Usutu virus (USUV) is an arthropod-borne, single-stranded RNA virus and belongs to the Japanese encephalitis virus group within the family Flaviviridae. This virus was first isolated in 1959 from *Culex neavei* mosquitoes collected in Ndumu, Natal, South Africa. Vectors of USUV are ornithophilic mosquitoes of the genera *Culex*, *Coquillettidia*, *Mansonia*, and *Culiseta*.

Wild birds are the principal reservoir for USUV and migratory birds play a key role in the introduction of USUV into new areas. Outside Africa, USUV emerged in 2001 in Vienna, Austria and caused deaths in blackbirds (*Turdus merula*) and great gray owls (*Strix nebulosa*). In 2002, USUV was still circulating in Austria, demonstrating that USUV has managed to overwinter in a local bird-mosquito cycle in central Europe. More recently, USUV-specific RNA or antigen was also detected in birds or mosquitoes in Hungary, Switzerland, Italy, and Spain. In the summer of 2009, USUV-related illness were reported in two immunocompromised patients in Italy. Despite these cases, the potential of USUV to cause human disease needs further investigations.

In 2002, an extensive analysis of serum samples from healthy birds captured in the United Kingdom was performed and demonstrated that a significant number of the serum samples contained neutralizing antibodies against USUV. This study was subsequently followed by a seroconversion study of chickens from the time of hatching until the age of eight or nine months and seroconversion to USUV was detected. Neutralizing antibodies against USUV were also demonstrated in serum samples of wild birds captured during 2002–2005 in Germany. In the United Kingdom and in Germany, no obvious reduction in the bird population was observed during this period. However, it was not known if USUV circulate in German mosquito populations. Therefore, the natural vectors of USUV in Germany also remain unknown.

Mosquitoes were collected during July–September 2009 and April–September 2010 at 11 sites in Germany (Figure 1). Trappings were made with CO₂-baited encephalitis vector surveillance traps (BioQuip, Compton, CA) and at the Weinheim trapping site (Figure 1) with gravid traps designed according to the Centers for Disease Control and Prevention (Atlanta, GA) gravid trap model 1712 (John W. Hock Company, Gainesville, FL). All collected mosquitoes were frozen at ~70°C and transported to the laboratory.

Mosquito identification, RNA extraction, and virus isolation were performed as described. Extracted RNA was analyzed by using a newly designed USUV-specific real-time reverse transcription–polymerase chain reaction (RT-PCR) with primers USUTU F (5’-CGTTTCTCGACTTGA-3’), nucleotide positions 3294–3311; nucleotide positions are given according to numbering in USUV strain Vienna, GenBank accession no. AY453411), USUTU R (5’-GCTAGTAGTAG TTCTTAAGA-3’), nucleotide positions 3384–3364, probe USUTU P (5’-FAM-ACCCTACCATGAAACAT-BHQ1-3’), nucleotide positions 3325–3346, FAM, 6-carboxyfluorescein; BHQ-1, black hole quencher 1). The target was a 91-nucleotide region of the nonstructural protein 1 gene. Real-time RT-PCR was used as described.

A total of 70,378 female mosquitoes (16,057 in 2009 and 54,321 in 2010) were captured, identified to species, and pooled according to species (25 mosquitoes per pool). The most abundant mosquito was *Aedes vexans* (46.6%) followed by *Ochlerotatus stricticus* (32.0%) and *Culex pipiens/Cx. torrentium* (15.0%) (Table 1). USUV-specific RNA was detected in one pool (pool no. 1477) of *Cx. pipiens/Cx. torrentium* captured in the middle of August 2010 at Weinheim (Figure 1). Compared with 2009, the number of collected *Cx. pipiens/Cx. torrentium* was nearly identical in 2010 (Table 1). The USUV infection rate was low because only 1 of 422 *Culex pipiens/Cx. torrentium* pools from Weinheim was positive for USUV.

For extensive phylogenetic analysis, a part of the envelope protein coding sequence (nt positions 1159–2527) was amplified by RT-PCR in two overlapping fragments with reported primers. Subsequent phylogenetic analysis by Bayesian inference showed a close relationship between novel USUV strain...
1477 and the USU338-04 strain from Austria, which detected in a dead blackbird (*Turdus merula*) in 2004 (Figure 1).

Our study demonstrates the potentially emerging USU in southwestern Germany. The virus most likely spread from Austria to Germany rather than being independently introduced from Africa. This finding is the first direct detection of USU in Germany, although neutralizing antibodies were detected in wild birds in Germany.11 No obvious increase in deaths of birds were detected in Germany in 2009 and 2010, which suggested a low prevalence or virulence of circulating USU. However, in Italy, USU-related illness in humans was reported even though the USU infection rate in mosquitoes was rather low.8

USU strain 1477 was detected in a pool of *Cx. pipiens pipiens* mosquitoes in Weinheim, a small city in the upper Rhine valley and the only trapping site that represented an urban ecosystem. The density of blackbirds and *Cx. pipiens pipiens* mosquitoes in such an urban ecosystems is much higher compared with an agro-ecosystems or forest. This might explain why USU was not found in other regions of Germany covered by our trapping sites.

In conclusion, after detection of several arboviruses that are new to Germany in 200912, 13 another important arbovirus was demonstrated to circulate in Germany in 2010. Thus, mosquito-based surveillance for arboviruses in Germany is important for public health because it provides data about arbovirus activity and distribution. Further studies will be conducted to estimate the medical importance of USU in southwestern Germany.

Received April 20, 2011. Accepted for publication June 10, 2011.

Authors' addresses: Hanna Jöst, German Mosquito Control Association, Ludwigstrasse 99, D-67165 Waldsee Germany, E-mail: hanna.joest@gmx.de. Alexandra Bialonski, Deborah Maus, Stephan Günther, and Jonas Schmidt-Chanasit, Department of Virology, Bernhard Nocht Institute for Tropical Medicine, Bernhard Nocht Strasse 74, D-20359 Hamburg, Germany, E-mails: bialonski@bni-hamburg.de, maus@bnitm.de, guenther@bni-hamburg.de, and jonassi@gmx.de. Vittorio Sambri, Microbiology Unit, Regional Reference Centre for Microbiological Emergencies, Azienda Ospedaliero–Universitaria di Bologna, Policlinico S.Orsola-Malpighi, Bologna Italy, E-mail: vittorio.sambri@unibo.it. Martin Eiden and Martin H. Groschup, Friedrich Loeffler Institute, Federal Research Institute for Animal Health, Institute for Novel and Emerging Infectious Diseases, Südufer 10, D-17493 Greifswald, Insel Riems, Germany, E-mails: martin.eiden@fli.bund.de and martin.groschup@fli.bund.de. Norbert Becker, German Mosquito Control Association, Ludwigstrasse 99, D-67165 Waldsee, Germany and University of Heidelberg, In Neuenheimer Feld 230 D-69120 Heidelberg, Germany, E-mail: norbertfbecker@web.de.


**REFERENCES**

1. Adam F, Diguette JP. *Virus d’Afrique (Base de Données). Centre Collaborateur OMS de Référence et de Recherche pour les Arbovirus et les Virus de Fièvres Hémorragiques (CRORA).*
USUTU VIRUS, GERMANY

553


