Usutu Virus in Bats, Germany, 2013

To the Editor: Usutu virus (USUV) is an arthropod-borne flavivirus that belongs to the Japanese encephalitis serocomplex (*1*). USUV circulates between ornithophilic mosquito vectors (mainly *Culex* spp. mosquitoes) and avian amplification hosts (*2*). Migratory birds play a key role in the introduction of USUV into new areas (*3*). USUV has recently been introduced from Africa into Europe, causing epizootics among wild birds and Usutu fever in humans (*4*–*6*). The detection and isolation of USUV from different bird and mammalophilic mosquitoes during the epizootic in Germany raise questions regarding the USUV host range (*2*,*3*). Bats have been considered natural reservoir hosts of a wide diversity of viruses, including several flaviviruses (*7*,*8*). Their ability to fly and their social behavior enable efficient maintenance, spread, and evolution of viruses.

In September and October 2013, in southwest Germany, 2 dead bats were found within ≈15 km of each other (bat 1, Ludwigshafen am Rhein, 49°28′34″N 8°26′46″E; bat 2, Waldsee, 49°23′44″N 8°26′27″E), corresponding to the previously described USUV-endemic area (*2*,*3*). A full necropsy was conducted on each bat, and samples were collected for virus detection, histologic analysis, and bat species determination.

Total DNA and RNA were extracted from tissue samples (brain, liver, lung, and heart) and subjected to reverse transcription PCR for rhabdovirus and flavivirus (*2*). Histologic analysis of the tissue samples was not successful because of autolysis. Use of a cytochrome b–specific PCR and direct sequencing of the PCR amplicons genetically identified each bat as a common pipistrelle (*Pipistrellus pipistrellus*) (*9*).

The bat samples were negative for rhabdoviruses but positive for flaviviruses (brain tissue only). Direct sequencing of the PCR amplicons revealed that the USUV sequences were related to the recently described bird-derived USUV strain BH65/11–02–03 from Germany (*2*). Attempts to isolate the bat USUV strains in cell culture were not successful, probably because of autolysis. However, the complete genome sequences of both bat USUV strains (BAT1USUTU-BNI, KJ859682; BAT2USUTU-BNI, KJ859683) were then determined directly from the brain samples by using primers (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/20/10/14-0909-Techapp1.pdf) designed from multiple alignments of USUV genomes obtained from databases.

The 2 genomes had an identical size of 11,065 nt with a 96-nt 5′ nontranslated region and a 664-nt 3′ nontranslated region. The single open reading frame encodes a polyprotein of 3,434 aa. Both bat USUV strains had 99.9% nt and 99.8% aa identity. We compared the 2 bat USUV strains with those detected in mosquitos, birds, and humans from Europe and Africa; the sequence identities of nucleotides varied from 78.3% to 99.3% and of amino acids from 90.8% to 99.3%. The sequence identity matrix with the USUV strain BH65/11–02–03 from Germany was 99.3% for nucleotides and 99.2% for amino acids. Comparison of the *Pipistrellus* bat USUV complete polyprotein sequence with mosquito and bird-derived strains showed 2 aa substitutions—one (A1236V) in the nonstructural protein (NS) 2a and the other (L1549F) in the NS3 gene—which were detected only in the bird-derived USUV strain BH65/11–02–03 from Germany. In addition, 2 additional unique amino acid substitutions (A1841V and K1870M) in the NS3 protein gene of the BAT1USUTU-BNI strain were also identified. Bayesian and maximum-likelihood phylogenetic analyses of the full-length sequences revealed the close relationship of the *Pipistrellus* bat–derived USUV strains with the 2011 bird-derived strain BH65/11–02–03 from Germany, forming a distinct group within the phylogenetic tree (group Europe 3) (Figure). A partial envelope and NS5-gene–based phylogenetic analysis that used more available sequences from databases yielded the same topology (data not shown).

Pipistrellus bats are highly prevalent in Germany. Their geographic range overlaps with that of the USUV epizootic. Thus, considerable interactions between birds, mosquitoes, and bats could have occurred. The amino acid replacements (A1236V and L1549F) detected in the NS genes of *Pipistrellus* bat–derived USUV strains and the bird-derived USUV strain from Germany suggest an adaptive evolution, which probably occurred during the introduction of the virus into Germany.

Although the role of these mutations is not known, similar mutations in the related West Nile virus modulated the host antiviral response by inhibition of interferon signaling (*10*). Our results suggest that bats probably contribute to the epizootic rather than act as a silent reservoir for the virus. In contrast, infections of bats might be merely coincidental to what may well be broader infections of vertebrates in the epizootic area. However, for confirmation of this hypothesis, further investigations are required. Although the detected bat-derived sequences are somehow distinct from sequences of other USUV strains, a spillover infection from birds or another, yet unrecognized, host cannot be ruled out. The detection of the virus exclusively in brain tissue suggests that USUV might have a higher tropism for the nervous system in bats, as opposed to the pantropism observed in birds (*2*). The detection of USUV in bats raises questions for further research, including the potential role of bats as reservoirs in Africa and transmission by mosquito vectors.

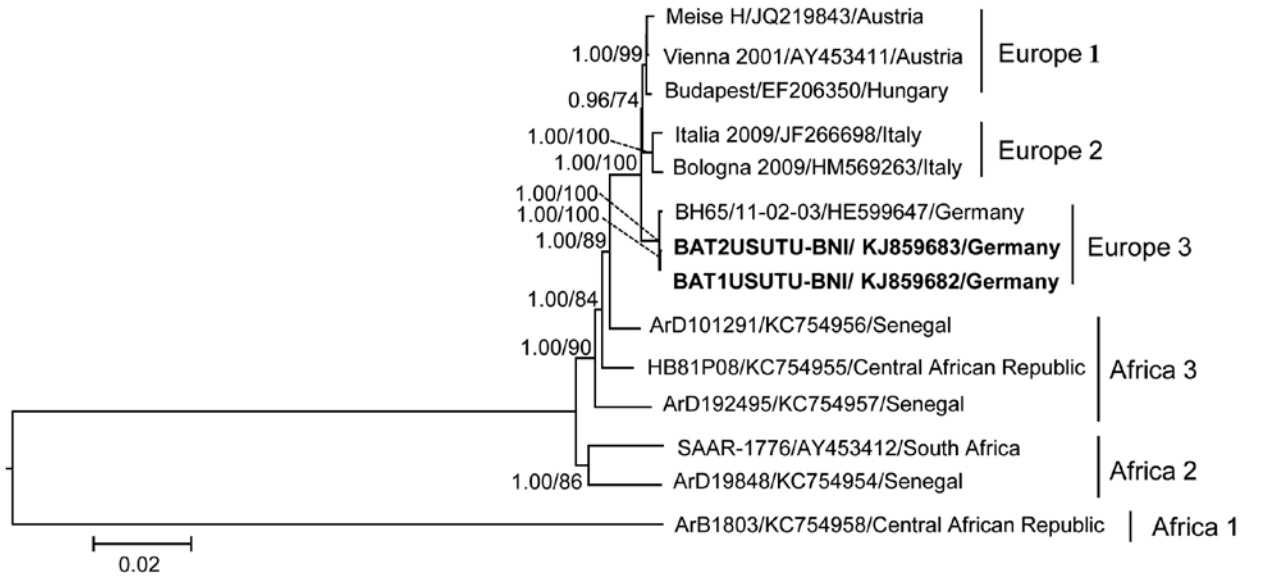

Figure. Maximum-likelihood phylogenetic tree of *Pipistrellus* bat Usutu viruses (USUV) based on full-length nucleotide sequences and showing the phylogenetic placement of the bat-derived USUV compared with human-, mosquito-, and bird-derived strains. The phylogenetic analyses were performed by using PhyML 3.0 (http://www.atgc-montpellier.fr/phyml/versions.php) with 1,000 pseudo-replicates and parallel Bayesian Markov chain Monte Carlo tree-sampling methods based on 2 runs consisting of 4 chains of 1,000,000 with a burn-in of 25% using MrBayes 3.1.2 (http://mrbayes.sourceforge.net/). The Akaike information criterion was chosen as the model selection framework and the general time-reversible model of sequence evolution with gamma-distributed rate variation among sites as the best model. Numbers at the nodes indicate maximum-likelihood bootstrap replicates (≥70%) and parallel the posterior probability values (clade credibilities ≥90%). Boldface indicates USUV strains from *Pipistrellus* bats in Germany in 2013 (this study). Strain names, GenBank accession numbers, and countries of origin for sequences used to construct the tree are indicated on the branches. Scale bar indicates mean number of nucleotide substitutions per site.

Acknowledgments

We thank Claudia Poggensee, Alexandra Bialonski, Birgit Hüsing, Corinna Thomè, Sabine Köhler, and Mathis Petersen for excellent technical assistance.

We dedicate this letter to the late Ursula Herrmann (1927–2014), who made this study possible.

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DOI: http://dx.doi.org/10.3201/eid2010.140909

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Chikungunya Virus Imported into French Polynesia, 2014

To the Editor: Chikungunya virus (CHIKV) is an emerging arthropodborne alphavirus of the family *Togaviridae* (*1*). The most common clinical manifestations of infection with CHIKV are abrupt onset of fever, headache, back pain, myalgia, and arthralgia affecting mainly the extremities; in ≈50% of patients, a rash develops, and relapsing and incapacitating arthralgia is common (*1*). Three CHIKV lineages have been characterized: West African, Asian, and East/Central/South African (*1*,*2*). The strain currently circulating in the Caribbean belongs to the Asian lineage (*2*).

In the Pacific region in 2011, a CHIKV outbreak was reported in New Caledonia (*3*). Additional outbreaks have been reported in Papua New Guinea in June 2012 (*4*), Yap State in August 2013 (*5*), and in the Kingdom of Tonga in April 2014 (*6*). In the Caribbean region, cases of CHIKV infection were reported in the French part of Saint Martin Island in December 2013, after which CHIKV rapidly spread to other Caribbean islands, including Guadeloupe (*2*), where by the end of May 2014 it had caused an estimated 23,100 infections.

On May 25, 2014, a healthy 60-year-old woman returned to French Polynesia after a 6-month stay with her husband's family in Guadeloupe, where she had been in contact with family members who reportedly had chikungunya. On the first night after arriving back home in French Polynesia, she noted headache, transient high fever, and mild arthralgia of the knees. The next day, she sought care from her general practitioner for weakness, headache, and severe polyarthralgia (wrists, fingers, knees, toes). Physical examination revealed only swollen fingers and toes; the patient was not febrile. Blood samples were collected, and the patient was administered acetaminophen and corticosteroids. Her headache persisted until day 3, and arthralgia persisted until day 4.

Laboratory tests revealed lymphopenia (589×10^6 cells/L) and slightly elevated C-reactive protein (14.2 mg/L) and liver enzyme levels (aspartate aminotransferase 44 IU/L, gamma-glutamyl transferase 58 IU/L). CHIKV infection was confirmed by a specific real-time reverse transcription PCR (rRT-PCR) with previously reported primers and probe (*7*) and by partial sequencing of the E1 gene (GenBank accession no. KJ939333). Phylogenetic analysis (Figure) showed that the virus strain isolated from the patient was most closely related to strains isolated in the British Virgin Islands in 2014 (VG14/99659, accession no. KJ451624), Yap State, Federated States of Micronesia, in 2013 (FM13/3807, accession no. KJ451622), and Zhejiang Province, China, in 2012 (CN12/chiksy, accession no. KF318729), with 100%, 99.89%, and 99.78% homology, respectively, thereby confirming its inclusion in the Asian lineage. A blood sample from the patient was inoculated into Vero and *Aedes albopictus* C6/36 cells. Cells were incubated for 6 days, after which time both supernatants were removed and tested. RT-PCR, as described above, gave positive results for CHIKV.

After CHIKV infection was confirmed, the case was immediately reported to health authorities in French Polynesia. Vector control measures were immediately implemented and included individual protection against mosquito bites (mosquito repellents) for the patient and her close social and family contacts and collective protection (insecticide spraying and breeding site elimination) targeting the house of the patient and the areas that she had visited. Written informed consent was obtained from the patient before publication of this case report.

Arbovirus diseases are endemic to French Polynesia. Dengue virus serotypes 1 and 3 have been cocirculating since 2013 (*8*); and, from October 2013 through April 2014, a large outbreak of Zika virus infection occurred (*9*). Because this case provides evidence of the possible emergence of CHIKV in French Polynesia, health authorities and health care workers in French Polynesia were immediately alerted and prepared to detect local transmission of CHIKV infection.

CHIKV is transmitted by mosquitoes of the *Aedes* species, especially *Ae. aegypti* and *Ae. albopictus* (*1*,*2*). The risk for emergence of a chikungunya outbreak in French Polynesia is high because of the presence of 2 potential vectors: *Ae. aegypti* mosquitoes, vectors of CHIKV in New Caledonia (*3*) and in Guadeloupe (*2*), and *Ae. polynesiensis* mosquitoes, potential CHIKV vectors as suggested by experimental infections (*10*).

The role of foreign travel in spreading arboviruses between French overseas territories is highlighted by the observation that the CHIKV-infected patient reported here returned from Guadeloupe and by a previous report that the 2013 outbreak of dengue virus type 3 in French Polynesia was caused by a virus introduced from French Guiana (*8*). Zika fever was reported in French Polynesia in October 2013; within the next 6

DOI: http://dx.doi.org/10.3201/eid2010.140909

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Technical Appendix

Technical Appendix Table. Primers used for the full-length amplification of Usutu virus

Name	Primer sequence	Position	Length
USUV_GF	5′-AGWYGTTSGYCTGYGTGAGC-3′	1-740	740
USUV_731R*	5′-CGCTTCGAGTGTCTGGTTCT-3′		
USUV_8F	5′-CGTCTGCGTGAGCTCTACTACTTA-3′	8-400	393
USUV_400R	5′-TTTTTGTGCCCCGATTGTT-3′		
USUV_52F	5′-TGAGATTAACACAGTGCCGG-3′	52-832	781
USUV_832R	5′-ATCTCGAAGCCTTGGTTGAG-3′		
USUV_712F	5′-AGGTGCACAAGAACCAGACA-3′	712-1727	1016
USUV_1727R	5′-GATTGCTTTGTGGCATGGGG-3′		
USUV_1560F	5′-GTTGAACACCGAGGCATACTACAT-3′	1560-2461	902
USUV_2434R	5′-TGGCGAGAAAGAGGAGCAC-3′		
USUV_2356F	5′-CAGGGTCTAATGGGAGCTCT-3′	2356-3320	965
USUV_3320R	5′-CCTGGGCAATAGTCAAAGTC-3′		
USUV_3241F	5′-CGGCGTGAAGGTTACAAAGT-3′	3241-4160	920
USUV_4160R	5′-GAGAACTGCCCTGTTGATGT-3′		
USUV_3776F	5′-CCTTCAAAATTCAACCAGGC-3′	3776-4778	1001
USUV_4778R	5′-ATAGCTGCCCCTCTTGTGGT-3′		
USUV_4541F	5′-GGACACCATGGGCAATAATACCT-3′	4541-5872	1332
USUV_5872R	5′-CTCTGCTGGCCCCAAAGTTCG-3′		
USUV_5669F	5′-ACACCGGGAAAACAGTCTGG-3′	5669-6802	1134
USUV_6802R	5′-TGAGCAGAGCCAGCAATARR-3′		
USUV_6661F	5′-GTTTTCTTGCTCCTCGTTCA-3′	6661-7457	797
USUV_7457R	5′-CCAATCAGTAAAATCTGGCC-3′		
USUV_7368F	5′-GGTAGATGGTTTGGTGGCTA-3′	7368-8160	793
USUV_8160R	5′-TTGTTCTTCCACCTCAGCAC-3′		
USUV_8038F	5′-TATGGCTGGAACCTTGTCAC-3′	8038-9057	1020
USUV_9057R	5′-CCCCATCATGTTGTAAATGC-3′		
USUV_8987F	5′-AAATGGTGGACGAAGAAAGG-3′	8987-9865	879
USUV_9865R	5′-TCCTTCCGTCCTTCATGATC-3′		
USUV_9643F	5′-GAGAACGGAGAAGAAAGGGT-3′	9643-10823	1181
USUV_10823R	5′-AACAGTTCGCATCACCGTCT-3′		
USUV_10673F	5′-GGGACCCTGCCTATTGG-3′	10673-11027	355
USUV_11027R	5′-GCGCTCTGTGCCTTGTGGTTGAT-3′		
USUV_ADF	5′-GAAAGCCCCTCAGAACCGTTTC-3′	10646-11066	420
USUV_11014R*	5′-AGATCCTGTGKTCTWSYYCMCCAYCAG-3′		

*Nucleotide positions are according to the Vienna strain genome (GenBank accession no. AY453411) (*1*).

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