Brief Report

First Detection of Rocahepevirus in Urban Wastewater from Guinea: A One Health Alert

Bakary Doukouré *, Yann Le Pennec , Cissé Fatoumata , Ramatoulaye Diallo , Issiaga Touré , Noël Tordo , Pierre Roques

Posted Date: 19 March 2026

doi: 10.20944/preprints202603.1340.v1

Keywords: *Rocahepevirus ratti*; RHEV; urban wastewater; phylogenetic analysis; zoonotic ris

 doi:10.20944/preprints202603.1340.v1

Brief Report

First Detection of *Rocahepevirus* in Urban Wastewater from Guinea: A One Health Alert

Bakary Doukouré [1,2,*], Yann Le Pennec [1], Cissé Fatoumata [1], Ramatoulaye Diallo [1], Issiaga Touré [1], Noël Tordo [1] and Pierre Roques [1]

[1] Institut Pasteur de Guinée, Conakry, Guinea.

[2] Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany.

* Correspondence: bakary.doukoure@pasteur-guinee.org; Tel.: 00224628642496

Abstract

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis worldwide, with zoonotic genotypes detected in humans and animals. In Africa, limited data exist on environmental HEV circulation. Here, we report the first detection of *Rocahepevirus ratti* (RHEV) in urban wastewater from Conakry, Guinea. From December 2024 to April 2025, *Rocahepevirus ratti* (RHEV) has been detected in 35 out of 180 urban untreated wastewater samples in Conakry, Guinea. Phylogenetic analysis of partial HEV ORF1 genome segments reveals clustering with African rodents RHEV strains, highlighting environmental contamination and potential zoonotic risk for human population in proximity. This finding underscores the need for integrated One Health surveillance to monitor HEV transmission at the human-animal-environment interface in West Africa particularly in Guinea.

Keywords: *Rocahepevirus ratti*; RHEV; urban wastewater; phylogenetic analysis; zoonotic risk

1. Introduction

Hepatitis E virus (HEV) or *Paslahepevirus balayani* is a major cause of acute viral hepatitis worldwide transmitted through fecal-oral routes. While specific human genotypes exist, zoonotic genotypes are also emerging from animal reservoirs with potential risk for human health. Zoonotic HEV genotype 3, mainly transmitted by pigs, has led to outbreaks in humans in Africa. HEV-3 circulation has been confirmed in Guinean pigs both by seroprevalence studies and molecular characterization of HEV 3c (OR283252.1) in feces as well as in water effluent from the pigs enclosure (PX441305) [1] . Beyond *Paslahepevirus balayani*, other hepeviruses belonging to distinct taxonomic groups circulate in animal populations. Although data on environmental circulation of hepeviruses in Africa remain limited [2], *Rocahepevirus ratti* (RHEV), primarily circulating in rodents, has been recently detected [3]. It is occasionally associated with human infections [4,5]. In Conakry, there was no wastewater treatment plan and beyond human, numerous rats and free circulation of pigs between open enclosure can be seen. Thus, this study aimed to investigate the presence of HEV in urban wastewater of the Conakry area, as part of a One Health surveillance program from the Institut Pasteur de Guinée for the ANSS (Agence National de Sécurité Sanitaire).

2. Materials and Methods

Between December and April 2025, grab raw wastewater samples (RWWS) of 200 mL were collected manually from 10 quarters in Conakry (Figure 1) using sterile polypropylene bottles once a week. Immediately after collection, samples were transported to the laboratory at 4°C to preserve their integrity. Sample processing was carried out within a maximum of three days post-collection. Samples that could not be processed within this timeframe were stored at −80°C to ensure optimal preservation of viral RNA for subsequent analyses.

 doi:10.20944/preprints202603.1340.v1

For viral detection, RWWS were first clarified by centrifugation (3000 rpm/30min) then were concentrated by polyethylene glycol (PEG 8000) precipitation. Briefly, 4 g of PEG 8000 (10%) and 0.9 g of NaCl (0.4 M) were added to 40 mL of clarified wastewater. The mixtures were stirred until fully dissolved and then incubated overnight at 4°C. After incubation, samples were centrifuged for 2 hours at 12,000 × g; the supernatant was discarded, and the pellet was resuspended in 500 µL of PBS. Concentrates obtained were aliquoted and stored at −80°C until RNA extraction. Viral RNA was extracted from 140 µL of concentrated wastewater (CWWS) samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. to account for the possible co-circulation of genetically distinct hepeviruses in environmental samples, the same RNA extracts were systematically screened using two genus-specific molecular assays.

First, *hepevirus*-specific HEV RNA detection was performed using a RT-qPCR targeting ORF3 [6] and positive samples were further confirmed by nested RT-PCR assays targeting ORF1 and ORF2 [7]; Then, RNA extracts were screened using a *Rocahepevirus*-specific nested RT-PCR targeting ORF1 [8]. To definitively confirm viral identity and exclude cross-reactivity between assays, ORF1 amplicons from positive samples were subjected to sequencing using primers specific for either Rocahepevirus or Paslahepevirus. PCR products were purified and quantified using the Qubit system (Thermo Fisher Scientific). Libraries were then prepared according to the manufacturer's instructions and loaded onto a flow cell for sequencing using the Oxford Nanopore Technologies MinION platform. Raw signal data were demultiplexed through barcode assignment and subjected to basecalling FASTA-format sequences were generated using the ARTIC nCoV bioinformatics pipeline. The resulting FASTA files were processed with a workflow adapted from the ARTIC protocol to generate consensus sequences. These consensus sequences were deposited in Genbank AC# PX408741-PX408746.

Finally, Hepatitis E virus Genotyping Tools (Software version 2.22.2 Tool version 1.16) were used to identify mutations and assign each sequence to the corresponding HEV variant.

3. Results

Of 180 water samples, 135 (75%) were positive using ORF3 Paslahepevirus specific RT-qPCR. Positive RT-qPCR signals were detected at all 10 sampling sites over the study period. Ct values varied between sites, with lower Ct values observed at some locations (Ct >32) whereas other sites showed higher Ct values (Ct < 32). Figure 1 illustrates the spatial distribution of these sampling sites and the frequency of HEV detection across the 18-week monitoring period.

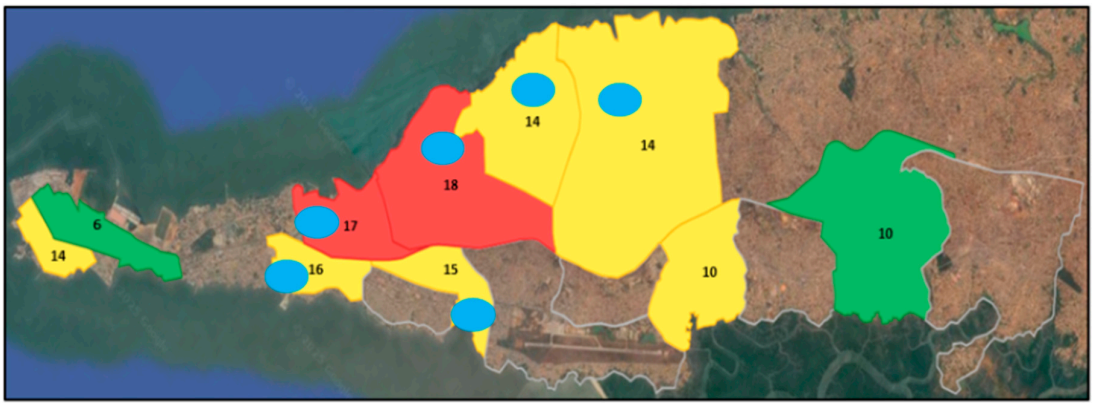

Figure 1. Spatial distribution of HEV detection sites in Conakry wastewater. For each site, the number of weeks (out of 18) during which HEV RNA was detected is indicated. The color code highlights the sites where high viral load was detected frequently (about 11 weeks) in **red**; moderately (1–4 weeks) in **yellow**, or sites with regular HEV circulation at lower viral load in **green**. The **blue** circles indicate the origine of sequenced samples.

 doi:10.20944/preprints202603.1340.v1

Thirty-five of the 135 positive samples were confirmed using ORF1 nested RT-PCR designed in 2006 by Jothikumar [6], none using ORF2 nested RT-PCR. These 35 samples were also positive using ORF1 nested RT-PCR specific for *Rocahepevirus ratti* [*8*].

Following ORF1 nested RT-PCR amplification, 12 positive samples were subjected to sequencing. Within the pipeline, either HEV-3h strain and RHEV strain V-105 genome as reference (JQ013794.1 and JX120573 respectively). From this reads processing, only the *Rocahepevirus* reference sequence provides consensus sequences of ±900 pb. From the 12 samples sequenced on the 10 sites, we retained 6 representative samples of the different areas at different time points from December to April.

The sequencing performance of all samples aligned to HEV reference JX120573.1 is summarized in Table 1, showing read numbers, coverage, and average base and mapping qualities.

Table 1. Sequencing coverage and quality metrics of the six sequenced samples aligned to HEV reference JX120573.

#Sequ ID	Numreads	Coverage bases	Coverage	Mean depth	Mean baseq	Mean mapq
PX408741	1697	777	77.08	1259.63	29.5	34.4
PX408742	1158	794	78.76	861.16	29.7	24.7
PX408743	528	775	76.88	391.72	29.4	25.7
PX408744	265	775	76.88	198.41	29.2	25.8
PX408745	510	780	77.38	379.33	29.3	28
PX408746	738	776	76.98	547.92	29.4	28.6

Reference sequence: HEV, GenBank accession JX120573.1). **Num reads**: Number of sequencing reads aligned to this region. **Coverage bases**: Number of bases covered by at least one read. **Coverage**: Average number of reads covering each base (or proportion of covered bases). **Mean depth**: Average sequencing depth across the region.**Mean baseq**: Mean base quality score (Phred scale), indicating confidence in base calls.**Mean mapq**: Mean mapping quality score, indicating confidence in read alignment to the reference.

Phylogenetic analysis based on these 900 nucleotides fragment of the ORF1 region showed that the Conakry wastewater sequences clustered within the sub-genotype RHEV-C1 of *Rocahepevirus ratti* (Figure 2). The Conakry's sequences (Accession numbers: PX408741-PX408746) clustered with HEV rodent sequences from Cameroon (GenBank PP764563.1 – PP764564.1) [3] with high bootstrap value (98%).

 doi:10.20944/preprints202603.1340.v1

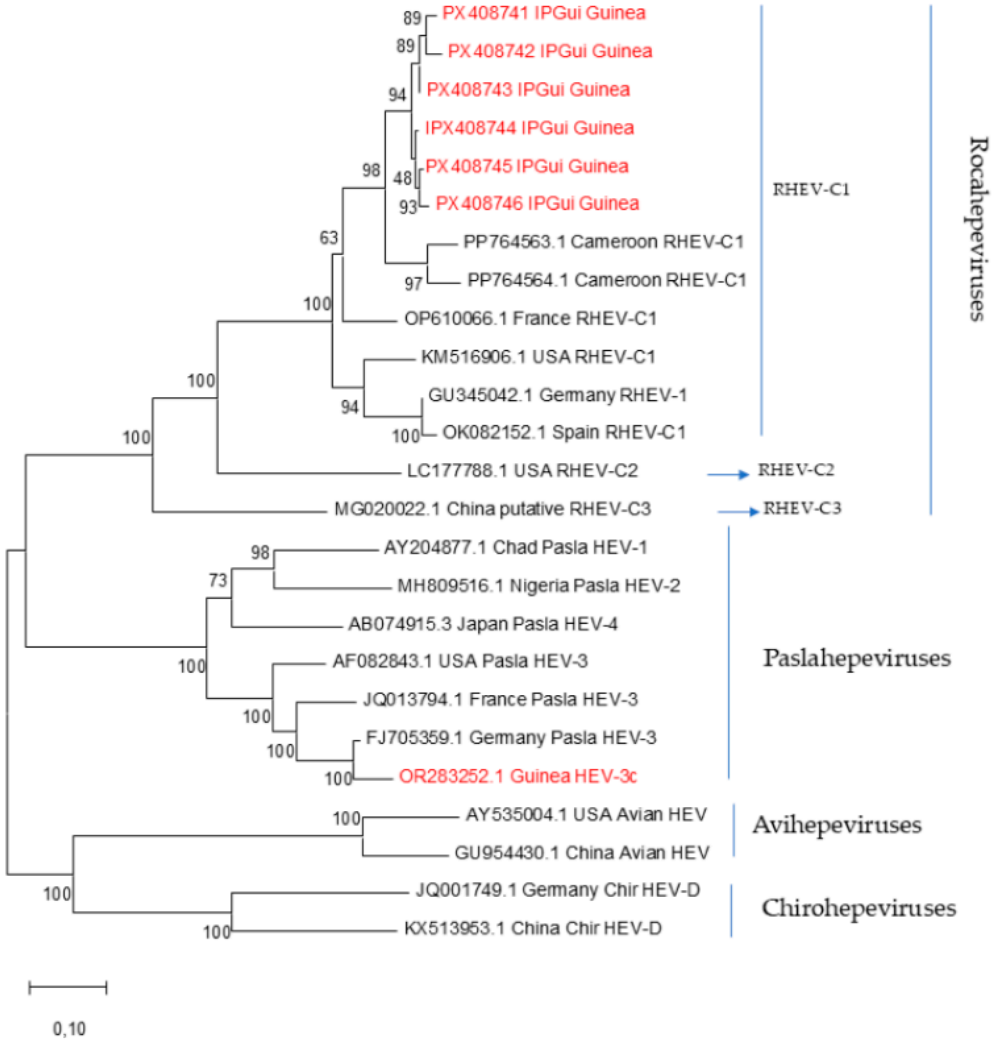

Figure 2. Phylogenetic tree of the Hepeviridae family based on 900 nucleotides of the ORF1 gene, constructed using the Neighbor-Joining method with 500 bootstrap replicates in MEGA version 12 (**https://www.megasoftware.net**). Six HEV sequences in Guinean wastewater and the HEV found in a Conakry pig were in red. Numbers correspond to the bootstrap percentage supporting each node.

4. Discussion/Conclusions

This is the first report of *Rocahepevirus ratti* RNA (RHEV-C1) circulation in Conakry wastewater. As *Paslahepevirus* of genotype 3c (OR283252.1) was detected in pigs feces and few effluent streams in 2023 in Conakry (PX441305) [1], we first tested wastewater samples with *Paslahepevirus* specific RT=PCR and confirmed 74% (135/180) of positive samples during the whole study period (4 months). However, we did not find any HEV-3 *Paslahepevirus* positive sample. Interestingly, 35 samples were also positive with *Rocahepevirus* specific RT-PCR and were further identified by NGS as the RHEV-C1 strain. These results suggest that *Rocahepevirus ratti* is circulating in a diffuse and prolonged way with hot spots suggesting local concentration of rat population.

The sequences obtained from wastewater samples that were positive with *Rocahepevirus ratti*-specific RT-PCR were included in a phylogenetic analysis alongside reference sequences from the Hepeviridae family. Care was taken to include representative sequences from *Rocahepevirus* and *Paslahepevirus* lineages to illustrate the relationship between the detected strains and previously described hepeviruses. As expected, sequences from HEV *Paslahepevirus* found in Guinean pigs were not included in the same clade as *Rocahepevirus ratti* (Figure 2).

 doi:10.20944/preprints202603.1340.v1

The detection of *Rocahepevirus ratti* in wastewater from Conakry reflects a heterogeneous spatial distribution. Areas where the virus was detected more frequently correspond to sectors that have less favorable sanitary conditions, potentially promoting the presence of rodents, which are *Rocahepevirus* reservoirs. These rodents often inhabit peri-domestic areas, facilitating frequent contact between humans, animals, and the environment. This pattern illustrates the One Health concept, emphasizing the interconnectedness of human, animal, and environmental health. This environmental contamination generates a potential risk of cross-species transmission to humans and/or pigs [9]. Despite the scarce information available, this risk must be assessed in Africa like in Asia (8) and Europe [10]. An integrated surveillance gathering regular investigation of rodent and swine reservoirs as well as related human exposure seems mandatory in West Africa and particularly in Guinea.

It is important to note that, while the data provide insights into the spatial distribution of *Rocahepevirus ratti* in Conakry wastewater, any interpretation regarding continuous circulation or transmission should be made with caution. Further genomic characterization would be required to fully resolve the taxonomic diversity of hepeviruses circulating in wastewater samples and additional survey in humans or trapping of rats are required to evaluate the true risk associated with this virus circulation.

Author Contributions: P.R., B.D.: Conceptualization of study; B.D, F.C, Y.L.P and R.D : laboratory analyses; B.D. and P.R.: Data curation; B.D., N.T and P.R.: Formal analysis; I;T. and Y.L.P.: Investigation; B.D., P.R., and N.T.: Writing and figures design. All authors read and approved the final version.

Funding: Sampling and Sequencing was supported by the Agence Française de Développement (AFD) through the ATLANTES project (grant CZ3246) to the Pasteur Network and AFROSCREEN project (grant CZZ3209) respectively. The AFROSCREEN Consortium (https://www.afroscreen.org/en/network/) was coordinated by ANRS-MIE in partnership with Institut Pasteur and IRD to improve pathogens' genomic surveillance in Africa. BD was supported by the Global Centre for Health and Pandemic Prevention CAIDERA (Project ID: 57592740), funded by the German Academic Exchange Service (DAAD) with funds from the German Federal Foreign Ministry (AA). YLP is funded by an ANRS-MIE PhD grant #ANRS0352b (ECTZ234766).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data supporting the findings of this study are available from the corresponding author, B.D., on request.

Acknowledgments: We gratefully acknowledge the support of the ANSS for fieldwork authorization. We also thank the National Reference Center for Hepatitis E (CNR-HEV) in France for their technical guidance and valuable discussions.

Conflicts of Interest: No conflicting financial interests exist.

Abbreviations

The following abbreviations are used in this manuscript:

ARTIC : Arctic Network protocol for pathogen sequencing
Ct : Cycle threshold
CWWS: Concentrated wastewater sample
FASTA : Format for nucleotide/amino-acid sequence files
HEV : Hepatitis E virus
HEV-3 : Hepatitis E virus genotype 3
RHEV-C1 : Rocahepevirus ratti (rat HEV) genotype C1
NGS : Next-Generation Sequencing
ORF : Open Reading Frame

 doi:10.20944/preprints202603.1340.v1

ORF1 / ORF2 / ORF3 : Open Reading Frame regions 1, 2, and 3
PEG : Polyethylene glycol
qPCR / RT-qPCR : Quantitative reverse-transcription polymerase chain reaction
PCR / RT-PCR / nested RT-PCR : (Nested) reverse-transcription polymerase chain reaction
RNA : Ribonucleic acid
RWWS: Raw wastewater sample

References

1. Doukouré B, Pennec Y Le, Troupin C, Grayo S, Eiden M, Groschup MH, et al. Seroprevalence and phylogenetic characterization of hepatitis E virus (Paslahepevirus balayani) in Guinean pig population. *Vector Borne Zoonotic Dis*. 2024;24:540–5.DOI: 10.1089/vbz.2023.0104.
2. Kanda T, Li TC, Takahashi M, Nagashima S, Primadharsini PP, Kunita S, et al. Recent advances in hepatitis E virus research and the Japanese clinical practice guidelines for hepatitis E virus infection. *Hepatol Res*. 2024;54:1–30. DOI: 10.1111/hepr.14062.
3. Meta-Djomsi D, Atsama-Amougou M, Ngamaleu MR, Godwe C, Maidadi-Foudi M, Tongo M, et al. Molecular surveillance of hepatitis E virus in wastewater in Yaoundé, Cameroon. *PLoS One*. 2025;20:e0322765. https:// doi.org/10.1371/journal.pone.0322765.
4. Caballero-Gómez J, Fajardo-Alonso T, Rios-Muñoz L, Cuadrado-Matías R, Somoano A, Panadero R, et al. Occurrence and genetic diversity of the zoonotic rat hepatitis E virus in small mammal species, Spain. *Vet Res*. 2025;56:68. https://doi.org/10.1186/s13567-025-01492-1.
5. Gherlan GS. Rocahepevirus ratti: an underrecognised cause of acute hepatitis. *World J Hepatol*. 2024;16:1084–90. https://doi.org/10.3390/ microorganisms11122996.
6. Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *J Virol Methods*. 2006;131:65–71. Doi:10.1016/j.jviromet.2005.07.004
7. Nguyen H, Le N, Pham DK, Le NP, Truong MN. Nested PCR optimization for detecting hepatitis E virus. *Int J Virol Res*. 2024;8:104–13. https://doi.org/10.32895/UMP.MPR.8.2.11.
8. Mulyanto, Depamede SN, Sriasih M, Takahashi M, Nagashima S, Jirintai S, et al. Frequent detection and characterization of hepatitis E virus variants in wild rats (Rattus rattus) in Indonesia. *Arch Virol*. 2013;158:87–96. DOI 10.1007/s00705-012-1462-0.
9. Yadav KK, Boley PA, Lee CM, Khatiwada S, Jung K, Laocharoensuk T, et al. Rat hepatitis E virus cross-species infection and transmission in pigs. *PNAS Nexus*. 2024;3:1–14. https://doi.org/10.1093/pnasnexus/pgae259.
10. Rouba A, Ansmant T, Chaqroun A, Challant J, Josse T, Schvoerer E, et al. First detection of hepatitis E virus (Rocahepevirus ratti) in French urban wastewater: potential implications for human contamination. *Sci Total Environ*. 2024;954:article 159835. https://doi.org/10.1016/j.scitotenv.2024.176805.