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First detection of hepatitis E virus in wastewater in Costa Rica

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Short Report

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Abstract

Hepatitis E virus (HEV) is an important cause of acute viral hepatitis worldwide, with both human-restricted (genotypes 1 and 2) and zoonotic lineages (genotypes 3 and 4). However, information regarding HEV circulation in Central America remains limited. In this study, wastewater-based epidemiological surveillance was conducted in San José, Costa Rica, during 2025 to investigate the presence of HEV in the main wastewater treatment plant serving approximately one million inhabitants. A total of 54 influent and effluent samples were analyzed. HEV RNA was not detected by quantitative RT-PCR; however, RT-nested PCR targeting the ORF1 region identified three positive influent samples. Sequencing yielded two high-quality partial ORF1 sequences suitable for phylogenetic analysis. Both Costa Rican sequences clustered together and showed a close relationship to genotype 3 sequences. To our knowledge, this represents the first report of HEV detected in wastewater in Costa Rica and the first scientific report of environmental detection in Central America. These findings highlight the value of wastewater-based surveillance for identifying circulating viral pathogens in regions where routine clinical testing is limited.

Introduction

Hepatitis E virus (HEV), belonging to the family *Hepeviridae* and the species *Palahepevirus balayani* (Purdy et al. 2022), is one of the causative agents of viral hepatitis, infecting around 20 million people annually worldwide (World Health Organization (WHO) 2025). HEV exists as a non-enveloped particle (27-34nm) in the feces of infected hosts and as a quasi-enveloped particle with a lipid membrane in circulating blood. The viral genome consists of a 7.2kb single-stranded positive-sense RNA, with three open reading frames (ORF 1–3) (Wang and Meng 2021). ORF-1 encodes a viral nonstructural polyprotein containing several functional domains, including an RNA-dependent RNA polymerase; ORF-2 encodes the viral capsid protein; and ORF-3 encodes a small phosphoprotein that interacts with various signaling proteins in the host cell, enhancing infected cell survival (Kenney and Meng 2019). Similarly to hepatitis A virus (HAV), HEV is predominantly spread via the fecal-oral route (Kamar et al. 2012).

Eight HEV genotypes have been identified. HEV1 and HEV2 infect only humans and are associated with waterborne outbreaks, particularly in Asia (HEV1), Africa, and Mexico (HEV2). In contrast, HEV3 and HEV4 are zoonotic and capable of infecting both humans and other mammals such as pigs (Kamar et al. 2012). HEV3 and HEV4 are typically linked to foodborne transmission through raw or undercooked meat and tend to cause mild or asymptomatic infections in immunocompetent individuals, though chronic infections may occur in immunocompromised hosts. HEV3 has a global distribution, whereas HEV4 is more common in Southeast Asia and parts of Europe (Ma et al. 2022). Conversely, HEV5 and HEV6 are associated with infections in wild boars; and HEV7 and HEV8 were recently identified in dromedaries and Bactrian camels, respectively (Sridhar et al. 2017). In addition, several other *Hepevirus* lineages have been identified, especially those affecting bats (Drexler et al. 2012), birds (avian HEV) (Huang et al. 2002), and rats (Johne et al. 2010).

In Latin America, HEV has emerged as a growing public health concern but remains underdiagnosed. A systematic review involving over 38,000 individuals estimated an anti-HEV IgG seroprevalence of approximately 9%, with substantial variation between countries (Magri et al. 2025). Evidence suggests that HEV3 predominates in South America, having been detected in humans, pigs, wild boars, and environmental samples (Pisano et al. 2020). Despite this, HEV is not routinely included in diagnostic panels for acute hepatitis, and infections are frequently misclassified as non-A, non-B, or non-C hepatitis, or reported as hepatitis of unknown etiology (Dalton et al. 2018; Peña Mesías et al. 2022). In Costa Rica, previous serological studies have detected anti-HEV antibodies in swine, indicating potential viral circulation in the country despite the lack of routine human testing and highlighting the need for alternative surveillance approaches (Kase et al. 2008).

Environmental surveillance has become a valuable complementary tool for understanding HEV circulation. Because infected individuals shed high viral loads in stool, HEV can enter sewage systems and be identified through wastewater-based epidemiology (WBE). This approach can detect community-level viral circulation even when few clinical cases are reported. Several environmental surveillance studies have detected HEV RNA in wastewater samples despite low numbers of confirmed human infections, highlighting ongoing but largely undetected viral circulation. WBE therefore provides early insight into HEV transmission dynamics and complements traditional clinical surveillance, particularly in regions where HEV testing is not routine (Fantilli et al. 2024).

In this work, we aimed to identify circulating HEV strains through WBE by sampling the main wastewater treatment plant (WWTP) serving the metropolitan area of Costa Rica. We report the first detection of HEV RNA in wastewater in Costa Rica, suggesting the circulation of a genotype with potential for human infection, based on partial ORF1 sequence analysis.

Methods

Sample collection, concentration and viral RNA extraction:

Wastewater sampling was conducted between March 7, 2025 and September 5, 2025 at the main wastewater treatment plant in San José, Costa Rica, which serves approximately one million residents in the Greater Metropolitan Area. Influent and effluent samples were collected weekly (every Wednesday) during this period, resulting in a total of 54 samples collected over 27 weeks. Following the methodology described by Morales et al. (2026) (Morales et al. 2026), one-liter influent and effluent samples were obtained using a 12-hour composite sampling strategy based on historical discharge patterns (15:00–22:00 and 05:00–08:00). Samples were transported at 4–8°C and processed immediately at the Institute for Health Research (INISA), University of Costa Rica.

Viral particles were concentrated using the polyethylene glycol-8000 (PEG-8000) precipitation method, following WHO guidelines and the Lewis & Metcalf method (Lewis and Metcalf 1988), with minor modifications. Briefly, 500 mL of wastewater was centrifuged at 4,000 × g for 20 min at 4°C to separate

the supernatant and sediment. The sediment was resuspended in 3% beef extract/2 M $NaNO_3$ (Titolo; pH 5.5), incubated at 4°C for 1 h under agitation, and centrifuged at 4,000 × g for 40 min. The resulting eluate was combined with the initial supernatant and adjusted to pH 7.2. PEG-8000 and NaCl were then added to a final concentration of 10% and 2% respectively (w/v), and the mixture was incubated overnight at 4°C under agitation, followed by centrifugation at 4,000 × g for 50 min at 4°C.

The resulting pellet was resuspended in 4 mL of phosphate-buffered saline (PBS; pH 7.4). Based on the initial sample volume (500 mL) and the final resuspension volume (4 mL), the estimated concentration factor was approximately 125×. The suspension was incubated for 1 h with occasional vortexing, and centrifuged at 4,000 × g for 40 min. Subsequently, a 300 µL aliquot of the concentrated supernatant was immediately used for RNA extraction, while the remaining volume was stored at − 80°C. RNA extraction was performed using the MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions, with a 1.5× increase in reagent volumes to accommodate the 300 µL input volume.

Viral detection and quantification by qPCR:

We performed a quantitative RT-PCR (qPCR) assay with the TaqMan™ Fast Virus One-Step Master Mix (ThermoFisher Scientific, Waltham, MA, USA) following manufacturer's instruction (fast protocol) to detect circulating HEV RNA in wastewater samples. For this purpose, primers and probe previously described by Jothikumar et al. (2006) (Jothikumar et al. 2006), targeting HEV genotypes 1–4, were used. The final primer concentration was 0.3 µM (forward: GGTGGTTTCTGGGGTGAC; reverse: AGGGGTTGGTTGGATGAA), and the final probe concentration was 0.2 µM (5'-VIC-TGATTCTCAGCCCTTCGC-MGB-NFQ-3'). In addition, Pepper Mild Mottle Virus (PMMoV) was quantified as an internal positive control due to its consistently high concentration in Costa Rican wastewater (Barrantes et al. 2023). PMMoV detection was performed using a final primer concentration of 0.4 µM (PMMV-FP1: GAGTGGTTTGACCTTAACGTTTGA; PMMV-RP1: TTGTCGGTTGCAATGCAAGT) and a final probe concentration of 0.3 µM (PMMV-Probe1: 5'-/56-FAM/CCTACCG AAGCAAATG/3BHQ_1/-3') (Haramoto et al. 2013).

Viral target sequences were synthesized as double-stranded DNA constructs (gBlocks) by Integrated DNA Technologies (IDT®, California, USA) and used for qPCR standard curves. Calibration curves for each gene were generated in triplicate during qPCR assays, spanning from 1-log10 to 5-log10 copies/reaction. The qPCR reactions were performed using the Step-One Plus qPCR instrument (ThermoFisher Scientific, Waltham, MA, USA) with recommended cycling conditions for the TaqMan™ Fast Virus One-Step Master Mix. Calculations were conducted using ThermoFisher's Design and Analysis Software v2.7.

HEV detection by nested PCR

For reverse transcription, 6 µL of the previously extracted RNA from influent samples only (27 samples in total) were used, as influent samples are considered more representative of community viral circulation than treated effluent. The extracted RNA was denatured at 95°C for 5 min. Reverse transcription was then performed using 14 µL of RT-mix, which included 1 µL of the M-MLV-RT enzyme (200 U/mL) (Thermo Fisher Scientific, Waltham, MA, USA), 1 µL of random primers (1 mg/mL), 2 µL of dNTPs (10 mM), 1.2 µL of DMSO, 2.5 µL of M-MLV-RT Buffer 5×, and 6.3 µL of nuclease-free water. The reaction was carried out under the following conditions: incubation at 25°C for 10 min, followed by 50 min at 37°C, and ending with 15 min at 75°C.

The resulting cDNA was amplified by PCR using the ConsORF1-s1/ConsORF1-a1 primer set (Wang et al. 1999), which flanks the ORF1 region of the HEV genome and generates a 418 bp amplicon (ConsORF1-s1: CTGGCATYACTACTGCYATTGAGC ; ConsORF1-a1: CCATCRARRCAGTAAGTGCGGTC). Each reaction was carried out in a final volume of 10 µL, containing 1 µL of cDNA, 0.1 µL of DreamTaq Hot Start DNA Polymerase (5 U/µL), 1 µL of 10× DreamTaq Buffer, 0.1 µL of dNTP mix (10 mM), 0.5 µL of each primer (5 µM), and 6.8 µL of nuclease-free water. The amplification protocol consisted of an initial denaturation at 94°C for 1 min, followed by 30 cycles of 92°C for 10 s, 42°C for 15 s, and 72°C for 1 min, with a final extension at 72°C for 5 min. A second-round (nested) PCR was then performed using the internal primer pair ConsORF1-s2/ConsORF1-a2 (Wang et al. 1999), which amplifies a 287 bp fragment (ConsORF1-s2 CTGCCYTKGCGAATGCTGTGG; ConsORF1-a2: GGCAGWRTACCARCGCTGAACATC). The reaction mixture and cycling conditions were identical to those described above, except that 1 µL of the first-round PCR product was used as template instead of cDNA. All thermocycling steps were performed on an Applied Biosystems Veriti 9902 thermocycler. The resulting amplicons from second PCR were visualized using a 2% agarose gel electrophoresis stained with the Invitrogen ^{TM} SYBR® Safe DNA Gel Stain (Thermo Fischer Scientific, Waltham, MA, USA).

Sequencing and phylogenetic analysis of HEV Costa Rican strains:
The PCR products generated with the ORF1-based ConsORF1-s2/ConsORF1-a2 primers were purified using ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced in both directions using the same primers. Sequencing was performed by Macrogen Inc. using the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions. Capillary electrophoresis was carried out on an Applied Biosystems SeqStudio Flex Genetic Analyzer. Forward and reverse sequences were assembled and manually curated using the Benchling web platform (https://benchling.com). A preliminary BLAST search was conducted against the GenBank RefSeq database to confirm sequence identity.

For the phylogenetic reconstruction of the HEV ORF1 region, representative sequences from HEV genotypes 1–8 were included based on reference sequences previously employed by Smith et al. (2020) (Smith et al. 2020), using an avian HEV sequence as outgroup. In addition, the three closest matches to our sequences identified by BLAST searches against the NCBI nucleotide core (nt) database (all belonging to HEV genotype 3) were included. Sequences were aligned using ClustalW and trimmed in MEGA v.12 (Kumar et al. 2024). Phylogenetic analyses were performed using the maximum-likelihood

method with IQ-TREE, employing the best-fit model of nucleotide substitution selected by ModelFinder according to the Bayesian information criterion (BIC). The robustness of the phylogenetic grouping was evaluated using the SH-like approximate likelihood ratio test (SH-aLRT) (1,000 replicates) (Guindon et al. 2010; Hoang et al. 2018) and the ultrafast bootstrap approximation (UFBoot) (10,000 replicates) (Hoang et al., 2018), as implemented in IQ-TREE v 3.0.1 (Minh et al. 2020).

Results and Discussion

We did not identify any HEV-positive samples using the qPCR method. However, positive control for PMMoV was successfully detected in all 54 samples, with Ct values ranging from 31.75 to 26.06, confirming the presence of amplifiable viral RNA in the extracts. In contrast, the endpoint HEV PCR assay detected three positive influent samples: A094 (March 27, 2025), A0102 (April 10, 2025), and A179 (July 18, 2025), each showing the expected 287 bp size. However, after sequencing, the A179 sequence showed extremely poor quality and was excluded from further analysis.

BLAST analysis of the ORF1 sequences obtained from samples A094 and A102 showed highest similarity with HEV genotype 3 (HEV3) strains (top three matches: EU718648.1, KF303502.2, AB196842.1 for A094 and EU718648.1, KF303502.2, MZ813444 for A0102). Phylogenetic reconstruction showed that our two Costa Rican (CR) sequences grouped together within HEV3, in close relationship with subtype HEV3a reference strains (Fig. 1).

The discordance observed between the qPCR and the nested PCR results may be related to differences in assay sensitivity and to the complexity of the wastewater matrix, which may affect viral detection efficiency (Barrantes et al. 2023). The qPCR assay used in this study was based on the protocol described by Jothikumar et al. (2006) (Jothikumar et al. 2006), targeting the ORF3 region of HEV genotypes 1–4, whereas the end-point nested PCR targets a fragment within the ORF1 region (Wang et al. 1999). Because the phylogenetic analysis showed that the detected sequences are not highly divergent from previously described HEV strains, the discrepancy between methods could be more likely associated with methodological sensitivity and matrix-related factors rather than extreme genetic divergence.

In addition, the use of a relatively small fragment, such as the ~ 280 bp ORF1 amplicon analyzed here, limits the phylogenetic resolution compared to full-length genome analyses. However, the topology obtained in the present study consistently placed both Costa Rican sequences within the HEV-3 clade. Although the sequences grouped near subtype 3a, additional genomic information, ideally including longer genomic regions or complete genome sequences, would allow a more precise subtype confirmation and a better understanding of the evolutionary relationship of the Costa Rican strains.

HEV3 is commonly associated with zoonotic transmission and has been reported in both human and animal hosts. This is particularly relevant because HEV3 strains are capable of infecting humans but are frequently linked to animal reservoirs, especially swine, suggesting a possible zoonotic origin of the strains detected in this study (Wang et al. 1999). Nevertheless, the sequences obtained clearly belong to

a member of the *Hepevirus* lineage and may represent strains with potential relevance for human infection.

To our knowledge, this study represents the first detection of HEV RNA in wastewater in Costa Rica and Central America. These findings highlight the value of wastewater-based epidemiological surveillance as a powerful tool for detecting circulating pathogens within populations, thereby supporting local and regional public health monitoring efforts. This is especially relevant for low- and middle-income countries, where clinical surveillance capacity may be limited (Mendoza-Guido et al. 2025).

The detection of HEV RNA in wastewater from the metropolitan area of San José suggests that the virus may be circulating silently in the population, which should alert public health authorities and supports the need to intensify the search for clinically compatible cases of hepatitis E in Costa Rica, especially those hepatitis cases of unknown etiology. These findings also highlight the importance of using multiple detection approaches in environmental surveillance studies, as differences in assay sensitivity and matrix-related inhibition may affect viral detection. Future studies should focus on obtaining full genome sequences of HEV and other human pathogens circulating in wastewater and river systems, and, when possible, comparing these sequences with local, regional, and global strains, particularly those associated with clinically relevant infections.

Declarations

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Competing Interests

The authors declare that they have no competing interests.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contribution

Bradd Mendoza-Guido participated in sample and data analysis and wrote the initial draft of the manuscript.Taimy Calvo and Luis Chacón performed laboratory analyses.María Belén Pisano conducted phylogenetic analyses and contributed to manuscript revision.Gisela Masachessi contributed to the

study design and critically reviewed the manuscript.Luz Chacón conceived and designed the study, obtained funding, and revised and finalized the manuscript.All authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Barrantes, K., Chacón-Jiménez, L., Rivera-Montero, L., et al. (2023). Challenges detecting SARS-CoV-2 in Costa Rican domestic wastewater and river water. *Science of The Total Environment*, *897*, 165393. https://doi.org/10.1016/j.scitotenv.2023.165393
2. Dalton, H. R., Kamar, N., Baylis, S. A., et al. (2018). EASL Clinical Practice Guidelines on hepatitis E virus infection. *Journal Of Hepatology*, *68*, 1256–1271. https://doi.org/10.1016/j.jhep.2018.03.005
3. Drexler, J. F., Seelen, A., Corman, V. M., et al. (2012). Bats Worldwide Carry Hepatitis E Virus-Related Viruses That Form a Putative Novel Genus within the Family Hepeviridae. *Journal Of Virology*, *86*, 9134–9147. https://doi.org/10.1128/JVI.00800-12
4. Fantilli, A. C., Masachessi, G., Cola, G., Di, et al. (2024). Integrated hepatitis e virus monitoring in central Argentina: a six-year analysis of clinical surveillance and wastewater-based epidemiology. *Water Research*, *261*. https://doi.org/10.1016/j.watres.2024.122004
5. Guindon, S., Dufayard, J-F., Lefort, V., et al. (2010). New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Systematic Biology*, *59*, 307–321. https://doi.org/10.1093/sysbio/syq010
6. Haramoto, E., Kitajima, M., Kishida, N., et al. (2013). Occurrence of pepper mild mottle virus in drinking water sources in Japan. *Applied And Environment Microbiology*, *79*, 7413–7418. https://doi.org/10.1128/AEM.02354-13
7. Hoang, D. T., Chernomor, O., von Haeseler, A., et al. (2018). UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology And Evolution*, *35*, 518–522.

https://doi.org/10.1093/molbev/msx281

8. Huang, F. F., Haqshenas, G., Shivaprasad, H. L., et al. (2002). Heterogeneity and Seroprevalence of a Newly Identified Avian Hepatitis E Virus from Chickens in the United States. *Journal Of Clinical Microbiology*, *40*, 4197–4202. https://doi.org/10.1128/JCM.40.11.4197-4202.2002
9. Johne, R., Heckel, G., Plenge-Bönig, A., et al. (2010). Novel Hepatitis E Virus Genotype in Norway Rats, Germany. *Emerging Infectious Diseases*, *16*, 1452–1455. https://doi.org/10.3201/eid1609.100444
10. Jothikumar, N., Cromeans, T. L., Robertson, B. H., et al. (2006). A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *Journal Of Virological Methods*, *131*, 65–71. https://doi.org/10.1016/j.jviromet.2005.07.004
11. Kamar, N., Bendall, R., Legrand-Abravanel, F., et al. (2012). Hepatitis E. *The Lancet*, *379*, 2477–2488. https://doi.org/10.1016/S0140-6736(11)61849-7
12. Kase, J. A., Correa, M. T., Luna, C., & Sobsey, M. D. (2008). Isolation, detection and characterization of swine hepatitis E virus from herds in Costa Rica. *International Journal Of Environmental Health Research*, *18*, 165–176. https://doi.org/10.1080/09603120701498311
13. Kenney, S. P., & Meng, X. J. (2019). Hepatitis E virus genome structure and replication strategy. *Cold Spring Harb Perspect Med*, *9*, 1–18. https://doi.org/10.1101/cshperspect.a031724
14. Kumar, S., Stecher, G., Suleski, M., et al. (2024). MEGA12: Molecular Evolutionary Genetic Analysis Version 12 for Adaptive and Green Computing. *Molecular Biology And Evolution*, *41*. https://doi.org/10.1093/molbev/msae263
15. Lewis, G. D., & Metcalf, T. G. (1988). Polyethylene glycol precipitation for recovery of pathogenic viruses, including hepatitis A virus and human rotavirus, from oyster, water, and sediment samples. *Applied And Environment Microbiology*, *54*, 1983–1988. https://doi.org/10.1128/aem.54.8.1983-1988.1988
16. Ma, Z., de Man, R. A., Kamar, N., & Pan, Q. (2022). Chronic hepatitis E: Advancing research and patient care. *Journal Of Hepatology*, *77*, 1109–1123. https://doi.org/10.1016/j.jhep.2022.05.006
17. Magri, M. C., Manchiero, C., Dantas, B. P., et al. (2025). Prevalence of hepatitis E in Latin America and the Caribbean: A systematic review and meta-analysis. *Public Health*, *244*. https://doi.org/10.1016/j.puhe.2025.105745
18. Mendoza-Guido, B., Montiel-Mora, J. R., Ureña-Salazar, C., et al. (2025). Molecular epidemiology of aquatic environments: challenges from sampling to implementation of surveillance programs. *Front Public Health*, *13*, 1–8. https://doi.org/10.3389/fpubh.2025.1652535
19. Minh, B. Q., Schmidt, H. A., Chernomor, O., et al. (2020). IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology And Evolution*, *37*, 1530–1534. https://doi.org/10.1093/molbev/msaa015
20. Morales, E., Rivera-Montero, L., Ramos-Montalvo, N., et al. (2026). Applying weighted quantile sum regression to wastewater surveillance: enteric viral mixtures and diarrheal disease trends in Costa Rica. *Journal Of Water And Health*, *00*, 1–15. https://doi.org/10.2166/wh.2026.197

21. Peña Mesías, M. A., Reyes Santana, B. G., Tomala Dueñas, M. F., & Castro Jalca, J. E. (2022). Formas de transmisión, consecuencias y prevalencia de la Hepatitis Viral: Un estudio Epidemiológico en Latinoamérica. Revista Científica Higía de la Salud 7:. https://doi.org/10.37117/higia.v7i2.756
22. Pisano, M. B., Mirazo, S., & Re, V. E. (2020). Hepatitis E Virus Infection: Is It Really a Problem in Latin America? *Clin Liver Dis (Hoboken)*, *16*, 108–113. https://doi.org/10.1002/cld.931
23. Purdy, M. A., Drexler, J. F., Meng, X-J., et al. (2022). ICTV Virus Taxonomy Profile: Hepeviridae 2022. *Journal of General Virology*, *103*. https://doi.org/10.1099/jgv.0.001778
24. Smith, D. B., Izopet, J., Nicot, F., et al. (2020). Update: proposed reference sequences for subtypes of hepatitis E virus (species Orthohepevirus A). *Journal of General Virology*, *101*, 692–698. https://doi.org/10.1099/jgv.0.001435
25. Sridhar, S., Teng, J., Chiu, T-H., et al. (2017). Hepatitis E Virus Genotypes and Evolution: Emergence of Camel Hepatitis E Variants. *International Journal Of Molecular Sciences*, *18*, 869. https://doi.org/10.3390/ijms18040869
26. Wang, B., & Meng, X-J. (2021). Structural and molecular biology of hepatitis E virus. *Comput Struct Biotechnol J*, *19*, 1907–1916. https://doi.org/10.1016/j.csbj.2021.03.038
27. Wang, Y., Ling, R., Erker, J. C., et al. (1999). A divergent genotype of hepatitis E virus in Chinese patients with acute hepatitis. *Journal of General Virology*, *80*, 169–177. https://doi.org/10.1099/0022-1317-80-1-169
28. World Health Organization (WHO) (2025). Hepatitis E. https://www.who.int/News-Room/Fact-Sheets/Detail/Hepatitis-e

Figures

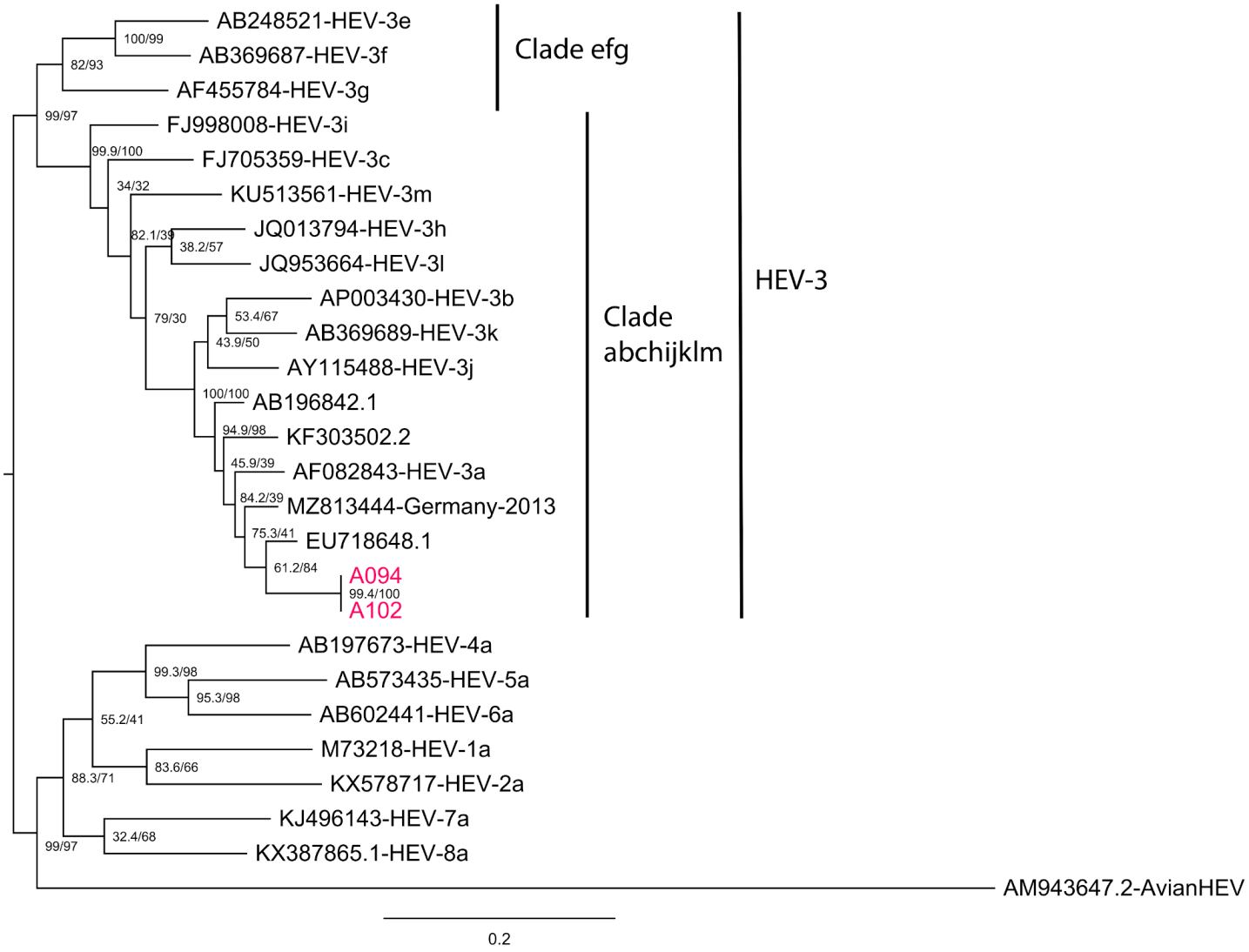


Figure 1

Phylogenetic tree showing the relationships between CR-HEV strains (in pink) and HEV genotypes 1–8. Avian HEV was used as the outgroup. Partial ORF1 sequences from the CR strains were aligned with complete genomes and partial ORF1 sequences from reference strains using ClustalW. The alignment was manually trimmed using MEGA 12 software, and maximum likelihood analysis was performed with IQ-TREE. Statistical support [SH-aLRT (%)/UFB (%)] values are indicated at nodes. Sequences EU718648.1, KF303502.2, MZ813444 and AB196842.1 were the top BLAST matches.