

Putative Lineage of Novel African Usutu Virus, Central Europe

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

[Sarah Gregory] The authors characterize the complete genome of the putative novel Usutu virus strain Usutu-BONN detected in a dead black bird from Germany. Genomic analysis revealed several unique amino acid substitutions among the polyprotein gene. Phylogenetic analysis demonstrated that Usutu bond constitutes a putative novel African Usutu virus lineage, which was probably, recently, introduced to Central Europe. Originally isolated from a *Culex neavei* mosquito in South Africa in 1959, Usutu virus was subsequently detected in different mosquito and bird species throughout sub-Saharan countries. Usutu virus has recently been introduced to Europe, where it caused wide spread deaths among resident bird populations, established a local transmission cycle, and became a resident pathogen. Usutu virus is maintained in an enzootic cycle involving mosquitos as vectors and birds as the main amplifying hosts. Humans are considered incidental or dead-end hosts. The authors have demonstrated that bats could also be infected with Usutu virus and might act as amplifying hosts, and there is increasing evidence that Usutu virus is pathogenic for humans, thus becoming a potential public health problem. On the basis of genetic differences, in comparison with Usutu virus strains from Africa, the Usutu virus strain from Europe, except those from Spain, form a distinct clade within Usutu virus phylogeny. The detection and isolation of Usutu virus from different bird species and mammalophilic mosquitos during the 2011 epizootic in Germany raised questions regarding the Usutu virus host range. Thus, as a part of the German Arbovirus Surveillance Program, the authors continued the monitoring of the mosquitoes, birds, and bats for the presence of Usutu virus.

During May through October of 2014, about 23,300 female mosquitoes from different parts of Germany were trapped, morphologically identified, and pooled. During January through November 2014, a total of 8 dead Pipistrellus bats and 32 dead birds, mainly blackbirds, from different regions of the country were subjected to complete necropsy, and samples were collected for virus detection. Total RNA and DNA from homogenized mosquito pools and tissue samples of brain, liver, lung, and heart from bats and birds were extracted. Extracted samples were analyzed for the presence of flavavirus RNA by using a modified pan-flavavirus reverse transcription PCR. Positive results were found for five mosquito pools, five blackbirds, and 1 bat. Direct sequencing of the pan-flavavirus PCR amplicons showed Usutu virus nucleic acid sequences in each sample. The positive samples were further subjected to PCRs for the amplification of a partial segment of the Usutu virus envelope and nonstructural 5 gene. Sequencing results showed that all samples, except one blackbird-derived Usutu virus sequence, were identical and originated from southwest Germany. The Usutu virus positive blackbird sample, which exhibited numerous nucleotide and amino acid changes compared with the other sequences, had been found outside of the Usutu virus endemic area. Full-length genome sequence of this putative novel Usutu virus strain, designated Usutu-BONN, was successfully attained.

In conclusion, the authors detected and genetically characterized a putative novel Usutu virus strain (Usutu-BONN) by determining its complete genome sequence and comparing it with Usutu virus strains for which complete polyprotein-encoding sequences are available. They

demonstrated that the Usutu-BONN strain from Germany constitutes a putative novel Usutu virus lineage. The unique synonymous mutations detected in the E and Non-structural 2a genes of Usutu-BONN strains may suggest an adaptive evolution. In this strain, 1 putative cleavage site of the viral polyprotein responsible for processing of structural proteins was changed. Given that Usutu-BONN has not led to massive deaths among birds and has not yet been found in other hosts or mosquito vectors, it seems evident that this strain was recently introduced into Germany and evolved in another geographic region, probably Africa. The possibility of an African origin of this virus strain is strengthened by the fact that phylogenetic analysis of complete polyprotein sequence established a separate basal lineage for the Usutu-BONN strain in a sister relationship with the African Usutu virus strains. This suggests that Usutu-BONN has evolved in parallel with strains from Africa, sharing a recent common ancestor. Similar to other strains, this putative novel Usutu virus strain was introduced into Europe by way of viremic migratory birds returning from winter migrations from Africa to Europe or through ship- or aircraft-borne transportation of Usutu virus infected mosquitos from Africa. However, identification of the possible sources of this new Usutu virus strain will require sequence information from neighboring countries where Usutu virus has been detected. The detection of Usutu virus in a Pipistrellus bat 1 year after the first detection of Usutu virus in bats from the same area in 2013 further strengthens the previous hypothesis that bats may contribute to the epizootic by serving as amplifying or reservoir hosts. The unique mutations in the Nonstructural-2a protein of the Usutu-BONN strain are located very close to the identical mutation observed in the previously described bat-derived Usutu virus strains. Although the biological consequences of these mutations are not known, similar mutations in West Nile virus were responsible for inhibition of interferon signaling.

You can read the entire September 2015 article, “Putative Lineage of Novel African Usutu Virus, Central Europe” online at cdc.gov/eid. I’m Sarah Gregory for Emerging Infectious Diseases.

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