

This case highlights 2 issues: the unknown epidemiology of CHIKV in Africa and the difficulty of diagnosing one arboviral infection during an outbreak of another arboviral infection. Further research is necessary to elucidate the true extent of CHIKV in African countries and to understand the public health implications of co-infection and co-distribution of multiple arboviruses.

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## Puumala Virus in Bank Voles, Lithuania

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Little is known about the presence of human pathogenic Puumala virus (PUUV) in Lithuania. We detected this virus in bank voles (*Myodes glareolus*) in a region of this country in which previously PUUV-seropositive humans were identified. Our results are consistent with heterogeneous distributions of PUUV in other countries in Europe.

Puumala virus (PUUV) (family *Bunyaviridae*) is an enveloped hantavirus that contains a single-stranded trisegmented RNA genome of negative polarity (1). PUUV harbored by the bank vole (*Myodes glareolus*) is the most prevalent human pathogenic hantavirus in Europe (2). A high population density of bank voles can lead to disease clusters and possible outbreaks of nephropathia epidemica, a mild-to-moderate form of hantavirus disease (3).

In contrast to the Fennoscandian Peninsula and parts of central Europe (4,5), little is known about the epidemiology of PUUV in Poland and the Baltic States. Recent investigations confirmed the presence of PUUV in certain parts of Poland (5,6). A molecular study of bank voles in Latvia identified 2 PUUV lineages (Russian and Latvian) (7). In Estonia, serologic and molecular screening provided evidence of the Russian PUUV lineage (8). For Lithuania, a previous serosurvey indicated the presence of PUUV-specific antibodies in humans from 3 counties (online Technical Appendix Figure 1, <http://wwwnc.cdc.gov/EID/article/23/1/16-1400-Techapp1.pdf>). However, molecular evidence of PUUV in humans or in voles is lacking (9).

We report a molecular survey of rodent populations in Lithuania at 5 trapping sites, including 2 sites in counties where PUUV-specific antibodies were previously detected in humans (online Technical Appendix Figure 1). A total of 134 bank voles, 72 striped field mice (*Apodemus agrarius*), and 59 yellow-necked field mice (*A. flavicollis*) were captured during 2015. Three trapping sites (Juodkrantė, Elektrėnai, and Lukštas) were located in forests at or near

a cormorant colony, and 2 trapping sites (Žalgiriai and Rusnė) were located in a wet forest and flooded meadows. All applicable institutional and national guidelines for the care and use of animals were followed.

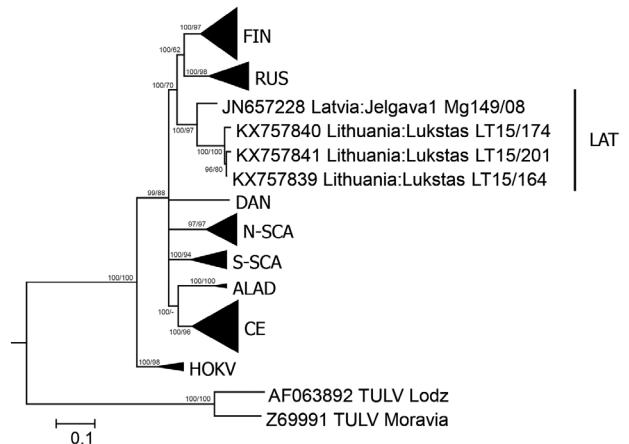
For PUUV detection, we extracted RNA from bank vole lung tissue samples by using the Qiazol Protocol (QIAGEN, Hilden, Germany) and conducting screening by using a small segment RNA-specific reverse transcription PCR (RT-PCR) and primers Pu342F and Pu1102R (6). We detected PCR products for 5 (LT15/164, LT15/165, LT15/166, LT15/174, and LT15/201) of 45 bank voles from the Lukštas trapping site. All 9 striped field mice and 2 yellow-necked field mice from Lukštas showed negative results for the PUUV RT-PCR.

We amplified the complete nucleocapsid protein-encoding region for 3 of the 5 samples positive by RT-PCR with 3 primer pairs: PuNCRS (5'-TAGTAGTAGACTCCTTGAA-3')/Pu255R (5'-TGGACACAGCATCTGCCA-3'), Pu40F (5'-CTGGAATGAGTGACTTAC-3')/Pu393R (5'-TATGGTAATGCTCTGATGT-3'), and Pu1027F (5'-ATGGCAGAGTTAGGTGCA-3')/Pu1779R (5'-TCAGCATGTTGAGGTAGT-3'). RT-PCR products were directly sequenced by using the BigDye Terminator Version 1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany). We deposited the sequences of the 5 samples in GenBank under accession nos. KX757839, KX757840, KX757841, KX751706, and KX751707 (Figure; online Technical Appendix Figure 2).

The 3 nucleocapsid protein-encoding nucleotide sequences showed identities of 98.2%–99.8%, and the 3 deduced nucleocapsid protein amino acid sequences showed identities of 99.8%–100% (online Technical Appendix Table). We found the highest similarity of the 3 nucleotide and corresponding amino acid sequences for the PUUV strain from Latvia (Jelgava1/Mg149/2008; JN657228): nucleotide sequence 89.8%–90.4% and amino acid sequence 99.8%–100% (online Technical Appendix Table).

We generated phylogenetic trees by using MrBayes 3.2.6 software (<http://mrbayes.sourceforge.net/download.php>) and MEGA6 software (<http://www.megasoftware.net/>) for complete (1,302 nt; Figure) and partial (465 nt; online Technical Appendix Figure 2) nucleocapsid protein-encoding sequences. Phylogenetic analysis confirmed results of pairwise nucleotide sequence divergence analysis, which indicated clustering of PUUV sequences from Lithuania with sequences from northern Poland (online Technical Appendix Figure 2) and the Jelgava 1 strain from Latvia (Figure). These sequences of the Latvian clade are well separated from the Russian and all other European PUUV clades.

To evaluate a potential association of PUUV with evolutionary lineages of the bank vole, we determined vole cytochrome b gene sequences, deposited them in GenBank



**Figure.** Phylogenetic tree based on complete nucleocapsid gene sequences of Puumala virus (PUUV) strains from Lithuania (LT), Latvia (Jelgava1), and other PUUV clades. Tula virus (TULV) was used as the outgroup. The tree was generated by Bayesian and maximum-likelihood analysis using MrBayes 3.2.6 (<http://mrbayes.sourceforge.net/download.php>) and MEGA6 software (<http://www.megasoftware.net/>). The optimal substitution model was calculated by using jModelTest 2.1.4 (<https://code.google.com/p/jmodeltest2>). The Bayesian tree was based on transition model 2 with invariant sites and gamma distribution and 4 million generations. For maximum-likelihood analysis, the Kimura 2-parameter model and 1,000 bootstrap replicates were used. Posterior probabilities are indicated before slashes, and bootstrap values are indicated after slashes. Scale bar indicates nucleotide substitutions per site. ALAD, Alpe-Adrian lineage; CE, Central European lineage; DAN, Danish lineage; FIN, Finnish lineage; HOKV, Hokkaido virus; LAT, Latvian lineage; N-SCA, North-Scandinavian lineage; RUS, Russian lineage; S-SCA, South-Scandinavian lineage.

under accession nos. KX769843 (LT15/164), KX769844 (LT15/165), KX769845 (LT15/166), KX769846 (LT15/174), and KX769847 (LT15/201), and compared them with cytochrome b prototype sequences of evolutionary lineages. Consistent with results for northern Poland (6), we identified 2 bank vole lineages at Lukštas, and the PUUV sequences were detected in 4 bank voles of the Carpathian phylogroup and in 1 vole of the Eastern lineage.

In conclusion, we detected PUUV in bank voles at 1 site (Lukštas) in Lithuania (prevalence of 11.1%). This site is located in a region where PUUV-seropositive persons were identified (9) and near the border with Latvia (online Technical Appendix Figure 1). The absence of PUUV in bank voles at 4 other sites might have been caused by the small number of voles tested. However, our results are consistent with heterogeneous distributions of PUUV in other countries (10).

Detection of this novel PUUV strain by using a specific RT-PCR confirms the reliability of this assay for molecular diagnostic and epidemiologic studies of this virus in Lithuania. Future large-scale monitoring studies are needed

to evaluate the geographic distribution and temporal fluctuation of PUUV in bank vole populations in Lithuania.

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## Loiasis in US Traveler Returning from Bioko Island, Equatorial Guinea, 2016

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The filarial parasite *Loa loa* overlaps geographically with *Onchocera volvulus* and *Wuchereria bancrofti* filariae in central Africa. Accurate information regarding this overlap is critical to elimination programs targeting *O. volvulus* and *W. bancrofti*. We describe a case of loiasis in a traveler returning from Bioko Island, Equatorial Guinea, a location heretofore unknown for *L. loa* transmission.

Loiasis (African eye worm disease) is caused by infection with *Loa loa*, a parasitic vector-borne filarial worm endemic to 10 countries in central and western Africa, including Equatorial Guinea (1). The worm, spread by the bite of *Chrysops dimidiata* and *C. silacea* flies, is of public health concern because of its geographic overlap with *Onchocerca volvulus* and *Wuchereria bancrofti* worms, which cause onchocerciasis and lymphatic filariasis, respectively (2). Mass drug administration programs for onchocerciasis and lymphatic filariasis often include ivermectin, which can cause serious and occasionally fatal adverse neurologic reactions in persons with high levels of circulating *L. loa* microfilariae (3). To avoid such reactions, an accurate picture of the geographic distribution of *L. loa* infection is needed. Given the importance of epidemiologic data in the management of filarial infections, we report a case of loiasis in a US woman who had traveled to Equatorial Guinea.

In May 2016, a 25-year-old woman sought care in Winston-Salem, North Carolina, USA, for fatigue, swelling of her left ankle, right knee pain, and intensely pruritic skin lesions on her lower extremities. She had lived on Bioko Island, Equatorial Guinea, during October 2015–March 2016 while studying local wildlife. On Bioko Island, she frequented local water sources to bathe and wash clothes and consistently took atovaquone/proguanil for malaria prophylaxis. She did not spend time on Equatorial Guinea’s mainland or travel to other nations in central or western Africa. Her flight from the United States to Bioko Island connected in Ethiopia; she did not leave the airport.

Symptoms developed soon after her return to North Carolina in late March 2016. Laboratory evaluations

# Puumala Virus in Bank Voles, Lithuania

## Technical Appendix

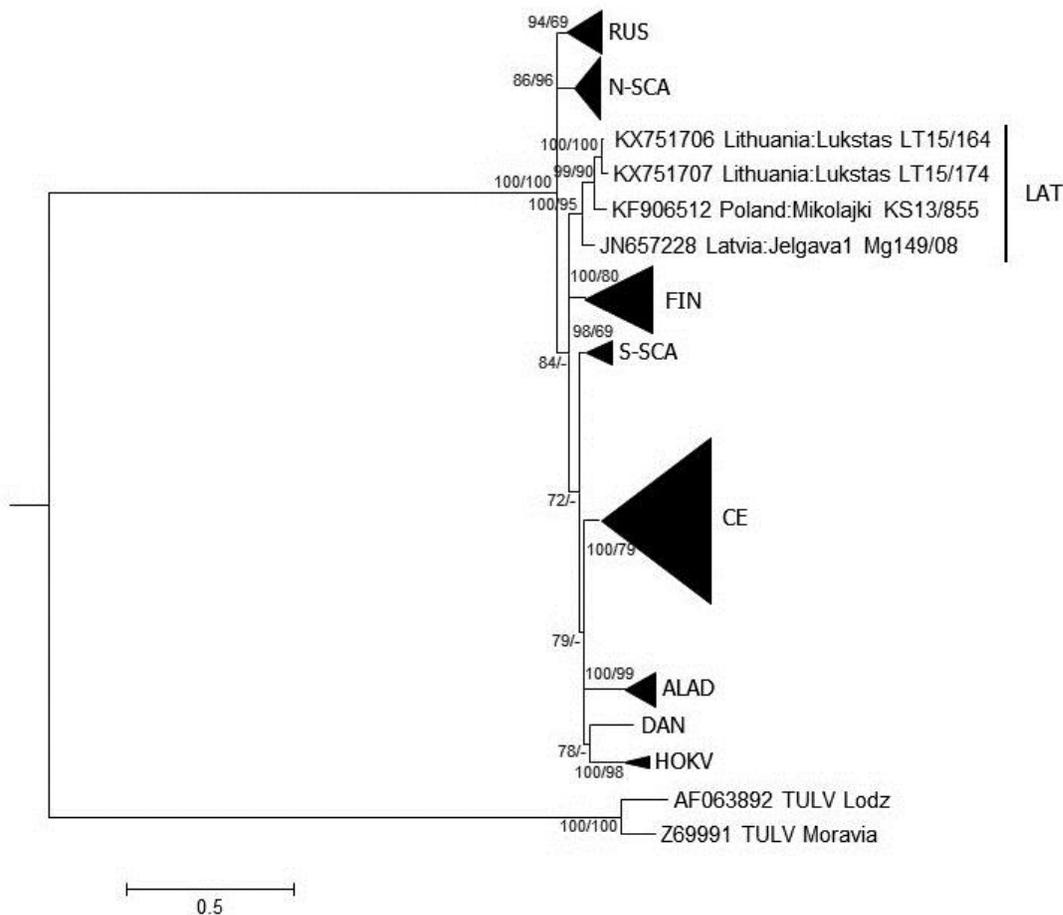
**Technical Appendix Table.** Percent pairwise identity of small segment RNA nucleotide sequences (above the diagonal) and amino acid sequences of nucleocapsid protein (below the diagonal) for Puumala virus strains\*

Strain	LT15/164	LT15/174	LT15/201	LAT	RUS1	RUS2	RUS3	FIN1	FIN2	N-SCA1	N-SCA2	S-SCA1	S-SCA2	CE1	CE2	DAN	ALAD
LT15/164		98.39	99.77	90.40	85.48	85.64	85.87	86.71	86.02	85.18	85.10	84.87	85.79	85.48	85.10	84.49	85.41
LT15/174	100.00		98.16	89.78	86.02	85.94	86.10	86.71	86.33	85.48	84.87	84.72	85.25	85.33	85.10	84.87	84.95
LT15/201	99.77	99.77		90.17	85.41	85.56	85.79	86.48	85.94	84.95	84.87	84.64	85.56	85.41	84.95	84.25	85.18
LAT	100.00	100.00	99.77		85.25	84.87	85.02	87.40	86.56	85.64	85.18	85.64	86.02	84.95	86.10	85.64	85.64
RUS1	96.77	96.77	96.77	96.77		95.47	86.48	84.49	85.10	84.72	83.33	84.25	84.72	82.03	83.72	82.64	83.72
RUS2	97.00	97.00	96.77	97.00	98.85		86.87	84.49	85.64	84.56	83.87	83.56	84.79	82.95	83.26	82.26	83.49
RUS3	96.77	96.77	96.54	96.77	96.54	97.23		85.64	84.95	83.87	84.56	83.18	84.49	83.64	83.56	83.72	84.18
FIN1	97.23	97.23	97.00	97.23	96.30	97.00	96.77		92.78	85.41	84.72	84.79	85.02	84.64	83.18	82.80	85.10
FIN2	97.00	97.00	96.77	97.00	96.07	96.54	96.77	98.61		84.10	83.79	84.79	84.87	84.41	83.26	82.95	85.18
N-SCA1	98.38	98.38	98.15	98.38	96.30	97.00	96.30	96.54	96.07		89.94	85.56	85.48	83.56	84.25	84.18	84.41
N-SCA2	98.15	98.15	97.92	98.15	95.61	95.84	95.15	95.84	95.61	98.38		84.79	83.87	83.87	84.18	84.49	83.72
S-SCA1	97.69	97.69	97.46	97.69	96.07	96.30	96.07	95.84	95.61	96.54	96.54		87.71	84.18	84.25	84.49	85.48
S-SCA2	98.61	98.61	98.38	98.61	95.84	96.54	96.77	97.23	96.77	97.46	96.77	98.15		85.71	85.18	84.18	84.56
CE1	99.08	99.08	98.85	99.08	96.30	96.77	96.54	97.00	97.23	97.69	97.23	97.00	97.92		87.56	83.56	85.48
CE2	98.85	98.85	98.61	98.85	96.54	97.00	96.30	97.23	97.46	97.92	97.46	97.46	98.15	98.85		84.33	86.48
DAN	98.15	98.15	97.92	98.15	95.38	95.84	95.84	95.61	95.61	97.00	97.00	95.84	97.00	97.92	97.69		84.10
ALAD	98.38	98.38	98.15	98.38	96.30	96.77	96.07	96.54	96.77	97.46	96.77	96.54	97.23	98.38	98.61	97.23	

\*Strains were from Lithuania (LT15/164, LT15/174, and LT15/201) and Latvia (LAT). We also used representative strains of Alpe-Adrian (ALAD), Central European (CE), Danish (DAN), Finnish (FIN), North-Scandinavian (N-SCA), Russian (RUS), and South-Scandinavia (S-SCA) lineages. ALAD, FN377821 Hungary; CE1, EU439968 Bavaria; CE2, KT247597 Jura; DAN, AJ238791 Fyn; FIN1, JQ319168 Konnevesi; FIN2, Z30702 Evo; LAT, JN657228 Jelgava1; N-SCA1, AY526219 Umea; N-SCA2, GQ339474 Kiviniemi; S-SCA1, AJ223369 Eidsvoll; S-SCA2, GQ339487 Munga; RUS1, JN657231 Jelgava2; RUS2, JN657232 Madona; RUS3, Z21497 Udmurtia.



**Technical Appendix Figure 1.** Lithuania and the surrounding countries (Poland and Latvia) showing 5 trapping sites (squares) for bank voles, which were tested for Puumala virus (PUUV). PUUV-positive localities (Jelgava 1, Jelgava 2, and Madona) in Latvia and (Mikołajki) in from Poland are indicated by circles. For the trapping site in Lukštas, 5 of 45 bank voles were positive for PUUV. At Juodkrantė (n = 28 voles), Elektrėnai (n = 27), Žalgiriai (n = 13), and Rusnė (n = 21), none of the bank voles were positive for PUUV. The 3 counties in Lithuania (Siauliai, Utena, and Vilnius), where previously PUUV-seropositive persons were detected (1), are indicated.



### Technical

**Appendix Figure 2.** Phylogenetic tree based on partial small segment RNA sequences of Puumala virus (PUUV) strains from Lithuania (LT), Latvia (Jelgava1), Poland (Mikolajki), and other PUUV clades. Tula virus (TULV) was used as the outgroup. Phylogenetic calculations were based on Bayesian and maximum-likelihood analyses using MrBayes 3.2.6 (<http://mrbayes.sourceforge.net/download.php>) and MEGA6 (<http://www.megasoftware.net/>) with the transition model with invariant sites and gamma distribution and 4,000,000 generations and with the Kimura 2-parameter model and 1,000 bootstrap replicates. A substitution model was determined by using jModelTest 2.1.4 software (<https://groups.google.com/forum/#!msg/jmodeltest/qPNGW0K6fdY/Xup7Xy6oAM4J0>). Posterior probabilities are indicated before slashes, and bootstrap values are indicated after slashes. Scale bar indicates nucleotide substitutions per site. LT15/165, LT15/166, and LT15/201 were identical to LT15/164 and were therefore excluded from phylogenetic analysis. ALAD, Alpe-Adrian lineage; CE, Central European lineage; DAN, Danish lineage; FIN, Finnish lineage; HOKV, Hokkaido virus; LAT, Latvian lineage; N-SCA, North-Scandinavian lineage; RUS, Russian lineage; S-SCA, South-Scandinavian lineage.

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