Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Evidence of rat hepatitis E virus across human and swine populations in Nigeria

Lucia Rios-Muñoz
Instituto Maimónides de Investigación Biomédica de Córdoba
Adeolu S. Oluremi
Ladoke Akintola University of Technology
Janet O. Aderibigbe
Ladoke Akintola University of Technology
Maria Casares-Jimenez
Instituto Maimónides de Investigación Biomédica de Córdoba
Javier Caballero-Gomez
Instituto Maimónides de Investigación Biomédica de Córdoba
Pedro Lopez-Lopez
Instituto Maimónides de Investigación Biomédica de Córdoba
Diana Corona-Mata
Instituto Maimónides de Investigación Biomédica de Córdoba
Claudia Ferreira-Tata
Instituto Maimónides de Investigación Biomédica de Córdoba
Olubisi D. Oluremi
Vintage Health Resources
Oladele O. Opaleye
Ladoke Akintola University of Technology
Oyebode A. T. Alli
Osun State University
David O. Ogbolu
Osun State University
Ana Belén Perez
Hospital Universitario Reina Sofía, Universidad de Córdoba (UCO)
Antonio Rivero
Hospital Universitario Reina Sofía, Universidad de Córdoba (UCO)
Antonio Rivero-Juarez
arjvet@gmail.com

Instituto Maimónides de Investigación Biomédica de Córdoba

Research Article

Keywords: rat hepatitis E virus, Rocahepevirus ratti, Nigeria, swine, seroprevalence, One Health

Posted Date: November 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11163265/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background.

Rocahepevirus ratti (rat hepatitis E virus, ratHEV) is an emerging zoonotic hepevirus maintained in rodents and increasingly recognized as a cause of acute and chronic hepatitis in humans. In Africa, available evidence is confined to rodent reservoirs and environmental matrices; neither human serological exposure nor infection of a domestic animal host has been documented.

Methods.

We conducted a cross-sectional study in southwestern Nigeria. Serum from 527 participants recruited into three cohorts with differing expected exposure (livestock-contact workers, rural villagers, students) was tested for ratHEV-specific IgG using a dot blot assay. Human sera were additionally screened for ratHEV RNA by multiplex real-time RT-PCR targeting two *ORF1* regions. In parallel, 544 swine fecal samples from the same setting were screened by the same assay, with confirmatory nested PCR and sequencing. Cohort seroprevalences were compared and potential associations were estimated by Firth penalized logistic regression with profile penalized-likelihood intervals.

Results.

RatHEV-specific IgG was detected in 15/527 participants (2.8%; 95% CI 1.7–4.6). Seropositivity was highest in the livestock-contact cohort (12/281; 4.3%; 95% CI 2.2–7.3), intermediate in students (2/79; 2.5%; 95% CI 0.3–8.9) and lowest in villagers (1/167; 0.6%; 95% CI 0.02–3.3). In the penalized model, livestock contact was associated with higher odds of seropositivity than village residence (OR 5.15; 95% CI 1.23–47.56; p = 0.022), whereas the student–villager contrast was not (OR 3.58; 95% CI 0.47–39.61; p = 0.212). No human serum pool was positive for ratHEV RNA. RatHEV RNA was detected in 4/544 swine fecal samples (0.7%; 95% CI 0.2–1.9). The four swine-derived sequences shared 98.3–99.7% nucleotide identity with each other and were assigned to genotype C1, subtype Ia, clustering with strains reported from a human case in France and from rodents in Sierra Leone.

Conclusions.

These findings extend African evidence on rat-associated hepeviruses from reservoir and environmental compartments to human serological exposure and molecular detection in domestic swine. The co-occurrence of human exposure and detection of ratHEV RNA in swine feces in a single setting supports integrated follow-up combining rodent, swine, environmental and clinically targeted human sampling in West Africa.

Introduction

Rocahepevirus ratti, commonly known as rat hepatitis E virus (ratHEV), has recently emerged as a zoonotic pathogen of increasing public health importance owing to its widespread distribution and recognized ability to cause human disease. Classified within the family Hepeviridae[1], ratHEV was originally identified in wild rodents[2, 3]; however, it has since been documented as a causative agent of both acute and chronic hepatitis in humans, including immunocompetent and immunocompromised individuals[4–7]. Although rats are considered the primary reservoir of ratHEV, increasing evidence indicates that ratHEV ecology extends beyond rodents to include environmental dissemination and occasional infection of non-rodent hosts[7]. Among these, domestic pigs are of particular interest for human exposure: natural infection has been reported in farmed animals[8, 9], and experimental inoculation confirms replication, fecal shedding and pig-to-pig transmission[10], supporting transient amplification outside the rodent reservoir. Together, these observations identify livestock environments as plausible interfaces for indirect human exposure[7]. However, the ecological pathways linking reservoir hosts, livestock and humans remain poorly understood, particularly outside Europe and Asia[7].

Despite increasing evidence of ratHEV circulation worldwide, knowledge from Africa remains remarkably limited. Most human infections reported to date originate from Asia and Europe, with additional reports from America[5, 6, 11]. Nevertheless, the second documented human case worldwide was acquired by a patient working in Gabon and the Democratic Republic of Congo, infected with a strain genetically distant from all other known ratHEV isolates[5]. Direct evidence from Africa remains restricted to reservoir and environmental surveillance. In the reservoir setting, viral metagenomic characterization of wild small mammals in Sierra Leone identified Rocahepevirus sp. among the zoonotic viral species detected[12], and molecular surveillance of synanthropic rodents and shrews in Kenya recovered ratHEV genomes clustering within rodent-associated lineages[13]. In the environmental setting, ratHEV was recently identified in untreated urban wastewater in Yaoundé (Cameroon) and Conakry (Guinea)[14, 15]. Whether ratHEV circulation documented in African rodent and environmental reservoirs extends to human and domestic animal populations remains unknown. Addressing this question requires integrated investigation of both human and domestic animal populations within the same epidemiological setting.

Nigeria combines a large and rapidly growing human population with extensive livestock production, including the largest pig population in Africa and a dominant share of pork production in West Africa[16]. Furthermore, the country has established endemicity for rodent-associated pathogens, and rodent–livestock contact is a documented feature of its smallholder production systems. Taken together, these characteristics make Nigeria an appropriate setting to investigate ratHEV at the human-livestock interface from a One Health perspective. Therefore, we conducted a cross-sectional study in southwestern Nigeria to investigate ratHEV simultaneously in human and livestock populations. Specifically, we aimed to determine human exposure, identify epidemiological correlates of seropositivity, and determine whether ratHEV RNA could be detected in domestic livestock from the same epidemiological setting.

Materials and methods

Study design and setting

We conducted a cross-sectional One Health study in southwestern Nigeria to investigate two previously unexplored aspects of ratHEV epidemiology in Africa: human exposure and molecular occurrence in domestic swine. he human study was selected to represent contrasting exposure profiles. Between January 2020 and February 2021, 527 participants were recruited into three cohorts: a livestock-contact cohort comprising individuals with regular occupational contact with domestic animals (n = 281), a villager cohort including residents of two rural communities without occupational animal contact (n = 167), and a student cohort with limited expected occupational or domestic animal exposure (n = 79). Whole blood was collected by venipuncture, centrifuged to obtain serum, transported on ice and stored at − 80°C until analysis. Age, sex and self-reported domestic animal contact were recorded using a structured questionnaire. Throughout this report, the three groups are referred to as the livestock-contact cohort, the villager cohort and the student cohort. Further details regarding recruitment procedures and cohort definitions have been described previously[17]. The present study included the 527 participants from that cohort for whom sufficient serum volume remained available for ratHEV testing.

A total of 544 domestic pigs were sampled from smallholder farms located within the same geographical setting as the human cohorts. Fecal samples were collected and processed for RNA extraction based on previous evidence demonstrating natural infection, fecal shedding and experimental transmission of ratHEV in swine[8–10].

Serological testing

Previous exposure to ratHEV was assessed using a ratHEV-specific dot blot assay previously developed and analytically validated by our group and subsequently applied in human seroepidemiological studies[18, 19]. Purified antigens from HEV, ratHEV and *Puumala orthohantavirus* were immobilized onto polyvinylidene difluoride membranes. The hantavirus antigen served as a heterologous negative substrate control, while an Escherichia coli lysate served as a positive substrate control. Membranes were blocked in TBS-T containing 5% skimmed milk, incubated overnight with serum samples diluted 1:5000, and subsequently incubated with recombinant protein A/G conjugated to horseradish peroxidase (Thermo Scientific, Schwerte, Germany). Immunoreactivity was visualized using Clarity Western ECL Substrate (Bio-Rad, Feldkirchen, Germany) on a ChemiDoc MP Imaging System (Bio-Rad). Each run included previously characterized ratHEV-seropositive and seronegative human sera as positive and negative controls, respectively. Detailed assay development, validation and analytical specificity have been reported previously[18, 19]. Seropositivity was defined a priori using the predefined interpretation criteria established during assay validation. Samples were classified as ratHEV-seropositive when reactivity against the ratHEV antigen was observed in the absence of reactivity against both the HEV antigen and the heterologous hantavirus control. Samples reactive against both hepevirus antigens were

classified as indeterminate and were not considered ratHEV-seropositive. Samples without reactivity against the E. coli positive substrate control were considered non-evaluable and excluded from the analysis. None of the ratHEV-seropositive samples showed reactivity against the hantavirus control antigen.

Molecular detection and sequencing

Nucleic acids were extracted from human serum and swine fecal samples. Human sera were analyzed in pools of four participants (100 µL per individual; 400 µL total), generating 132 pools (131 pools of four sera and one pool of three sera). RNA was extracted using the QIAamp MinElute Virus Spin Kit on the QIAcube automated platform (QIAGEN, Hilden, Germany) and eluted in 50 µL. RNA from swine fecal samples was extracted individually using the IndiSpin Pathogen Kit (Indical Bioscience, Leipzig, Germany) according to the manufacturer's instructions. RatHEV RNA was detected using a validated multiplex real-time RT-PCR assay targeting two independent regions within ORF1 using FAM- and HEX-labelled probes. The assay corresponds to the molecular diagnostic workflow previously developed and validated by our group following comparative evaluation of twelve published RT-qPCR assays and subsequent clinical validation in patients with acute hepatitis of unknown origin[20]. RT-qPCR-positive samples were confirmed by nested RT-PCR and Sanger sequencing as previously described. Positive controls consisted of RNA extracted from the liver of sequencing-confirmed ratHEV-positive rodents (GenBank accessions OR282813 and OR977609).

Phylogenetic analysis

RatHEV genotype assignment was performed using the ratHEV subtyping tool[21], and confirmed by BLASTn analysis. Sequences were aligned using MEGA version 11. Phylogenetic relationships were reconstructed using the neighbour-joining method with 1,000 bootstrap replicates. Complete ratHEV genomes available in GenBank at the time of analysis were included together with animal- and human-derived sequences previously characterized by our group. Two independent alignments spanning the 3' region of ORF1 were analyzed: one comprising 791 nucleotide positions across 90 sequences, including the longest sequence generated in the present study, and a second alignment of 278 nucleotide positions across 92 sequences including all swine-derived sequences.

Statistical analysis

The primary outcome was ratHEV IgG seropositivity. Seroprevalence was calculated as the proportion of seropositive participants with corresponding 95% confidence intervals (CI), using the Wilson score method for the overall estimate and the Clopper–Pearson exact method for stratum-specific estimates. The frequency of ratHEV RNA detection in swine was likewise expressed as a proportion with exact 95% CI. Categorical variables were compared using Fisher's exact test. Because only 15 seropositive events were observed, associations with seropositivity were estimated using Firth penalized logistic regression

to reduce small-sample bias. Confidence intervals were derived from the penalized profile likelihood, and statistical significance was assessed using penalized likelihood ratio tests. Cohort was retained as the main explanatory variable throughout the analysis. Age and sex are presented descriptively but were not included in the multivariable model because the limited number of events did not support additional covariates and age was structurally associated with cohort by study design. Conventional maximum-likelihood estimates are presented alongside penalized estimates for comparison. All tests were two-sided, and statistical significance was defined as $P \leq 0.05$.

Ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Oyo State Hospital Management Board (AD13/479/585) and the Babcock University Health Research Ethics Committee (BUHREC 529/21). All participants provided written informed consent. Export of biological samples to Spain was authorized by the General Directorate of Public Health of the Spanish Ministry of Health.

Results

Study population

A total of 527 participants and 544 domestic pigs were included in the study. Among participants, 281 (53.3%) belonged to the livestock-contact cohort, 167 (31.7%) to the villager cohort and 79 (15.0%) to the student cohort. Overall, 275 participants (52.2%) were male and 252 (47.8%) were female. The age distribution differed substantially between cohorts, with the student cohort comprising exclusively participants aged ≤ 40 years, whereas older individuals predominated in the livestock-contact cohort (Table 1).

Table 1
Demographic characteristics of the population and seroprevalence of rat hepatitis E virus IgG antibodies.

Variable	Global	Livestock contact	Villagers	Students
N	527	281	167	79
Gender, n (%)				
Male	275 (52.2)	143 (50.9)	89 (53.3)	43 (54.4)
Female	252 (47.8)	138 (49.1)	78 (46.7)	36 (45.6)
Age (years), n (%)				
> 50	132 (25)	88 (31.3)	44 (26.3)	0
41–50	91 (17.3)	65 (23.1)	26 (15.6)	0
31–40	108 (20.5)	69 (24.6)	39 (23.4)	0
21–30	133 (25.2)	39 (13.9)	47 (28.1)	47 (59.5)
≤ 20	63 (12)	20 (7.1)	11 (6.6)	32 (40.5)
ratHEV IgG antibodies, n (%)				
Negative	512 (97.2)	269 (95.7)	166 (99.4)	77 (97.5)
Positive	15 (2.8)	12 (4.3)	1 (0.6)	2 (2.5)
Legend: Number of individuals (N or n); percentage (%); ratHEV (rat hepatitis E virus).				

Human seroprevalence

RatHEV-specific IgG antibodies were detected in 15 of 527 participants, corresponding to an overall seroprevalence of 2.8% (95% CI 1.7–4.6) (Table 1; Supplementary Figure S1). Seroprevalence differed across cohorts (overall Fisher exact test, p = 0.077), being highest in the livestock-contact cohort (12/281; 4.3%; 95% CI 2.2–7.3), followed by the student cohort (2/79; 2.5%; 95% CI 0.3–8.9) and the villager cohort (1/167; 0.6%; 95% CI 0.02–3.3). Pairwise comparison showed a significantly higher seroprevalence in the livestock-contact cohort than in villagers (p = 0.037). Seroprevalence did not differ according to sex (p = 0.795) or between the two rural communities (p = 0.419).

Association between cohort and seropositivity

Firth penalized logistic regression showed that participants in the livestock-contact cohort had significantly higher odds of ratHEV seropositivity than villagers (OR 5.15; 95% profile-likelihood CI 1.23–47.56; p = 0.022). No significant difference was observed between the student and villager cohorts (OR 3.58; 95% CI 0.47–39.61; p = 0.212), and the overall cohort effect did not reach statistical significance (p = 0.073). Conventional maximum-likelihood logistic regression yielded similar estimates with wider confidence intervals (Table 2).

Table 2
Multivariate analysis for rat hepatitis E virus IgG antibody prevalence.

Variable	IgG + (n/N)	OR	OR, 95% CI	p-value
Gender				
Male	7/275	1.000		
Female	8/252	1.57	0.49–4.96	0.443
Age (per year increase)		0.96	0.92–0.99	0.029
Population				
Villagers	1/167	1.000		
Livestock contact	12/281	8.4	1.07–65.89	0.043

Legend: Number of individuals (N or n); odds ratio (OR); percentage (%); 95% confidence interval (95% CI); significance value (p).

Molecular detection of ratHEV

None of the human serum samples tested positive for ratHEV RNA. RatHEV RNA was detected in 4 of 544 swine fecal samples (0.7%; 95% CI 0.2–1.9). The four sequences shared 98.3–99.7% nucleotide identity (GenBank accession nos. PZ019511, PZ683210-PZ683212) and were assigned to genotype Ia by phylogenetic analysis (Fig. 1). They clustered with ratHEV strains previously identified in rodents from Sierra Leone and a human case from France. BLASTn analysis of the shorter amplicons showed the highest sequence similarity with ratHEV sequences recovered from wastewater in Guinea.

Discussion

Knowledge of the ecology of ratHEV in Africa remains extremely limited. Direct evidence has so far been restricted to detection in wild rodents from Sierra Leone and Kenya[12, 13], and more recently to environmental surveillance, with ratHEV identified in untreated urban wastewater from Guinea and closely related rodent-associated hepeviruses detected in wastewater from Cameroon[14, 15]. Consequently, whether viral circulation extended beyond reservoir and environmental compartments into human and domestic animal populations remained unknown. At the same time, current ecological frameworks for rodent-borne zoonoses emphasize that spillover is rarely determined by the reservoir alone but instead emerges from interactions between rodent ecology, environmental contamination, domestic animals and human activities [22]. Within this framework, the demonstration of human exposure together with molecular detection of ratHEV in domestic pigs substantially expands the recognized ecology of ratHEV in Africa and identifies the human-livestock interface as a previously unexplored epidemiological compartment.

The overall seroprevalence observed in our study was 2.8% (95% CI 1.6–4.7). Although comparisons between studies should be interpreted cautiously because of differences in study populations and serological assays, this estimate lies among the highest reported in surveys performed with ratHEV-specific methods. It exceeded the prevalence described in urban populations from Hong Kong and in people living with HIV in Spain[18, 23], while remaining comparable to cohorts selected a priori because of increased occupational or environmental exposure, including people who use drugs in Spain and rural residents from Hainan, China[19, 24]. Together, these comparisons indicate that human exposure to ratHEV in southwestern Nigeria is not anecdotal and occurs at frequencies similar to those reported in populations already recognized as being at increased risk. The distribution of seropositivity within our cohorts provides additional epidemiological information. Participants with regular occupational livestock contact showed the highest seroprevalence and significantly higher odds of seropositivity than villagers without occupational animal exposure. Although based on a limited number of events, this association is biologically coherent. Livestock production systems frequently provide favorable conditions for synanthropic rodents through the availability of feed, shelter and water, while occupational activities increase opportunities for indirect exposure to rodent excreta through contaminated surfaces, dust, feed and water rather than through direct contact with rodents themselves[22]. These findings support the hypothesis that livestock production environments represent ecological interfaces where human exposure to ratHEV is more likely to occur.

Evidence of human exposure was accompanied by molecular detection of ratHEV in domestic pigs from the same geographical setting. Experimental studies have demonstrated that pigs are susceptible to ratHEV infection, support viral replication, shed viral RNA in feces and transmit infection to contact animals, while natural infection has also been documented under field conditions in Europe[8–10]. Our findings extend these observations to sub-Saharan Africa and indicate that this ecological interaction is not confined to Europe or Asia. Although viral shedding was detected in only four pigs (0.7%), naturally infected animals demonstrate that ratHEV reaches domestic livestock under field conditions in Africa. This observation supports the hypothesis that pigs participate in the ecology of ratHEV at the human–livestock interface. Whether they function as true bridge hosts facilitating indirect human exposure remains to be determined, but their demonstrated susceptibility identifies them as relevant components of the transmission network. The phylogenetic analysis further supports this ecological interpretation. All four Nigerian sequences belonged to genotype C1, subtype Ia, and clustered with strains previously identified in rodents from Sierra Leone, a human case from France and wastewater from Guinea[5, 12, 15]. The observed nucleotide identities are compatible with the substantial genetic diversity already recognized within ratHEV and do not indicate direct epidemiological linkage between these cases. Collectively, however, these findings document the presence of genetically related ratHEV lineages across wildlife reservoirs, environmental matrices, domestic livestock and humans in West Africa. Whether these observations represent a single interconnected transmission network or multiple independent ecological cycles cannot be resolved with the available data and should be addressed through integrated longitudinal investigations.

Our study has several limitations. The cross-sectional design precludes establishing temporal relationships between human exposure and infection in pigs. Rodents were not sampled, preventing direct comparison between the reservoir population and the human and livestock compartments within the same ecological setting. Molecular investigation in pigs relied exclusively on fecal samples and therefore reflects active viral shedding rather than cumulative exposure. In addition, exposure assessment in humans was limited to demographic characteristics and self-reported livestock contact, without detailed information on housing conditions, rodent infestation or food storage practices, all of which are recognized determinants of indirect rodent-borne transmission[22]. Finally, the relatively small number of seropositive individuals limits the precision of the estimated associations. Nevertheless, this study represents the first integrated investigation of ratHEV simultaneously evaluating human exposure and domestic livestock infection in Africa using ratHEV-specific serological and molecular methods previously developed, analytically validated and clinically applied by our group[18–20].

In conclusion, the present study provides the first evidence of human exposure to ratHEV together with molecular detection in domestic pigs in sub-Saharan Africa. Considered alongside previous reports from rodents and wastewater[12–15], these findings substantially broaden the recognized ecological distribution of ratHEV on the continent and identify the human-livestock interface as a key component of its transmission ecology. Defining how wildlife reservoirs, domestic animals, environmental contamination and human populations interact should now become a priority for One Health research and surveillance aimed at understanding the public health significance of this emerging zoonotic virus.

Declarations

Funding:

This work was funded by the Andalusian General Secretariat for Research, Development and Innovation in Health (PI-0287-2019), the Spanish Ministry of Health (RD12/0017/0012) and the Instituto de Salud Carlos III (PI21/00793, PI22/01098, PI24/00557), with co-funding from the European Union. ARJ is supported by a contract from the Spanish Junta de Andalucía (Nicolas Monardes Program: C1-0001-2023). MCJ and LRM are recipients of a CONTRATOS PREDOCTORALES DE FORMACIÓN EN INVESTIGACIÓN EN SALUD (PFIS) (FI22/00180 and FI25/00103) from the Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union. DCM is the recipient of a Juan Rodes (JR24/00047) grant from the Instituto de Salud Carlos III (ISCIII) and co-funded by the European Union. CFT is the recipient of a Río Hortega grant by the Carlos III Health Institute (Instituto de Salud Carlos III-ISCIII; CM24/00099). PLL is the recipient of a Sara Borrell Research Contract program (CD24/00007) by Carlos III Health Institute and co-funded by the European Union.

The funders did not play any role in the design, conclusions, interpretation of the study, or decision to publish.

Author Contribution

Author contributions: A.R.-J. and A.R. conceptualized the study and designed the work. A.S.O., J.O.A., O.D.O., O.O.O., O.A.T.A. and D.O.O. contributed to sample and data collection in Nigeria. L.R.-M., P.L.-L., J.C.-G. and M.C.-J. performed the molecular and phylogenetic analyses. J.C.-G., P.L.-L., M.C.-J., D.C.-M., J.O.A. and A.B.P. managed samples. L.R.-M., A.R.-J. and A.R. analyzed the data. L.R.-M., A.R.-J., J.O.A. and A.S.O. drafted the manuscript. All authors revised and approved the final version.

Acknowledgement

We thank Laura Ruiz Torres and Ismael Zafra Soto for technical support in sample processing and analysis, and the Humboldt Research Hub - Centre for Emerging and Re-emerging Infectious Diseases (HRH-CERID), LAUTECH, for assistance with sample storage.

Data availability:

All data generated or analyzed during this study are included in this article and its supplementary material. The raw datasets underlying the analyses are available from the corresponding author on reasonable request. Nucleotide sequences generated in this study were deposited in GenBank under accession numbers PZ019511 and PZ683210-PZ683212.

References

1. Wu H, Zhou L, Wang F, Chen Z, Lu Y. Molecular epidemiology and phylogeny of the emerging zoonotic virus Rocahepevirus: A global genetic analysis. Infect Genet Evol. 2024;118:105557. https://doi.org/10.1016/j.meegid.2024.105557.
2. Johne R, Plenge-Bonig A, Hess M, Ulrich RG, Reetz J, Schielke A. Detection of a novel hepatitis E-like virus in faeces of wild rats using a nested broad-spectrum RT-PCR. J Gen Virol. 2010;91:750–8. https://doi.org/10.1099/vir.0.016584-0.
3. Johne R, Heckel G, Plenge-Bönig A, Kindler E, Maresch C, Reetz J, et al. Novel Hepatitis E Virus Genotype in Norway Rats, Germany. Emerg Infect Dis. 2010;16:1452–5. https://doi.org/10.3201/eid1609.100444.
4. Sridhar S, Yip CCY, Wu S, Cai J, Zhang AJ-X, Leung K-H, et al. Rat Hepatitis E Virus as Cause of Persistent Hepatitis after Liver Transplant. Emerg Infect Dis. 2018;24:2241–50. https://doi.org/10.3201/eid2412.180937.
5. Andonov A, Robbins M, Borlang J, Cao J, Hatchette T, Stueck A, et al. Rat Hepatitis E Virus Linked to Severe Acute Hepatitis in an Immunocompetent Patient. J Infect Dis. 2019;220:951–5. https://doi.org/10.1093/infdis/jiz025.

6. Rivero-Juarez A, Frias M, Perez AB, Pineda JA, Reina G, Fuentes-Lopez A, et al. Orthohepevirus C infection as an emerging cause of acute hepatitis in Spain: First report in Europe. J Hepatol. 2022;77:326–31. https://doi.org/10.1016/j.jhep.2022.01.028.
7. Rivero-Juarez A, Johne R, Sridhar S. Rocahepevirus ratti: molecular evolution, zoonotic potential and public health impact. Nat Commun. 2026;17:4853. https://doi.org/10.1038/s41467-026-71382-3.
8. Rios-Muñoz L, Gonzálvez M, Caballero-Gomez J, Castro-Scholten S, Casares-Jimenez M, Agulló-Ros I et al. Detection of Rat Hepatitis E Virus in Pigs, Spain, 2023. Emerg Infect Dis 2024;30. https://doi.org/10.3201/eid3004.231629
9. Caballero-Gómez J, García-Bocanegra I, Cano-Terriza D, Casares-Jiménez M, Jiménez-Ruiz S, Risalde MA, et al. Zoonotic rat hepatitis E virus infection in pigs: farm prevalence and public health relevance. Porcine Health Manag. 2025;11:34. https://doi.org/10.1186/s40813-025-00450-9.
10. 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. https://doi.org/10.1093/pnasnexus/pgae259.
11. Situ J, Wong TC, Wu S, Li Z, Shun EHK, Ho SFS, et al. Epidemiology and clinical characteristics of rat hepatitis E virus infection in humans. J Infect. 2026;92:106667. https://doi.org/10.1016/j.jinf.2025.106667.
12. Zhang X-A, Zhang M-Q, Liu Y-W, Lin L, Zhang J-T, George T, et al. Virome characterization of wild small mammals provides new insight into zoonotic pathogens in West Africa. Microbiome. 2025;13:242. https://doi.org/10.1186/s40168-025-02195-7.
13. Ochola G, Pulkkinen E, Ogola JG, Mäkelä H, Masika M, Vauhkonen H et al. Rat hepatitis E virus and novel paramyxoviruses in synanthropic rodents and shrews in Kenya. 2026. https://doi.org/10.64898/2026.04.21.719784
14. 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.
15. Doukouré B, Pennec Y, Le, Fatoumata C, Diallo R, Touré I, Tordo N, et al. First Detection of Rocahepevirus in Urban Wastewater from Guinea: A One Health Alert. Pathogens. 2026;15:385. https://doi.org/10.3390/pathogens15040385.
16. Adesehinwa AOK, Boladuro BA, Dunmade AS, Idowu AB, Moreki JC, Wachira AM. — Invited Review — Pig production in Africa: current status, challenges, prospects and opportunities. Anim Biosci. 2024;37:730–41. https://doi.org/10.5713/ab.23.0342.
17. Oluremi AS, Casares-Jimenez M, Opaleye OO, Caballero-Gomez J, Ogbolu DO, Lopez-Lopez P, et al. Butchering activity is the main risk factor for hepatitis E virus (Paslahepevirus balayani) infection in southwestern Nigeria: a prospective cohort study. Front Microbiol. 2023;14. https://doi.org/10.3389/fmicb.2023.1247467.
18. Casares-Jimenez M, Rivero-Juarez A, Lopez-Lopez P, Montes ML, Navarro-Soler R, Peraire J et al. Rat hepatitis E virus (*Rocahepevirus ratti*) in people living with HIV. Emerg Microbes Infect 2024;13.

https://doi.org/10.1080/22221751.2023.2295389

19. Casares-Jimenez M, Corona-Mata D, Garcia-Garcia T, Manchado-Lopez L, Rios-Muñoz L, de Guia-Castro M, et al. Serological and molecular survey of rat hepatitis E virus (*Rocahepevirus ratti*) in drug users. Emerg Microbes Infect. 2024;13. https://doi.org/10.1080/22221751.2024.2396865.
20. Caballero-Gómez J, Casares-Jiménez M, Gallo-Marín M, Pereira-Pardo S, Beato-Benítez A, Poyato A, et al. Rat hepatitis E virus as an aetiological agent of acute hepatitis of unknown origin. J Hepatol. 2025. https://doi.org/10.1016/j.jhep.2025.02.027.
21. Lo KH-Y, Ho S-FS, Shun EH-K, Wu S, Yip CC-Y, Situ J, et al. Enhanced surveillance, subtyping, and host adaptation analysis reveal genotype-wide zoonotic potential of rat hepatitis E virus. J Hepatol. 2026;84:51–61. https://doi.org/10.1016/j.jhep.2025.07.004.
22. Friant S, Mistrick J, Luis AD, Harden C, Simons D, Fichet-Calvet E, et al. Reducing the threats of rodent-borne zoonoses requires an understanding and leveraging of three key pillars: disease ecology, synanthropy, and rodentation. Lancet Planet Health. 2025;9:101300. https://doi.org/10.1016/j.lanplh.2025.101300.
23. Situ J, Hon-Yin Lo K, Cai J-P, Li Z, Wu S, Hon-Kiu Shun E, et al. An immunoassay system to investigate epidemiology of Rocahepevirus ratti (rat hepatitis E virus) infection in humans. JHEP Rep. 2023;5:100793. https://doi.org/10.1016/j.jhepr.2023.100793.
24. Cui X, Situ J, Tang T, Li Z, Chen D, Ho SS-F, et al. Prevalence of Rocahepevirus ratti (rat hepatitis E virus) in humans and rats in China. JHEP Rep. 2025;7:101370. https://doi.org/10.1016/j.jhepr.2025.101370.

Figures

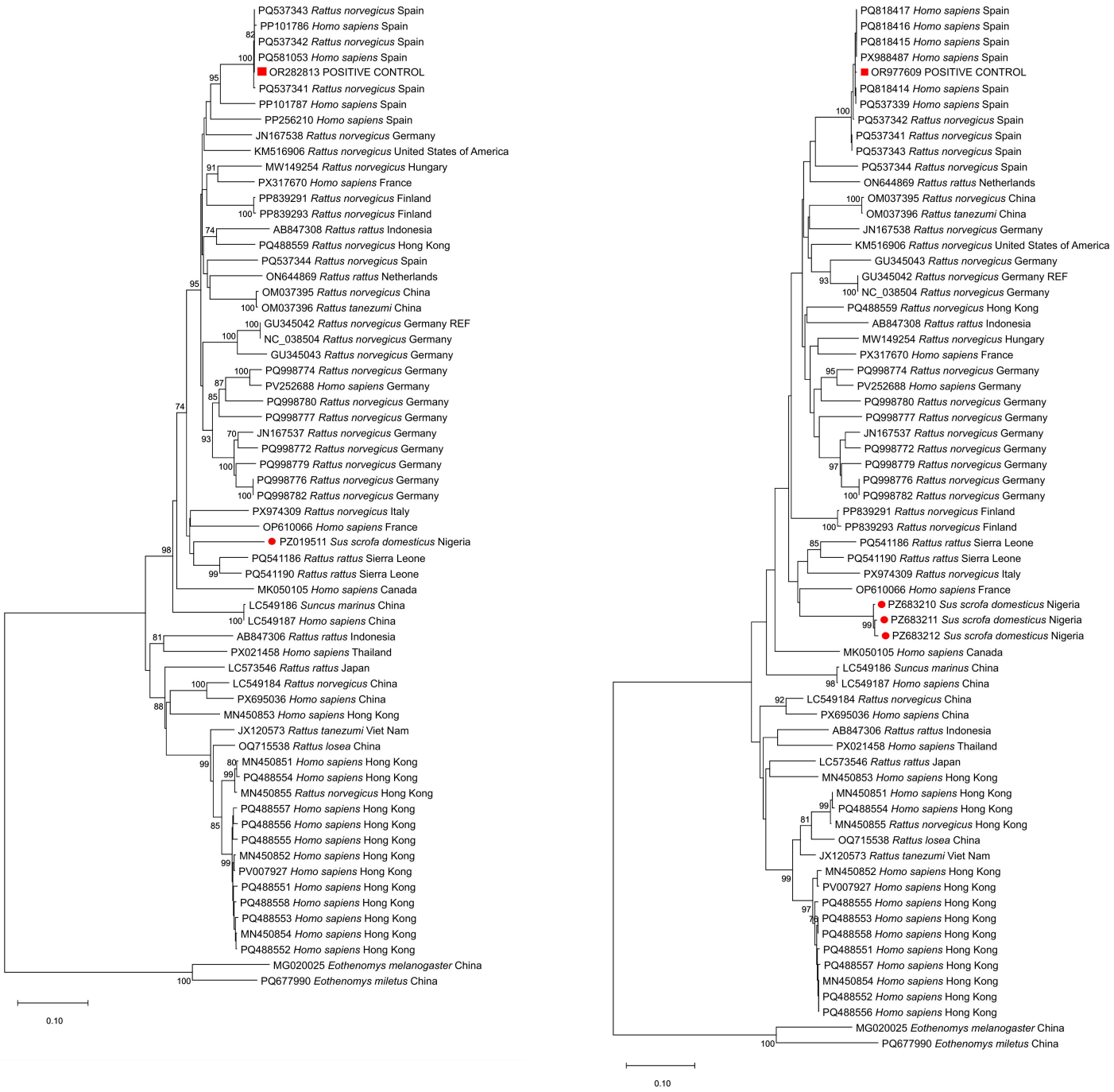


Figure 1

Phylogenetic tree constructed using the Neighbor Joining method (1,000 replicates). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Complete RatHEV sequences available in GenBank at the time of analysis and human-derived sequences previously characterized by our group.were included in both phylogenetic trees. **(A)** The analysis involved 63 nucleotide sequences and 719 positions located at the end of ORF1 in the final dataset. The sequence obtained in the present study is marked with a red dot and the positive control with a red square. **(B)** The analysis involved 67 nucleotide sequences and 282 positions located at the

end of ORF1 in the final dataset. The sequences obtained in the present study are marked with a red dot and the positive control with a red square.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Table2.docx
- SupplementaryMaterial.docx