obtained from the patient during her hospital stay and at the time of autopsy were utilized to extract and sequence fragments from the L and S genome segments.

Phylogenetic analysis of the L-segment sequence fragment (346 nt) revealed that the isolate from Komsomolsk-on-Amur is consistent with the same SEOV strain that has previously been identified in patients and brown rats from Khabarovsk and surrounding areas. However, it differs from the VDV strain of SEOV identified in Vladivostok (Fig.). The nucleotide sequence divergence between SEOV isolates from Komsomolsk and Vladivostok was 4.7–5.0 %. The nucleotide differences between the Khabarovsk, Komsomolsk, and Chinese SEOV isolates were 0.3–1.2 %. The encoded amino acid sequences were identical for all isolates.

Genetic analysis of the S-segment fragment (760 nt) has also revealed a close relationship between the SEOV virus isolated from a patient in Komsomolsk-on-Amur and isolates from Norway rats and patients in Khabarovsk and China. A comparison of nucleotide sequences revealed the highest level of similarity with isolates from Khabarovsk patient samples, HU977/Russia/2019, and Priamursk patient samples, HU1619/Russia/2018, with 99.7 % and 100 % identity, respectively, at the amino acid level of the nucleocapsid protein encoded by these sequences. Similarity with isolates from China reached 99.5 % at the nucleotide level and 100 % at the amino acid level.

The data presented allow us to propose two hypotheses regarding the origin of the previously unreported urban focus of SEOV in Komsomolsk-on-Amur: the importation of infected rats with food products from either Khabarovsk or China, and the possible existence of this mainland variant of the virus in a wide area, including the eastern regions of China and the Khabarovsk region.

DISCUSSION

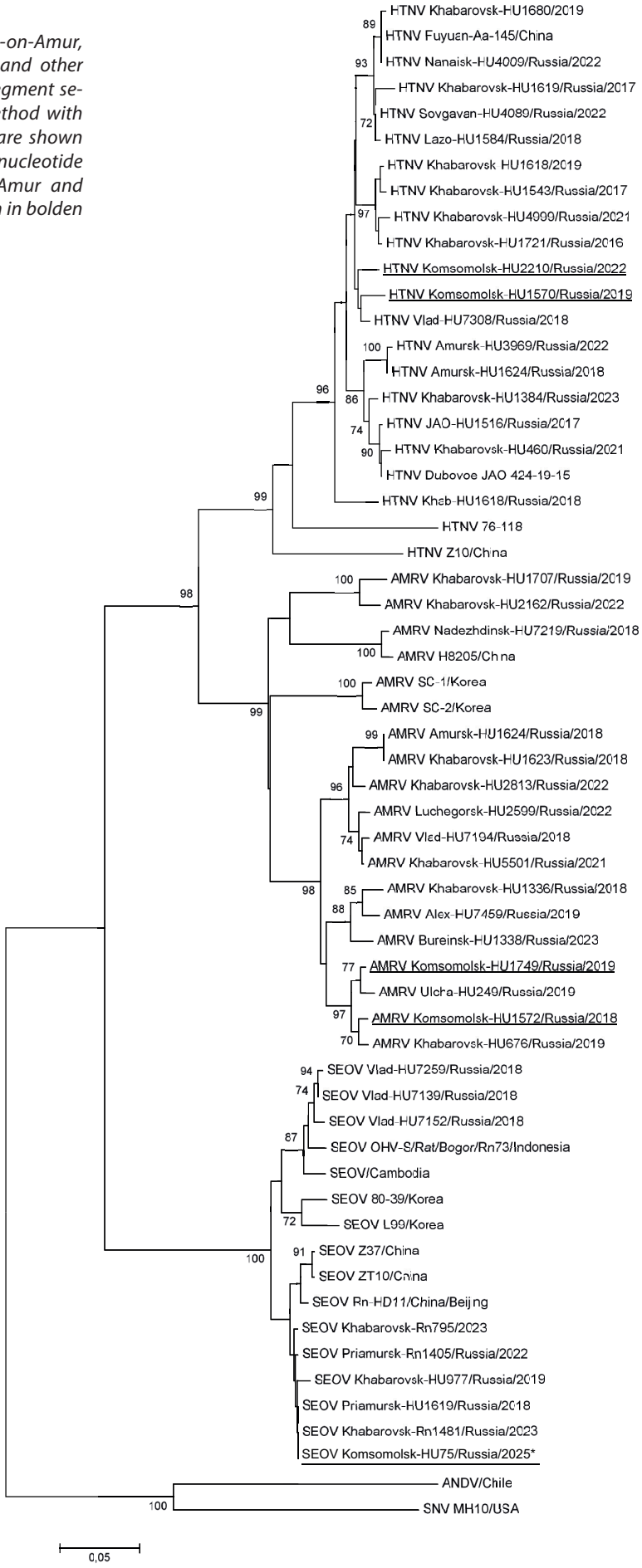
The Russian Far East is part of the high-risk area for HFRS, where three pathogenic orthohantaviruses have been identified as responsible for HFRS cases: HTNV, AMRV, and SEOV [2, 3]. Only two of these viruses, AMRV (isolates Komsomolsk-HU1572/Russia/2018 and Komsomolsk-HU1749/Russia/2019) and HTNV (Komsomolsk-HU1570/Russia/2019 and Komsomolsk-HU2210/Russia/2022), have been previously identified in infected residents of Komsomolsk-on-Amur and the surrounding area.

This is the first documented case of SEOV hantavirus infection in a patient with HFRS who had not travelled outside of Komsomolsk-on-Amur, and it has been genetically confirmed.

Over 80 % of HFRS cases associated with HTNV and AMRV have a severe or moderate course, whereas in cases of SEOV infection the proportion of severe forms does not exceed 5.7 %, with a mortality rate in Asia of less than 1 % [6, 9, 11]. However, the clinical case presented demonstrates a severe and complicated form of HFRS caused by SEOV, with a fatal outcome on the sixth day. Most likely, the fatal course of the disease was related to an aggravated pre-morbid background. It is known that hyperproduction of pro-inflammatory cytokines in combination with suppression of the cellular immune response plays a crucial role in the pathogenesis of severe HFRS forms, contributing to the development of systemic immune inflammation in the microvasculature of the kidneys, lungs, liver, myocardium, and brain. The rapid development of coagulopathy, accompanied by platelet depletion and disruption of microcirculation and central hemodynamics, as well as uncontrolled vascular responses (toxic shock), leads to multiple organ failure and irreversible destructive processes [12, 13]. Chronic ethanol intoxication, in addition to toxic multiple organ dysfunction, has also been shown to suppress Th1 lymphocyte activity and lead to an increase in blood

FIG.

Phylogenetic tree of new strain identified in Komsomolsk-on-Amur, in relationship to hantavirus strains from far-eastern and other regions of the world. Tree was based on the partial L-segment sequences (346 bp). The trees were generated by NJ method with 1000 bootstrap replicates, bootstrap values (> 70 %) are shown at relevant nodes. The scale bar depicts the number of nucleotide substitutions per site. Strains from Komsomolsk-on-Amur and Komsomolsk district are underlined, new strain is shown in bolden lettering and asterisk (*)



levels of pro-inflammatory (IL-6) cytokines and a decrease in anti-inflammatory cytokine levels (IL-10) [14, 15]. This can enhance the systemic immune-mediated damage caused by the etiological agent of HFRS.

Previously, cases of HFRS caused by SEOV infection had not been reported in Komsomolsk-on-Amur. This is likely due to the fact that standard laboratory diagnostics using ELISA do not allow for differentiation of pathogen types, and the more recently implemented differential diagnostic system based on RT-PCR has not yet been widely adopted in practical healthcare. It has also been demonstrated that the reported incidence of HFRS does not fully represent the actual prevalence of the pathogens, primarily due to undiagnosed cases of mild or asymptomatic infections, which constitute a significant portion of the epidemic process [16, 17]. This is particularly true for SEOV-associated variants of HFRS, which often present as mild clinical forms [6, 8, 11].

CONCLUSION

Therefore, for the first time, we have obtained genetic confirmation of human infection with SEOV circulating in Komsomolsk-on-Amur. We have also determined that the identified SEOV isolate belongs to a genotype previously identified in Khabarovsk and its surrounding areas, which is prevalent in China.

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Conflicts of interest

No potential conflict of interest relevant to this article reported.

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