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Puumala Virus Infection in Family, Switzerland

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We report 3 cases of Puumala virus infection in a family in Switzerland in January 2019. Clinical manifestations of the infection ranged from mild influenza-like illness to fatal disease. This cluster illustrates the wide range of clinical manifestations of Old World hantavirus infections and the challenge of diagnosing travel-related hemorrhagic fevers.

Puumala orthohantavirus (PUUV), a species of the genus *Orthohantavirus* within the *Hantaviridae* family, is an enveloped single-strand negative-sense RNA virus (*1*). The case-fatality ratio of Old World hantaviruses ranges from 1%–10% for Dobrava-Belgrade and Hantaan orthohantaviruses to <1% for PUUV. Infection is transmitted by direct inhalation of virion-containing aerosols from rodent urine and feces. PUUV causes nephropathia epidemica, a limited form of hemorrhagic fever with renal syndrome (*1*). In Russia, 6,000–8,000 cases of hemorrhagic fever with renal syndrome are reported annually. Most cases occur in Western Russia and are caused by PUUV and Dobrava-Belgrade orthohantaviruses (*2*).

Asthenia, fever, chills, diffuse myalgia, and lumbar pain developed in a man 45 years of age 4 days after he returned to Switzerland from Samara, his hometown in central Russia (Appendix, https://wwwnc.cdc.gov/EID/article/27/2/20-3770-App1.pdf). Four days later, he sought treatment at the Geneva University Hospitals (Geneva, Switzerland) for septic shock with disseminated intravascular coagulation and kidney and liver failure. He had severe thrombocytopenia and elevated levels of C-reactive protein, procalcitonin, and leukocytes (Appendix Table 2). We transferred him to the intensive care unit for mechanical ventilation and hemodynamic support because of severe metabolic acidosis and confusion. We began treatment with broad-spectrum antimicrobial drugs, including doxycycline for possible leptospirosis. The day after admission, the patient tested positive for PUUV by real-time reverse transcription PCR (*3*) with a cycle threshold of 28. His serum sample tested positive for IgM and IgG against hantaviruses (Appendix Table 1). Shortly after his diagnosis, we administered 2 doses of 30 mg subcutaneous icatibant 6 hours apart. The patient died of multiple organ failure ≤60 hours after admission.

The next day, fever, lymphopenia, moderate thrombocytopenia, and hepatitis developed in the index patient's daughter, who was 12 years of age (Appendix). She was hospitalized and tested positive for PUUV by PCR with a cycle threshold of 26. We prescribed a 5-day course of oral ribavirin starting with an initial dose of 30 mg/kg followed by 15 mg/kg every 6 hours (*4*). The viral load in plasma rapidly decreased. We did not detect viral RNA in urine (Appendix Table 3). Interstitial nephropathy briefly developed and subsided; she was discharged without sequelae after 7 days.

The wife of the index patient had had influenza-like symptoms in Russia during the week before her husband's illness. Her serum sample tested positive for IgM and IgG against hantaviruses. We used a pseudovirus-based neutralization assay to confirm serologic results (Appendix Figure 1).

We sequenced the viral genome from blood samples taken from the father (GenBank accession no. MT822196) and the daughter (GenBank accession no. MT822195) using high-throughput sequencing (Appendix Figure 2). Both sequences showed a 100% s segment match and were related to PUUV sequences in GenBank from Samara (Figure), confirming that the patients were exposed there. Regular outbreaks occur in Samara (*5*), where annual rodent control measures were delayed in 2019. In Switzerland, local acquisition of PUUV is rare (*6*).

This familial cluster highlights the wide spectrum of clinical manifestations of PUUV, which can range from an influenza-like illness (mother) to the classical nephropathy (daughter) to a rapidly fatal hemorrhagic fever with shock and multiple organ failure (father). Such a large spectrum of disease might be caused by the viral inoculum or host factors. Uncontrolled immune response and subsequent cytokine storm have been identified as key factors in the development of critical disease (*7*). Smoking, enzymatic polymorphisms, and gene variants such as HLA-B8 DRB1*03:02 (*8*) might be risk factors for severe disease, whereas HLA-B57 might have a protective effect (*9*). High procalcitonin levels, severe thrombocytopenia, increased interleukin 6 levels, and leukocytosis are known markers for severe disease.

Although specific antimicrobial drugs have been tested against PUUV infections, treatment is limited to supportive care. A small trial in Russia showed no effect of ribavirin on PUUV viral load or risk for death (*10*). We decided to treat the daughter with ribavirin because of her early diagnosis and treatment, the potential genetic factors that might predispose her to severe disease, and the emotional context of her father's death. We treated the father with icatibant, a selective antagonist of the bradykinin type 2 receptor that reduces capillary leakage. This treatment has been used with apparent success in 2 patients with severe PUUV infection (Appendix).

PUUV usually causes limited renal disease but has a broad spectrum of clinical manifestations. Human hantavirus infections are rare in Switzerland and mostly acquired outside of the country. Physicians should consider viral hemorrhagic fevers when a patient has worsening influenza-like illness, thrombocytopenia, renal and hepatic impairment, and a plausible epidemiologic link to a region to which these viruses are endemic.

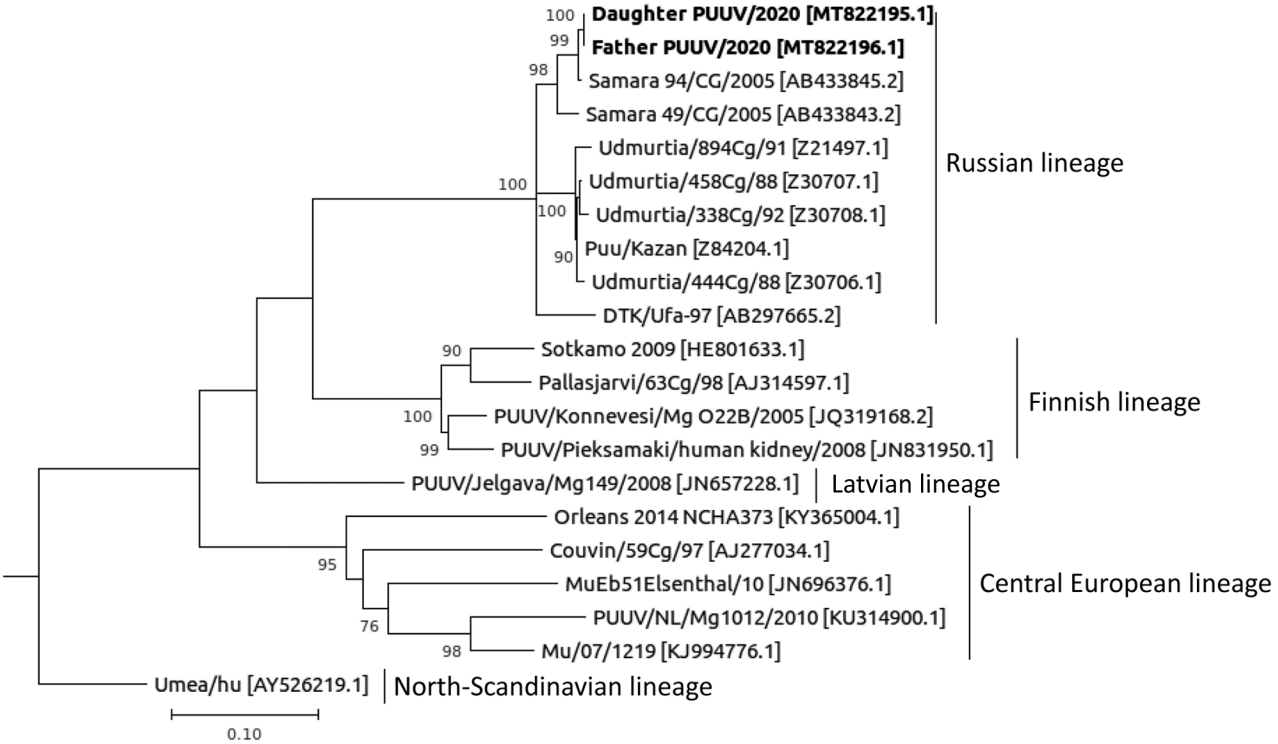

Figure. Phylogenetic tree of Puumala virus using S segment nucleotide sequences. Bold text indicates sequences isolated from family in Switzerland. GenBank accession numbers are provided in brackets. Lineages are indicated at right. Scale bar indicates number of substitutions per site.

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Protective Immunity and Persistent Lung Sequelae in Domestic Cats after SARS-CoV-2 Infection

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Severe acute respiratory syndrome coronavirus 2 readily transmits between domestic cats. We found that domestic cats that recover from an initial infection might be protected from reinfection. However, we found long-term persistence of inflammation and other lung lesions after infection, despite a lack of clinical symptoms and limited viral replication in the lungs.

Previous studies have demonstrated the transmissibility of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by direct or indirect contact between domestic cats (*1*,*2*). Given the

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Puumala Virus Infection in Family, Switzerland

Appendix

Supplementary Case Description

The index patient was not taking any medication, was a nonsmoker, and had no known relevant medical history, except for untreated limited cutaneous psoriasis. Two days before admission, he sought medical attention because of asthenia, and was diagnosed with hypertension and discharged without treatment. A few hours later, he developed an influenza-like illness characterized by fever, chills, diffuse myalgia, and lumbar pain. The next day, during a second visit, blood tests showed hemoconcentration and severe thrombocytopenia (50 G/L). Blood cultures were collected and intravenous ceftriaxone was started for a suspected urinary tract infection because of proteins and hemoglobin at urinary dipstick, despite the absence of leukocytes and nitrites. The patient was admitted 24 hours later, 4 days after symptom onset, to Geneva University Hospitals' emergency room presenting with signs of septic shock, with disseminated intravascular coagulation marked by profound thrombocytopenia in addition to kidney and liver failure.

Both C-reactive protein and procalcitonin were elevated and white blood cell count increased progressively (Appendix Table 1). Because of severe metabolic acidosis and confusion, the patient was immediately transferred to the intensive care unit for orotracheal intubation, mechanical ventilation, and hemodynamic support. Broad-spectrum antibiotic therapy, including meropenem, vancomycin, and clindamycin was initiated because septic shock of bacterial origin was suspected. The patient's wife reported a rodent invasion around Samara, their hometown in Russia, so doxycycline was added to treat potential leptospirosis.

Supplementary Case Investigations

The bacterial analysis of urine and blood cultures had negative results. A screening for multidrug resistant bacteria in stools and skin swabs also returned negative results. Samples

tested negative for the Asian hantavirus panel and Crimean–Congo hemorrhagic fever virus by RealStar CCHF RT-PCR Kit 1.0 (Altona, https://www.altona-diagnostics.com).

A full body computed tomography (CT) scan showed bi-basal pulmonary consolidations and diffuse ground-glass opacities associated with “crazy-paving” infiltrates and bilateral pleural effusions. No abdominal lesions were observed. Parieto-occipital leptomeningeal enhancements were observed on the cerebral CT scan.

A transthoracic echocardiography showed hyperdynamic cardiac function with a left ventricular ejection fraction estimated between 70% and 75%. No sign of valvulopathy and no pericardial effusion were observed.

Methods

Sequencing and Phylogenetic Tree Construction

Viral genome sequences were recovered from the father’s (GenBank accession no. MT822196) and the daughter’s (GenBank accession no. MT822195) blood by high-throughput sequencing on a HiSeq 4000 platform (Illumina, https://www.illumina.com) by using an RNA protocol previously published (*2*). To construct the phylogenetic tree (Figure 1), the maximum likelihood method and the Tamura 3-parameter model (*3*) were used and analyses were conducted in MEGA X (*4*). The tree is rooted using the “JN657228.1” sequence (Latvian genetic lineage).

Immunofluorescence Assay

We used Hantavirus Mosaic 1, (Euroimmun, https://www.euroimmun.com) according to the manufacturer’s instructions to perform immunofluorescence assays (Appendix Table 1). Samples from the mother were taken approximately 3 weeks after the onset of symptoms.

Puumala Virus Neutralization Assay

Serum samples were inactivated by incubation for 30 min at 56°C and serial 2-fold dilutions were incubated with vesicular stomatitis virus with Puumala virus glycoprotein pseudotype virus (*5*). Residual infectivity was determined by counting green fluorescent protein–positive VeroE6 cells and expressed as a percentage of infected cells (Appendix Figure 1).

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Appendix Table 1. Immunofluorescence assay results of family members infected with Puumala virus, Switzerland

Patient	Immunoglobulin	Dilution
Father	IgG	1:100
	IgM	1:100
Mother	IgG	1:100
	IgM	1:100
Daughter	IgG	1:100
	IgM	1:100

Appendix Table 2. Laboratory values of father with Puumala virus infection, Switzerland*

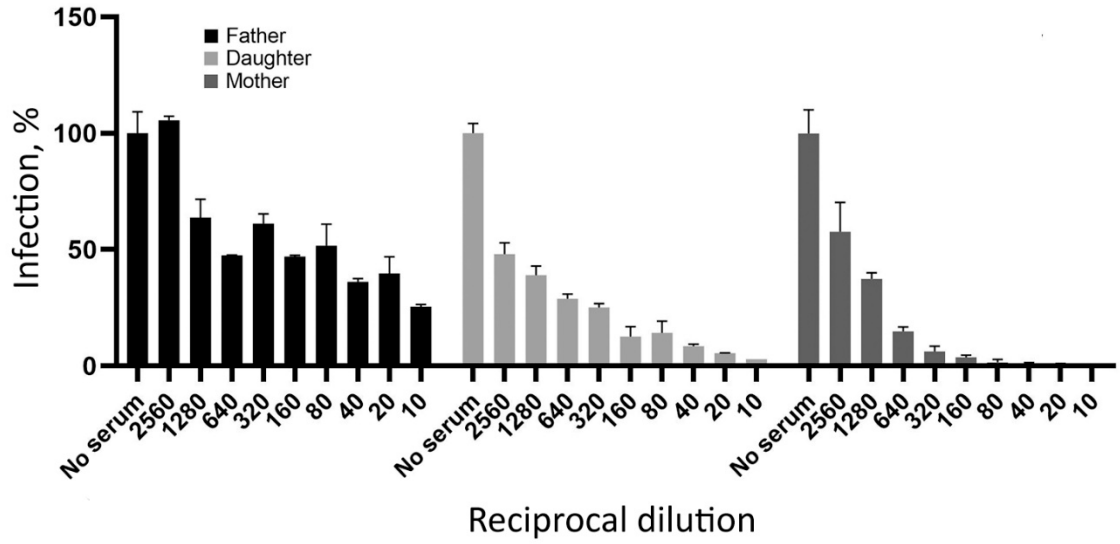
Parameter	Reference range	Time after symptom onset, d			
		4	5	6	7
Hemoglobin, g/L	140–180	**194**	**181**	133	**83**
Hematocrit, %	40.0–52.0	**53.9**	50.2	**38.5**	**25.3**
Leukocytes, 10^9 cells/L	4.0–11.0	8.6	**21.8**	**50.2**	**21.8**
% Segmented neutrophils (absolute no., 10^9 cells/L)	33.0–75.0 (1.50–7.50)	56.0/4.82	**78.5/17.11**	29.5/**14.81**	-
% Lymphocytes (absolute no., 10^9 cells/L)	15.0–60.0 (1.00–4.50)	**6.0/0.52**	**8.0**/1.74	18.5/9.29	
Platelets, 10^9 cells/L	150–350	**16**	**11**	**43**	**28**
INR		25	1.77	6.38	8.4
PTT, s	26.0–37.0	51	**65.1**	**159.8**	**>160**
Fibrinogen, g/L	1.5–3.5	2.9	1.8	**0.3**	**<0.4**
Activity Factor V, %	>70	91	**65**	**13**	**<10**
C-reactive protein, mg/L	0.00–10.00	**154**	**85**	**46**	**34**
Sodium, mmol/L	136–144	**125**	**131**	**145**	**130**
Potassium, mmol/L	3.6–4.6	4.4	4.6	4.6	**7.8**
BUN, mmol/L	3.2–7.5	4.6	**11.7**	**8.6**	5.2
Creatinine, µmol/L	62–106	108	**258**	**266**	**251**
CK total, U/L	47–222		**836**	**2'875**	**44061**
ASAT, U/L	14–50	**242**	**936**	**37'736**	N/A
ALAT, U/L	12–50	**111**	**339**	**8'811**	**6064**
Alkaline phosphatase, U/L	25–102	48	37	**121**	**235**
GGT, U/L	9–40	**115**	**47**	**46**	**103**
Total bilirubin, µmol/L	7–25	17	23	**57**	**61**
Conjugated bilirubin, µmol/L	0.5–9.5	<22	**13.9**	**33.9**	**26**
Lipase, U/L	13–60	**103**	**134**	**197**	**>600**
Lactate, mmol/L	<2	**8.4**	**5.3**	**23**	**19**
Procalcitonin, µg/L	<0.25	-	**9.5**	**1.7**	-

*Bold text indicates values outside the reference range. On day 7, no complete blood count was conducted. ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatinine kinase; GGT, gamma glutamine transpeptidase; INR, international normalized ratio; PTT, partial thromboplastin time.

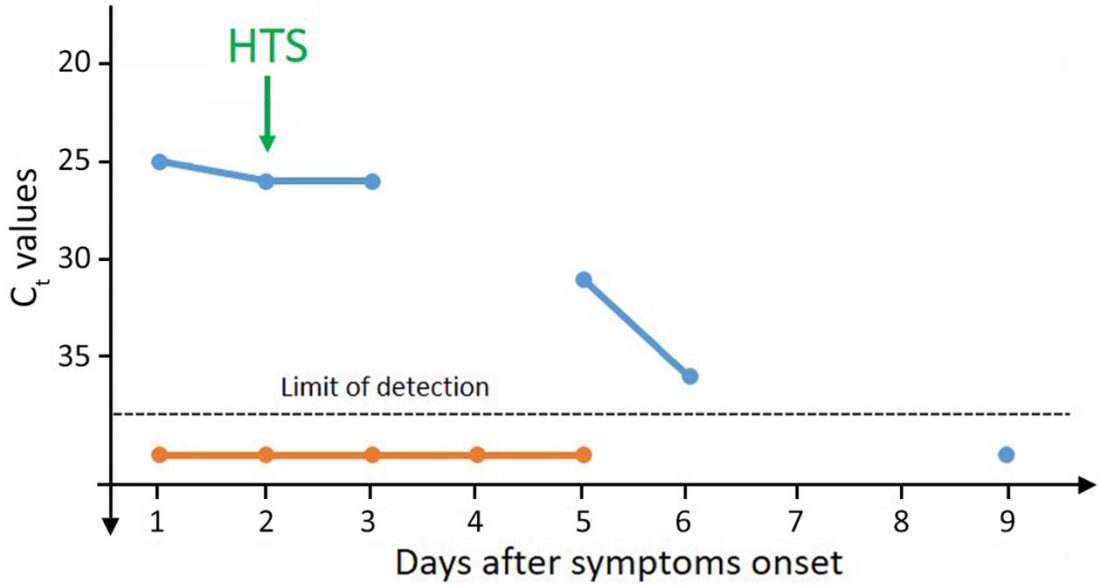
Appendix Table 3. Laboratory values of daughter with Puumala virus infection, Switzerland*

Parameter	Reference range	Time after symptom onset, d			
		1	3	5	7
Hemoglobin, g/L	115–155	143	140	142	130
Hematocrit, %	35.0–45.0	41.7	40.6	41.2	36.1
Leukocytes, 10^9 cells/L	4.5–13.5	**3.2**	5.6	5.2	**4.4**
% Segmented neutrophils (absolute no., 10^9 cells/L)	31.0–67.0 (1.50–7.50)	68.0/2.18	55.0/3.08	36.0/1.87	-
% Lymphocytes (absolute no., 10^9 cells/L)	22.0–51.0 (1.50–6.50)	**16.0/0.51**	23.0/**1.29**	41.0/2.13	50/2.21
Platelets, 10^9 cells/L	168–392	**100**	**67**	**113**	239
INR		1.28	1.15	1.05	1.04
PTT, s	26.0–37.0	**40.4**	**38.5**	32.6	30.4
Fibrinogen, g/L	1.5–3.5	2.9	2.9	**4.1**	3.4
C-reactive protein, mg/L	0.00–10.00	6.00			
Sodium, mmol/L	133–143	137	138	**144**	139
Potassium, mmol/L	3.5–5.1	4.3	3.8	3.7	3.7
Creatinine, µmol/L	25–52	50	57	**82**	66
ASAT, U/L	0–33	**73**	**46**	25	**50**
ALAT, U/L	0–19	28	22	23	**50**
Alkaline phosphatase, U/L	129–417	248	186	154	150
GGT, U/L	4–16	13	**26**	**29**	**27**
Total bilirubin, µmol/L	0–8	3	6	**10**	**10**
Procalcitonin, µg/L	<0.25	**0.61**	**0.68**	**0.33**	-
Urine					
Albumin, mg/L	0–10	**15**	**1002**	**367**	**11**
Creatinine, mmol/L		7.3	2.1	1.9	5.6
Protein, g/L		0.17	1.55	0.7	0.05

*Bold text indicates values outside the reference range. On day 7, no complete blood count was conducted. ALAT, alanine aminotransferase; ASAT, aspartate aminotransferase; BUN, blood urea nitrogen; CK, creatinine kinase; GGT, gamma glutamine transpeptidase; INR, international normalized ratio; PTT, partial thromboplastin time.



Appendix Figure 1. Neutralizing antibody levels in serum samples from members of a family with Puumala virus infection, Switzerland. Error bars indicate 95% CIs.



Appendix Figure 2. Cycle threshold (C_t) values of PCR of whole blood and urine samples of a girl with Puumala virus infection, Switzerland. Blue indicates whole blood samples. Orange indicates urine samples. Green arrow indicates the day of sample collection for high-throughput sequencing (HTS).