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A metagenomic survey reveals rhabdo and negevvirus sequences in mosquito pools from Turkey

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Purpose: Mosquitoes are among the most frequently-observed arthropod vectors, with the potential to transmit several viral diseases of significant human health impact. In addition to the viruses associated with vertebrate infections, several viruses belonging to diverse viral families have been identified in mosquito populations, suggesting the frequent circulation of these agents. In this study, a metagenomic sequencing approach was utilized to investigate the abundance and diversity of viruses that replicate in mosquitoes, collected during a field surveillance campaign in Turkey.

Methods & Materials: Four mosquito pools that comprise 13-45 individuals of *Culex pipiens* sensu lato (n=2) and *Aedes caspius* (n=2) mosquitoes, collected in Mediterranean and Thrace regions during 2015 were evaluated. The pools were processed via standard protocols employed for virus isolation and subjected to sequencing on an Illumina HiSeq 1500 platform. Sequences were analyzed with a workflow for the identification of highly divergent viruses that include trimming for quality reads, substraction of irrelevant sequences, alignment, taxonomic binning and visualization steps, and further assembled into contigs and near-complete genomes.

Results: In an *Ae. caspius* pool, previously positive for flavivirus screening, sequences belonging to an Ochlerotatus caspius flavivirus were identified. Negevvirus sequences were detected in two *Culex pipiens* pools, originating from different regions. In the *Culex pipiens* pool from Thrace region, complete genome of a rhabdovirus, closely-related to Merida virus, a putative novel rhabdovirus recently-identified in *Cx. quinquefasciatus* mosquitoes and in the Yucatan Peninsula of Mexico, was characterized. The assembled sequence comprised over 11800 nucleotides and demonstrated 83% sequence similarity to Merida virus in pairwise comparison.

Conclusion: Viral metagenomic analysis can overcome the limitation of standard methods in virus screening and proves to be an effective approach for investigating the potential viral diversity associated with field-collected mosquitoes. The employed approach have resulted in characterization of negevvirus and *Culex* rhabdoviruses for the first time in mosqutoes from Turkey.

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High diversity of replication-associated protein encoding circular viruses in guano samples of European bats

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Purpose: Bats are increasingly recognized as hosts and sources of viruses with important evolutionary relevance or zoonotic capability even from Europe. Based on a recent study of virome analyses of bats in China, circular single-stranded DNA viruses along with members of the family *Parvoviridae* constitute the main groups of DNA viruses within bat faecal virome. In the last couple of years several novel circular replication-associated protein encoding single-stranded (CRESS) DNA viruses were described in bats from Brazil, China, USA and Tonga, Oceania. Over decades, metagenomic studies of bat samples along with surveillance studies using degenerate, group specific primers have expanded the number of newly described, often unclassified, viruses within the family *Circoviridae.* Other CRESS DNA viruses were also described from bat fecal samples, such as Gemycircularviruses of the family *Mycodnaviridae.* The aim of our study was to reveal the diversity of CRESS DNA viruses in Central-Eastern European bat fecal samples collected in Ukraine, Hungary, Romania, Serbia and Georgia.

Methods & Materials: Faecal samples were collected from multiple localities in Georgia, Hungary, Romania, Serbia and Ukraine by trained chiropterologists during bat ringing and bat rehabilitation activities. In addition 42 guano samples, collected for previous studies in Hungary were randomly selected and subjected to random primed reverse transcription PCR and semiconductor sequencing by using the Ion Torrent PGM platform. Samples from Georgia, Romania, Serbia and Ukraine were further tested with nested-PCR, targeting the *rep* gene of *Circoviridae* family. The identified viral sequences were used for inverse PCR (iPCR) primer design, using Geneious software v9.1. iPCR amplicons which corresponded to the size of 1.5-3 kb were purified, than PCR products were sequenced by genome walking method with Big Dye Terminator Cycle Sequencing Ready Reaction on ABI-PRISM 3100 Genetic Analyzer sequencing platform.

Results: Several novel viruses were described, with a potentially novel viral species within the family *Circoviridae*. Niminivirus from the family *Geminiviridae* was also described at the first time from Europe. CRESS DNA viruses of the family *Mycodnaviridae* were also detected.

Conclusion: The results of this study provide the first dataset on CRESS DNA viruses, circulating among European bat species and discuss their phylogenetic relationship with other previously described viruses.

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