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Rabbit Hepatitis E Virus, Ukraine, 2024

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We identified hepatitis E virus (HEV) 3ra, a potentially zoonotic subtype, in rabbits in Ukraine, which highlights their potential role as reservoirs. Screening of rabbit fecal samples identified HEV-3ra. Public health services in this country should improve HEV surveillance and expand HEV sampling.

Hepatitis E virus (HEV), family Hepeviridae, species *Paslahepevirus balayani*, is a major cause of acute enterically transmitted hepatitis worldwide, particularly in developing nations, where its public health impact is substantial (*1*). *P. balayani* has 8 genotypes (*2*). Zoonotic genotypes 3 and 4 are globally distributed and transmitted to humans through consumption of infected animal products or contact with infected animals, particularly pigs (*3*). Rabbits (*Oryctolagus cuniculus*) have been identified as reservoirs for HEV, specifically the potentially zoonotic HEV-3ra subtype linked to human infections, with cases reported during 2015–2016 (*4*). Despite widespread detection of HEV in Western and Central Europe, its prevalence and the role of animals in transmission remain underexplored in Eastern Europe, especially Ukraine. That knowledge gap is concerning because of the zoonotic risk from rabbit meat consumption or environmental exposure (*5*). The risk might be heightened in situations of armed conflict, where greater reliance on bushmeat could increase the chances of spillover events. We investigated the prevalence and molecular characterization of HEV in domestic rabbits from Ukraine.

In April 2024, we collected 108 fresh fecal samples from clinically healthy Californian domestic rabbits (*O. cuniculus*) born and raised on private family farms in the Sumy region, northeastern Ukraine. We suspended samples in 10% phosphate-buffered saline (pH 7.2) and centrifuged at 8,000 × g for 5 minutes. We extracted total nucleic acids from 200 μL of the supernatant by using the QIAamp Cador Pathogen Mini Kit (QIAGEN, https://www.qiagen.com) on a QIAcube automated system, according to the manufacturer's protocol. For HEV RNA, we used a pangenotypic quantitative reverse transcription PCR (RT-PCR) targeting the conserved open reading frame (ORF) 3 region (*6*), then used a broad-spectrum nested RT-PCR targeting the ORF1 region (*7*). We used the World Health Organization PEI 6329/10 subgenotype 3a (GenBank accession no. AB630970) as a positive control. We performed real-time PCR on a CFX96 Real-Time PCR System (Bio-Rad Laboratories, https://www.bio-rad.com) by using the NZYSupreme One-Step RT-qPCR Probe Master Mix (2×) kit plus ROX dye (NZYTech, https://www.nzytech.com). We performed nested RT-PCR on a T100 thermocycler (Bio-Rad Laboratories) by using the Xpert One-Step RT-PCR Kit (GriSP, https://grisp.pt) in the first round and the Xpert Fast Hotstart Mastermix 2× with dye (GriSP) in the second round, according to the manufacturer's instructions. We used Sanger sequencing to sequence the suspected positive amplicons

Table. Details of HEV genome fragments obtained and analyzed in study of rabbit hepatitis E virus, Ukraine, 2024*

Sequence ID	Length, bp	Nucleotide start position	Nucleotide end position	GenBank accession no.
5	235	4263	4498	PQ541214
5	150	1765	1915	PQ541215
5	314	5836	6150	PQ541216

*Nucleotide start and end positions are relative to the NC_001434 reference sequence.

in both directions. We aligned trimmed sequences by using Sequence Alignment Editor version 7.1.9 (BioEdit, https://bioedit.software.informer.com) and compared with nucleotide sequences from GenBank.

To further analyze positive samples, we used sequence-independent single-primer amplification with a previously described protocol (*8*), and the sequence-independent single-primer amplification samples underwent next-generation sequencing at Novogene (https://www.novogene.com) by using the NovaSeq 6000 platform (Illumina, https://www.illumina.com). We trimmed data by using BBduk

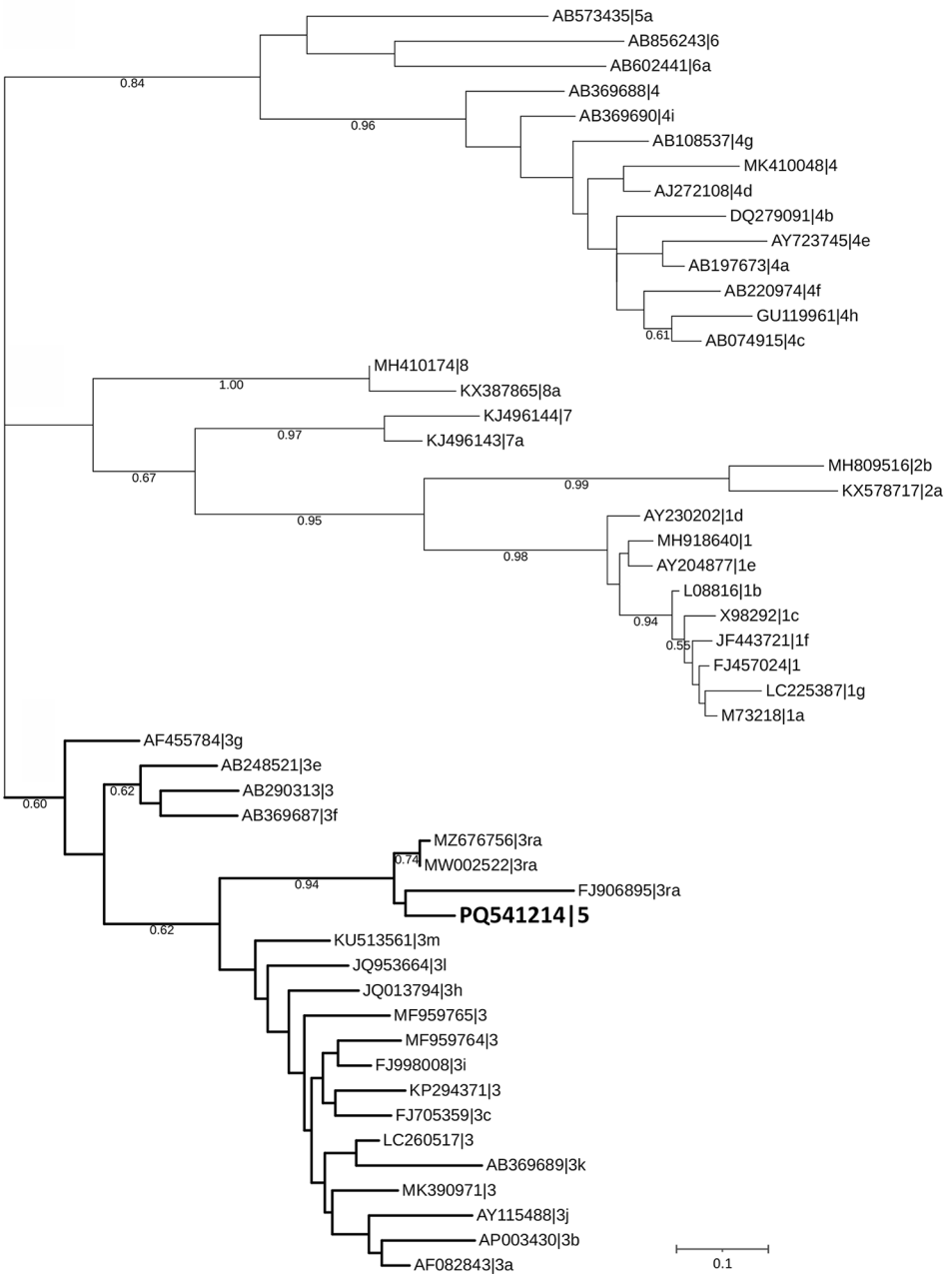

Figure. Phylogenetic tree of the RNA-dependent RNA polymerase sequence of rabbit hepatitis E virus (HEV), Ukraine, 2024. Bold indicates the HEV sequence identified in this study. The tree was inferred using MEGA X software (https://www.megasoftware.net) with the Kimura 2-parameter substitution model and visualized using the Interactive Tree of Life (https://itol.embl.de). The tree includes 51 HEV nucleotide sequences, with reference sequences retrieved from GenBank, displayed with their accession number, genotype, and subgenotype. The tree is structured into distinct clusters corresponding to different HEV genotypes and subgenotypes; the detected sequence groups within the HEV-3ra cluster. This placement indicates its close relationship to previously reported rabbit HEV sequences. Sequence analysis confirmed the genome identity as HEV. The ORF2 fragment (GenBank accession no. PQ541216) showed identity with 2 human sequences from Switzerland: 92.04% identity with GenBank accession no. OX044324 and 91.72% with GenBank accession no. OV844765. One ORF1 fragment (GenBank accession no. PQ541214) showed identity with rabbit sequences from Australia: 91.42% with GenBank accession no. MW002522 and 90.99% with GenBank accession no. MZ676756. Scale bar indicates nucleotide substitutions per site.

version 38.86 (https://jgi.doe.gov/data-and-tools/software-tools/bbtools) to remove adaptor sequences and low-quality bases. We mapped the trimmed reads to the HEV reference genome by using BBMap version 38.86 (https://jgi.doe.gov/data-and-tools/software-tools/bbtools), processed the reads with Samtools version 1.18 (http://www.htslib.org) to generate binary alignment map files, and created a consensus FASTA sequence. We conducted phylogenetic analysis by using MEGA X software version 10.2 (https://www.megasoftware.net) and applied the maximum-likelihood method with 1,000 bootstrap replicates. We used the HEVnet tool (https://www.rivm.nl/en/hevnet) for further genotyping (*9*).

We detected HEV RNA in 1 (0.93%, 95% CI 0.02%–5.05%) rabbit stool sample from the 108 samples tested. We obtained 1 genome fragment from ORF2 and 2 fragments from ORF1 (Table). Sequence analysis confirmed the genome identity as HEV. The ORF2 fragment (GenBank accession no. PQ541216) showed identity with 2 human sequences from Switzerland: 92.04% identity with GenBank accession no. OX044324 and 91.72% with GenBank accession no. OV844765. One ORF1 fragment (GenBank accession no. PQ541214) showed identity with rabbit sequences from Australia: 91.42% with GenBank accession no. MW002522 and 90.99% with GenBank accession no. MZ676756. The other ORF1 fragment (GenBank accession no. PQ541215) displayed lower similarity to sequences from human samples from France (87.59% with GenBank accession no. MF444074) and rabbit samples from China (86.57% with GenBank accession no. KX227751).

Phylogenetic analysis confirmed clustering of the obtained RNA-dependent RNA polymerase genome fragment to the rabbit-associated HEV-3ra (Figure). The HEV-3ra prevalence in rabbits reported in this study (0.93%) is lower than in another study from Europe, in which rabbit-associated HEV prevalence ranged from 7% to 23% in domestic and wild rabbits (*5*). However, the absence of HEV RNA has also been reported in other regions of Europe, such as in Portugal (*10*). Those differences could be attributed to regional variations in sampling and detection methods, environmental factors, and surveillance intensity.

In summary, this study provides evidence for HEV circulation in Ukraine, specifically the potentially zoonotic HEV-3ra in a domestic rabbit, addressing a key gap in its epidemiology. The genetic similarity of the detected HEV-3ra strain to those found in humans and animals elsewhere highlights the subgenotype's zoonotic potential and risk for cross-border transmission. Future research should expand sampling across species and regions using molecular and serologic studies to clarify transmission dynamics and public health risks. The findings in this study emphasize the need for robust surveillance in animals and humans to clarify HEV circulation.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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All authors contributed to the study's conception and design, commented on previous versions of the manuscript, and read and approved the final manuscript. Material preparation, data collection, and analysis were performed by S.S.-S. and J.R.M. The first draft of the manuscript was written by S.S.-S. and J.R.M.

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Human Adenovirus B55 Infection in Patient without Recent Travel History, France

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We report a rare case of pneumonia caused by human mastadenovirus (HAdV) B55 in France in a patient without recent travel history. HAdV-B55 infection was identified retrospectively after being detected in feces during an investigation for concomitant diarrhea. This case suggests possible silent endemic circulation of HAdV-B55 in France.

Human adenovirus (HAdV) is a nonenveloped DNA mastadenovirus classified into 7 species (HAdV-A–G) with >100 identified genotypes (HAdV Working Group, http://hadvwg.gmu.edu). HAdV diversity arises from mutations or recombinations of different viral strains (*1*).

HAdV species B genotype 55 (HAdV-B55) is a recombinant virus derived from HAdV-B11 and HAdV-B14. HAdV-B55 has emerged as a major acute respiratory disease pathogen, and endemic circulation has been reported in China and South Korea (*2*). We report a case of pneumonia caused by HAdV-B55 in France.

A 51-year-old woman was hospitalized in April 2024 for febrile emesis and cough. She had no peculiar medical history, was on no medications, and was an occasional smoker. At admission to the emergency department on day 1 (D1), she was febrile at 39°C and experiencing continuous emesis. Her symptoms had started 5 days before and she had experienced no improvement, preventing her from maintaining hydration or alimentation. One episode of diarrhea had occurred 3 days previously without relapse. The patient had no recent travel, did not work in the tourism sector, and resided in a nontouristic area of Paris with her son, who had experienced influenza-like symptoms and fever 1 week earlier. No other potential exposure was identified.

Biologic testing on D1 indicated an inflammatory syndrome with elevated C-reactive protein at 135 mg/L (reference ≤5 mg/L) and hepatic cytolysis (aspartate aminotransferase 131 U/L [reference ≤32 U/L] and alanine aminotransferase 124 U/L [reference ≤33 U/L]). Results of blood cultures and nasopharyngeal PCR tests for SARS-CoV-2, influenza A and B, and respiratory syncytial virus were negative. Pulmonary examination found crackles in the right basilar area. A thoraco-abdomino-pelvic computed tomography scan revealed a right lower lobe consolidation. A probabilistic antibiotic therapy with amoxicillin/clavulanic acid and supplemental oxygen (1 L/min) was initiated on D1 because of a peripheral capillary oxygen saturation of 94%.