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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References

1. Filipe AF, Pinto MR. Arbovirus studies in Luanda, Angola. 2. Virological and serological studies during an outbreak of dengue-like disease caused by the chikungunya virus. Bull World Health Organ. 1973;49:37–40.
2. World Health Organization. Situation report: yellow fever outbreak in Angola W30, 29 July 2016 [cited 2016 Aug 18]. http://www.afro.who.int/en/yellow-fever/sitreps/item/8866-situation-report-yellow-fever-outbreak-in-angola-29-july-2016.html
3. World Health Organization. Rift Valley fever in China [cited 2016 Aug 18]. http://www.who.int/csr/don/02-august-2016-rift-valley-fever-china/en/
4. Ross RW. The Newala epidemic. III. The virus: isolation, pathogenic properties and relationship to the epidemic. J Hyg (Lond). 1956;54:177–91. http://dx.doi.org/10.1017/S0022172400044442
5. Moyen N, Thiberville SD, Pastorino B, Nougairede A, Thirion L, Mombouli JV, et al. First reported chikungunya fever outbreak in the republic of Congo, 2011. PLoS One. 2014;9:e115938. http://dx.doi.org/10.1371/journal.pone.0115938
6. Ochieng C, Ahenda P, Vittor AY, Nyoka R, Gikunju S, Wachira C, et al. Seroprevalence of infections with dengue, Rift Valley fever and chikungunya viruses in Kenya, 2007. PLoS One. 2015;10:e0132645. http://dx.doi.org/10.1371/journal.pone.0132645
7. Gudo ES, Pinto G, Vene S, Mandlaze A, Muianga AF, Cliff J, et al. Serological evidence of chikungunya virus among acute febrile patients in southern Mozambique. PLoS Negl Trop Dis. 2015;9:e0004146. http://dx.doi.org/10.1371/journal.pntd.0004146
8. Centers for Disease Control and Prevention. Geographic distribution. Where has chikungunya virus been found? [cited 2016 Aug 18]. https://www.cdc.gov/chikungunya/geo/index.html
9. Furuya-Kanamori L, Liang S, Milinovich G, Soares Magalhaes RJ, Clements AC, Hu W, et al. Co-distribution and co-infection of chikungunya and dengue viruses. BMC Infect Dis. 2016;16:84. http://dx.doi.org/10.1186/s12879-016-1417-2
10. Parreira R, Centeno-Lima S, Lopes A, Portugal-Calisto D, Constantino A, Nina J. Dengue virus serotype 4 and chikungunya virus coinfection in a traveller returning from Luanda, Angola, January 2014. Euro Surveill. 2014;19:20730. http://dx.doi.org/10.2807/1560-7917.ES2014.19.10.20730

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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′-CTGGAATGAGTGACTTAAC-3′)/Pu393R (5′-TATGGTAATGTCCTTGATGT-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, KX 757841, 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

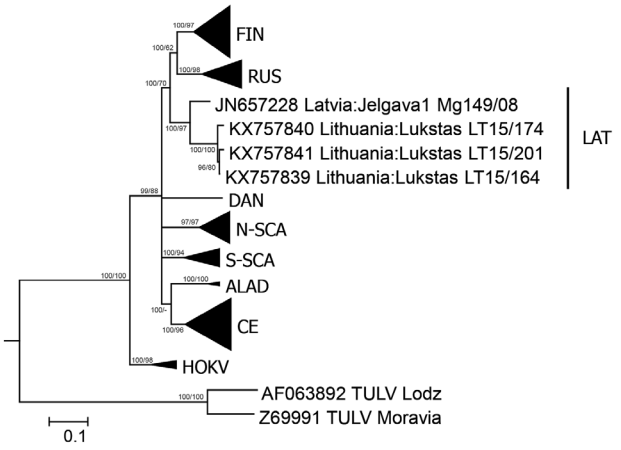

Figure. Phylogenetic tree based on complete nucleocapsid gene sequences of Puumala virus (PUUV) strains from Lithuania (LT), Latvia (Jelgava1), and other PUUV clades. Tula virus (TULV) was used as the outgroup. The tree was generated by Bayesian and maximum-likelihood analysis using MrBayes 3.2.6 (http://mrbayes.sourceforge.net/download.php) 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 linage; 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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References

1. Plyusnin A, Beaty BJ, Elliot RM, Goldbach R, Kormelink R, Lundkvist A, et al. Family *Bunyaviridae*. In: King AM, Adams MJ, Carstens EB, Lefkowitz EJ, editors. Virus taxonomy: ninth report of the international committee on taxonomy of viruses. San Diego: Elsevier Academic Press; 2012. p. 725–41.
2. Heyman P, Ceianu CS, Christova I, Tordo N, Beersma M, João Alves M, et al. A five-year perspective on the situation of haemorrhagic fever with renal syndrome and status of the hantavirus reservoirs in Europe, 2005-2010. Euro Surveill. 2011;16:19961.
3. Clement J, Maes P, van Ypersele de Strihou C, van der Groen G, Barrios JM, Verstraeten WW, et al. Beechnuts and outbreaks of nephropathia epidemica (NE): of mast, mice and men. Nephrol Dial Transplant. 2010;25:1740–6. http://dx.doi.org/10.1093/ndt/gfq122
4. Klempa B, Radosa L, Krüger DH. The broad spectrum of hantaviruses and their hosts in central Europe. Acta Virol. 2013;57:130–7. http://dx.doi.org/10.4149/av_2013_02_130
5. Michalski A, Niemcewicz M, Bielawska-Drózd A, Nowakowska A, Gaweł J, Pitucha G, et al. Surveillance of hantaviruses in Poland: a study of animal reservoirs and human hantavirus disease in Subcarpathia. Vector Borne Zoonotic Dis. 2014;14:514–22. http://dx.doi.org/10.1089/vbz.2013.1468
6. Ali HS, Drewes S, Sadowska ET, Mikowska M, Groschup MH, Heckel G, et al. First molecular evidence for Puumala hantavirus in Poland. Viruses. 2014;6:340–53. http://dx.doi.org/10.3390/v6010340
7. Razzauti M, Plyusnina A, Niemimaa J, Henttonen H, Plyusnin A. Co-circulation of two Puumala hantavirus lineages in Latvia: a Russian lineage described previously and a novel Latvian lineage. J Med Virol. 2012;84:314–8. http://dx.doi.org/10.1002/jmv.22263
8. Golovljova I, Sjölander KB, Lindegren G, Vene S, Vasilenko V, Plyusnin A, et al. Hantaviruses in Estonia. J Med Virol. 2002;68:589–98. http://dx.doi.org/10.1002/jmv.10231
9. Sandmann S, Meisel H, Razanskiene A, Wolbert A, Pohl B, Krüger DH, et al. Detection of human hantavirus infections in Lithuania. Infection. 2005;33:66–72. http://dx.doi.org/10.1007/s15010-005-4058-8
10. Drewes S, Turni H, Rosenfeld UM, Obiegala A, Strakova P, Imholt C, et al. Reservoir-driven heterogeneous distribution of recorded human Puumala virus cases in South-West Germany. Zoonoses and Public Health. In press 2016.

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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