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Novel Ozark Orthohantavirus in Hispid Cotton Rats (*Sigmodon hispidus*), Arkansas, USA

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We report a novel orthohantavirus, putatively named Ozark orthohantavirus, in hispid cotton rats captured within the Ozark Plateau in Arkansas, USA. This virus phylogenetically clusters with other orthohantaviruses that cause severe human disease. Continued orthohantavirus surveillance and virus sequencing are needed to address the potential public health threat of this virus.

Orthohantaviruses (family Hantaviridae, genus *Orthohantavirus*) are a group of zoonotic viruses primarily found in muroid rodents; many of the viruses are pathogenic in humans (*1*). Pathogenic orthohantaviruses in the Americas are hosted by rodents in subfamilies Sigmodontinae and Neotominae and cause hantavirus cardiopulmonary syndrome (HCPS) in humans, which has a 30%–40% case-fatality rate (*2*,*3*). Although several pathogenic orthohantaviruses have been identified in the Americas, the specific etiologic virus is unknown for many HCPS cases (*2*).

We report a novel orthohantavirus species, putatively named Ozark orthohantavirus or Ozark virus (OZV), in hispid cotton rats (*Sigmodon hispidus*) in Arkansas, USA. Hispid cotton rats are a reservoir host of a notable pathogenic orthohantavirus, Black Creek Canal virus (BCCV) (*4*), in the United States and have also been identified as the host of the proposed Muleshoe virus (*5*). Despite the wide distribution of hispid cotton rats in North America (22 US states and northern Mexico), previously published orthohantavirus surveillance and detection in this rat species has been limited to only Florida and Texas in the United States.

We analyzed frozen lung tissue samples collected from euthanized hispid cotton rats previously captured during 2020 and 2021 in the Ozark Plateau region of Arkansas, USA (*6*). Of 338 rat samples previously tested, 26 (7.7%) were orthohantavirus-seropositive; seropositive rats had been captured in 5 distinct grassland sites (*6*).

We performed homogenization, filtration, and nuclease pretreatment of available lung tissue samples from 13 orthohantavirus-seropositive rodents captured in 3 of the 5 unique grassland sites (Appendix Table, https://wwwnc.cdc.gov/EID/article/29/12/23-0549-App1.pdf) (*7*,*8*). We then extracted RNA by using Invitrogen TRIzol (Thermo Fisher Scientific, https://www.thermofisher.com) according to manufacturer guidelines. We used the NEBNext rRNA Depletion Kit (human/mouse/rat) to remove host rRNA, then the NEBNext Ultra II RNA Library Prep Kit (both from New England Biolabs, https://www.neb.com) to construct libraries. We performed next-generation sequencing by using the Illumina NovaSeq system (https://www.illumina.com). We quality filtered and de novo assembled the raw data and annotated the contigs by using LazyPipe (*9*).

We obtained complete genome sequences of OZV coding regions for small (S), medium (M), and large (L) segments from 2 rat samples and partial genome sequences from 6 other rat samples that included 3 additional complete S and 4 additional complete M segment sequences (Appendix Table). We used Open Reading Frame (ORF) Finder (https://www.ncbi.nlm.nih.gov/orffinder) to detect ORFs and the Expasy translate tool (https://www.expasy.org) to translate ORFs to amino acid sequences. We compared corresponding nucleic acid and protein phylogenies of

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each OZV genome segment with BCCV and other related orthohantavirus sequences obtained from GenBank by using IQ-TREE2 (http://www.iqtree.org). We then used the Sequence Demarcation Tool version 1.2 program (http://web.cbio.uct.ac.za/~brejnev) to compare protein sequence pairwise identities of each OZV segment with those of closely related orthohantaviruses. Finally, we performed pairwise evolutionary distance (PED) analyses by using TREE-PUZZLE version 5.2 (http://www.tree-puzzle.de) with a PED cutoff value of 0.1 for species classification (*10*).

OZV nucleotide sequences most closely clustered with other sigmodontine-borne orthohantaviruses, particularly BCCV and Bayou virus (BAYV), which are pathogenic to humans, and Catacamas virus (CATV), which is not known to cause human infections (*2*). OZV S segment contig lengths were 1,988 and 1,884 nt and were 80.84% similar to BCCV, 81.15% similar to BAYV, and 80.93% similar to CATV S gene segments (Appendix Figures 1). OZV M segment contig lengths were 3,690 and 3,709 nt and were 77.91% similar to BCCV and 78.11% similar to BAYV (Appendix Figures 2). OZV L segment contig lengths were 6,523 and 6,462 nt and were 80.32% similar to BCCV, 80.16% similar to BAYV, and 80.01% similar to CATV (Appendix Figures 3). Pairwise relationships for protein sequences among OZV and related viruses were similar to those observed for nucleotide sequences (Figure, https://wwwnc.cdc.gov/EID/article/29/12/23-0549-F.htm; Appendix Figures 4–6). PED results for sigmodontine- and neotomine-borne orthohantaviruses indicated that OZV is a novel species with a PED value >0.1 and is closely related to BCCV, BAYV, and CATV (Appendix Figure 7).

OZV is the second definitive orthohantavirus species identified in hispid cotton rats. This discovery also expands the geographic distribution of orthohantavirus-carrying hispid cotton rats in the United States, previously limited to Florida and Texas; because of OZV's similarity to BCCV and BAYV, which cause severe disease, this discovery provides crucial public health information. OZV identification also informs broader orthohantavirus evolution, especially for within-host evolution and divergence. Although uncommon, multiple orthohantaviruses in a single reservoir host species have been observed, particularly in cricetid-borne orthohantaviruses in the Americas (*3*).

In conclusion, hispid cotton rats are primarily found in grassland and agricultural habitats, and their range comprises the entire state of Arkansas. At least 1 HCPS case has been recorded in Arkansas; because of its close phylogenetic relationship with known human pathogens, OZV should be considered a potential cause of future HCPS cases in Arkansas, surrounding states, and other areas that harbor hispid cotton rats. Continued surveillance is needed to address the potential public health threat of OZV throughout the distribution range of the hispid cotton rat host.

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Zoonotic Marine Nematode Infection of Fish Products in Landlocked Country, Slovakia

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Fish products in Slovakia have been heavily infected with *Anisakis* spp. larvae, which causes human anisakiasis. We found larvae in all tested samples of frozen Atlantic herring. Anisakid allergen t-Ani s7 testing revealed 2 positive cases in humans, signaling need for health authorities to closely monitor zoonotic marine parasites, even in inland areas.

Food safety is an unquestioned global public health imperative. Strict controls on food before release to markets are intended to prevent disease caused by agents of infectious diseases, including parasitoses such as human anisakiasis. Anisakiasis, an emerging zoonosis, is caused mainly by marine nematodes of the genus *Anisakis*. They mature in dolphins and whales, but their third-stage larvae, which reside in the flesh, gonads, and body cavities of marine fish, can infect humans who consume raw or undercooked fish (*1*). Most human cases are reported in Japan, Spain, and South Korea (*1*).

Zoonotic diseases caused by marine parasites have been largely confined to coastal regions, but surveillance by health authorities in landlocked countries is lacking. However, global trade and the increasing popularity of raw fish consumption have contributed to emergence of that disease. We provide data on extensive infection of fish products with *Anisakis* larvae in Slovakia, a landlocked country in central Europe. We also report seropositive cases in a group of volunteers regularly eating fish products. We conducted this study in accordance with ethics standards in the 2013 revision of the Declaration of Helsinki of 1975. It was approved by the ethics committee of the Institute of Parasitology, Slovak Academy of Sciences (EC/01/2018; December 14, 2018).

We examined 100 frozen Atlantic herring (*Clupea harengus*) provided by a fish product supplier and 18 packages of ready-to-eat pickled herring from local supermarkets for anisakid larvae. We found 4,163 larvae in frozen Atlantic herring at an intensity of infection of 2–368 (mean 42) larvae/fish (Figure, panels A–C, E–H). Although we found most larvae in the abdominal cavity, we also found them in the muscles of 1/3 and the gonads of 1/5 of fish we examined. Although all larvae were dead, even dead parasites or their residues in contaminated fish products can cause allergic reaction in sensitized persons (*2*,*3*). In addition, we found anisakids in 1/3 of ready-to-eat pickled herring (1–9 larvae/fish) (Figure, panel D).

We morphologically identified larvae as *Anisakis* spp. and used several larvae for genotyping based on the ≈800 bp–long internal transcribed spacer region. Sequences of all isolates were identical to those of *A. simplex* sensu stricto (Appendix, https://wwwnc.cdc.gov/EID/article/29/12/23-0674-App1.pdf), considered the predominant agent of human anisakiasis (*4*).

We used a Trisakis 170, *A. simplex* IgE-ELISA kit (Laboratorio de Parasitología, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela, Spain) to test IgE sensitization to *Anisakis* spp. in a human population. The kit detects IgE to the recombinant allergens r-Ani s 1 and t-Ani s 7 in human serum, which we collected in 2020 from 91 volunteers who regularly ate fish products. The allergen tests are highly specific (up to 100%), as deduced also from primary amino acid sequences of both allergens that have no significant homologies with other allergens to which humans are known to be sensitized. Sensitivity reaches 61.1% for Ani s 1 and 93.9% for Ani s 7; many researchers consider serum truly