Epidemiology of Hepatitis E in Guinea: a One Health Approach

Dissertation

der Mathematisch-Naturwissenschaftlichen Fakultät
der Eberhard Karls Universität Tübingen
zur Erlangung des Grades eines
Doktors der Naturwissenschaften
(Dr. rer. nat.)

vorgelegt von
Bakary Doukoure
aus Conakry, Guinea

Tübingen
2025

Gedruckt mit Genehmigung der Mathematisch-Naturwissenschaftlichen Fakultät der Eberhard Karls Universität Tübingen.

Tag der mündlichen Qualifikation:	27.02.2026
Dekan:	Prof. Dr. Thilo Stehle
1. Berichterstatter/-in:	Prof. Dr. Nadine Ziemert
2. Berichterstatter/-in:	apl. Prof. Dr. Thirumalaisamy P. Velavan

Statutory Declaration

I hereby declare that the doctoral thesis submitted with the title:
“Epidemiology of Hepatitis E in Guinea: a One Health Approach”
has been composed by myself independently, using only the sources and tools indicated. All passages that are quoted or paraphrased from other works have been clearly marked as such.

I confirm that the ethical and scientific guidelines for good research practice of the **University of Tubingen** and the collaborating institution have been strictly observed.
I solemnly declare that the information provided is true and complete, and that I have withheld nothing.
My individual contributions to joint publications are described in the section **“Declaration of Author Contribution.”**

Acknowledgements

I would like to express my sincere gratitude to my thesis supervisors:

Dr Pierre ROQUES, for giving me the opportunity to conduct this research within his unit. I am deeply grateful to him for his constant support throughout my studies, from my master's degree to my PhD, as well as for his insightful advice and unwavering support.

I would also like to thank Professor Nadine ZIEMERT and Professor Steffen BORRMANN for their diligent supervision throughout the various stages of the thesis evaluation and for writing the annual progress reports.

My special thanks go to Dr Noel TORDO. Without your guidance, I would never have discovered my true passion for viruses and virology. I thank you from the bottom of my heart for allowing me to complete my Master's thesis and spend time at the Pasteur Institute of Cameroon, an experience that laid the foundations for my scientific career and my current work on HEV. Your support and encouragement have been invaluable, and I will never forget it.

I would like to express my warmest thanks to the University of Tübingen, through the Caidera project, for funding my research and for the enriching conferences organised as part of the CAIDERA and DAAD seminars.

I would also like to express my gratitude to my CAIDERA research team: all the coordinators, and especially Alejandra Duque (thank you, Alejandra), as well as my colleagues Maradona, Emmanuel, Caroline and Barbara. Working with you has been an exceptional experience, and I hope that our collaboration will continue.

I would like to thank everyone who contributed to the smooth running of this project, from Germany to Guinea, via Gabon.

Special thanks go to the staff of the INSTITUT PASTEUR DE GUINÉE. Thank you from the bottom of my heart for your support, your availability and your friendship. Without you, this thesis would not have been possible. You have been exceptional colleagues and friends.

Thank you to Isabelle, Sagno, Salimatou and Noumouké Traoré (may you rest in peace) for your invaluable help and the unforgettable moments we shared; I hope these moments will continue forever.

Reine, Fatoumata, Siba and Issiaga, thank you for your constant support in the laboratory and beyond.

I would also like to thank Dr Solène, Dr Aissata, Dr Jean Mathieu Bart and Dr Brice for their scientific and technical support in the laboratory.

Dr Cécile Troupin, despite your departure from the Institute, your presence has been very important to me. Thank you for the pleasant moments we spent together and for the beautiful memories we will continue to share.

Noumouké KANTE, you have been a valuable colleague from day one and will always remain so. Thank you for your wise advice, your attentive listening and your ability to reassure me when stress was mounting.

My deepest gratitude also goes to my family for their unwavering support in all my projects. Without you, none of this would have been possible, and I will be eternally grateful to you.

Finally, a special thank you to my father and mother. Dad, you have always been my motivation in everything because you were the solution to all my problems and stresses, and you always managed to guide me in everything I undertook. You are gone, but I know you are watching over us from heaven.

Mum, thank you for being there and thank you for everything you have done and continue to do for us. We cannot thank you enough. I wish you a long and healthy life.

My brothers Yeli and Mamadou Bobo, my sister Aissata, my sister-in-law Mariame, thank you for your support.

To my dear wife Mariame Ciré Barry, thank you for coming into my life and giving me my son Mamadou Bobo and thank you for your constant support.

Thank you to God Almighty.

This thesis is dedicated to two important people I have lost: my father Nima DOUKOURE and my little sister Aissata DOUKOURE. May your souls rest in peace, Amen!

Abstract

The hepatitis E virus (HEV) is an emerging pathogen with zoonotic and environmental transmission, representing a major public health concern in West Africa. Its circulation involves complex interactions between humans, animals, and environmental populations. In Guinea, few data are available to understand these interactions, and the dynamics of HEV remain insufficiently characterized, particularly among animal reservoirs and especially in high-risk populations such as pregnant women.

This thesis objective is to investigate HEV circulation within a One Health framework in Guinea, combining human, animal, and environmental data to characterize circulating viral types, and factors influencing transmission.

Serological surveys were conducted in pigs, with molecular analyses targeting HEV on fecal and blood samples. Urban wastewater in Conakry was analyzed to detect and identify potential environmental sources of HEV infection. Other side, a study among pregnant women assessed IgG/IgM seroprevalence and the presence of viral RNA. The data were analyzed to examine regional variations and ecological or anthropogenic factors associated with viral circulation. The mean serological prevalence in pigs was 22%, with significant regional disparities: 43% in the Forest region and 7% in Lower Guinea. A complementary analysis revised the national prevalence to 35%. Molecular detection indicated low active circulation of HEV-3c in pigs. Environmental analyses identified Rocahepevirus ratti (HEV-C1) in Conakry wastewater, suggesting the existence of an epidemiological cycle linked to rodents. Besides, among pregnant women, IgG seroprevalence was low (2.4%) and no RNA detection was observed, showing limited active transmission. The results assessed two epidemiological cycles: a porcine HEV-3c cycle and an environmental HEV-C1 cycle associated with rodents. The spatial distribution of pig seroprevalence reflects the influence of farming practices and ecological factors. These results demonstrate the complexity of HEV transmission mechanism and the importance of integrated surveillance integrating serological, molecular, and environmental approaches to better understand risks to human health. This work shows the necessity of a One Health approach to characterize HEV dynamics in Guinea and guide strategies of prevention. The combination of animal, human, and environmental data provides essential insights to identify reservoirs, understand spatial variability, and prepare targeted interventions to reduce the risk of transmission to humans. Integrated surveillance combining serological, molecular, and environmental approaches is needed to better understand risks to human health. This thesis demonstrates the necessity of a One Health approach to characterize HEV dynamics in Guinea and guide prevention strategies. The combination of animal, human, and environmental data provides essential insights to identify reservoirs, understand spatial variability, and tailor targeted interventions to reduce the risk of transmission to humans.

Zusammenfassung

Das Hepatitis-E-Virus (HEV) ist ein neu auftretender Erreger, der zoonotisch und über die Umwelt übertragen wird und in Westafrika ein großes Problem für die öffentliche Gesundheit darstellt. Seine Verbreitung ist mit komplexen Wechselwirkungen zwischen Menschen, Tieren und Umweltpopulationen verbunden. In Guinea liegen nur wenige Daten vor, um diese Wechselwirkungen zu verstehen, und die Dynamik des HEV ist nach wie vor unzureichend charakterisiert, insbesondere bei Tierreservoirs und vor allem bei Hochrisikogruppen wie schwangeren Frauen.

Das Ziel dieser Arbeit ist es, die Verbreitung des HEV in Guinea im Rahmen eines One-Health-Ansatzes zu untersuchen, indem Daten zu Menschen, Tieren und der Umwelt kombiniert werden, um die zirkulierenden Virustypen und die Faktoren, die die Übertragung beeinflussen, zu charakterisieren.

Es wurden serologische Untersuchungen an Schweinen durchgeführt, wobei molekulare Analysen auf HEV in Stuhl- und Blutproben abzielten. Das städtische Abwasser in Conakry wurde analysiert, um potenzielle Umweltquellen für HEV-Infektionen zu erkennen und zu identifizieren. Andererseits wurde in einer Studie unter Schwangeren die IgG/IgM-Seroprävalenz und das Vorhandensein von viraler RNA untersucht. Die Daten wurden analysiert, um regionale Unterschiede und ökologische oder anthropogene Faktoren im Zusammenhang mit der Viruszirkulation zu untersuchen. Die durchschnittliche serologische Prävalenz bei Schweinen betrug 22 %, mit erheblichen regionalen Unterschieden: 43 % in der Waldregion und 7 % in Niederguinea. Eine ergänzende Analyse korrigierte die nationale Prävalenz auf 35 %. Der molekulare Nachweis zeigte eine geringe aktive Zirkulation von HEV-3c bei Schweinen. Umweltanalysen identifizierten Rocahepevirus ratti (HEV-C1) im Abwasser von Conakry, was auf die Existenz eines epidemiologischen Zyklus im Zusammenhang mit Nagetieren hindeutet. Außerdem war die IgG-Seroprävalenz bei schwangeren Frauen gering (2,4 %) und es wurde keine RNA nachgewiesen, was auf eine begrenzte aktive Übertragung hindeutet. Die Ergebnisse bewerteten zwei epidemiologische Zyklen: einen Schweine-HEV-3c-Zyklus und einen Umwelt-HEV-C1-Zyklus in Verbindung mit Nagetieren. Die räumliche Verteilung der Seroprävalenz bei Schweinen spiegelt den Einfluss landwirtschaftlicher Praktiken und ökologischer Faktoren wider. Diese Ergebnisse zeigen die Komplexität des HEV-Übertragungsmechanismus und die Bedeutung einer integrierten Überwachung, die serologische, molekulare und umweltbezogene Ansätze umfasst, um die Risiken für die menschliche Gesundheit besser zu verstehen. Diese Arbeit zeigt die Notwendigkeit eines One-Health-Ansatzes, um die HEV-Dynamik in Guinea zu charakterisieren und Präventionsstrategien zu entwickeln. Die Kombination von Daten zu Tieren, Menschen und der Umwelt liefert wichtige Erkenntnisse, um Reservoirs zu identifizieren, räumliche Variabilität zu verstehen und gezielte Maßnahmen zur Verringerung des Übertragungsrisikos auf den Menschen vorzubereiten. Eine integrierte Überwachung, die serologische, molekulare und umweltbezogene Ansätze kombiniert, ist erforderlich, um die Risiken für die menschliche Gesundheit besser zu verstehen. Diese Arbeit zeigt die Notwendigkeit eines One-Health-Ansatzes, um die HEV-Dynamik in Guinea zu charakterisieren und Präventionsstrategien zu entwickeln. Die Kombination von Tier-, Human- und Umweltdaten liefert wichtige

Erkenntnisse, um Reservoirs zu identifizieren, räumliche Variabilität zu verstehen und gezielte Maßnahmen zur Verringerung des Übertragungsrisikos auf den Menschen zu entwickeln.

.

Table of content

List of sigles and abbreviations

eHEV : enveloped Hepatitis E Virus

ER : Endoplasmic Reticulum

ESCRT : Endosomal Sorting Complex Required for Transport

Hel : Helicase

HEV : Hepatitis E Virus

HSC70 : Heat Shock Cognate 70

HSPGs : Heparan Sulfate ProteoGlycans

HVR : Hyper Variable Region

ICTV : International Committee on Taxonomy of Viruses

IFN-γ : Interferon-gamma.

IgG : Immunoglobulin G

IgM : Immunoglobulin M

kb : kilobase

Met : Methyltransferase

neHEV : naked Hepatitis E Virus

NK cells : Natural Killer cells

ORF : Open Reading Frame

PCP : Papain-like Cysteine Protease

PSAP motif : Proline-Serine-Alanine-Proline motif

RdRp : RNA-dependent RNA polymerase.

RNA : Ribonucleic Acid

sgRNA : subgenomic RNA.

TSG101 : Tumor Susceptibility Gene 101 protein

TNF-α : Tumor Necrosis Factor-alpha

WHO : World Health Organization

ELISA: Enzyme-Linked Immunosorbent Assay

PCR: Polymerase Chain Reaction

RTPCR: Reverse Transcription Polymerase Chain Reaction

RTqPCR: Quantitative Reverse Transcription PCR

FLI: Friedrich Loeffler Institute

DNA: Deoxyribonucleic Acid

HIV: Human Immunodeficiency Virus

RNA Seq: RNA Sequencing

CI: Confidence Interval

CDC: Centers for Disease Control and Prevention

List of figures and tables

List of publications

Part I Accepted publication in decreased date of publication

1) Epidemiological situation of hepatitis E in Africa. **Doukouré B**, Tordo N, Roques P. Virologie (Montrouge). 2025 Oct 29;29(5):0. doi: 10.1684/vir.2025.1112

2) Eloiflin R, Pérez-Antón E, Camara A, Dujeancourt-Henry A, Boiro S, Djetchi MN, Traoré MB, Koffi M, Kaba D, Le Pennec Y, **Doukouré B**, Camara AD, Kagbadouno M, Campagne P, Camara M, Jamonneau V, Thévenon S, Bart JM, Glover L, Rotureau B. A SHERLOCK toolbox for eco-epidemiological surveillance of African trypanosomes in domestic pigs from Western Africa. *Elife.* 2025 Sep 22;14:RP106823. doi: 10.7554/eLife.106823. PMID: 40981773.
3) **Doukouré B**, Le Pennec Y, Troupin C, Grayo S, Eiden M, Groschup MH, Tordo N, Roques P. Seroprevalence and Phylogenetic Characterization of Hepatitis E Virus (Paslahepevirus balayani) in Guinean Pig Population. *Vector Borne Zoonotic Dis*. 2024 Aug;24 (8):540-545. doi: 10.1089/vbz.2023.0104. Epub 2024 Apr 23. PMID: 38651618
4) Grayo S, Camara A, **Doukouré B**, Ellis I, Troupin C, Fischer K, Vanhomwegen J, White M, Groschup MH, Diederich S, Tordo N. Geographic Disparities in Domestic Pig Population Exposure to Ebola Viruses, Guinea, 2017-2019. *Emerg Infect Dis.* 2024 Apr;30 (4):681-690. doi: 10.3201/eid3004.231034. PMID: 38526081.

5) Grayo S, Troupin C, Diagne MM, Sagno H, Ellis I, **Doukouré B**, Diallo A, Bart JM, Kaba ML, Henry B, Muyisa BS, Sow MS, Dia N, Faye O, Keita S, Tordo N. SARS-CoV-2 Circulation, Guinea, March 2020-July 2021. *Emerg Infect Dis*. 2022 Feb;28 (2):457-460. doi: 10.3201/eid2802.212182. Epub 2021 Dec 14.

6) Troupin C, Ellis I, **Doukouré B**, Camara A, Keita M, Kagbadouno M, Bart JM, Diallo R, Lacôte S, Marianneau P, Groschup MH, Tordo N. Seroprevalence of brucellosis, Q fever and Rift Valley fever in domestic ruminants in Guinea in 2017-2019. *BMC Vet Res*. 2022 Feb 4;18 (1):64. doi: 10.1186/s12917-022-03159-x. PMID: 35120506.

Part II Manuscript ready for publication

7) 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], N Tordo[1], P Roques[1].
8) Epidemiological characteristics of Hepatitis E virus (HEV) infection in West Africa. Komi Victor-Mari Setondji[1†] ,Kuan Abdoulaye Traoré[1, 2] ,Bakary Doukouré[3, 4] ,Jean-Bienvenue Ouoba[1] ,Madou Sanou[1] ,Essodolom Taale[5] ,Bruno Lalidia Ouoba[1] ,Pierre Roques[3] ,Simplice Damintoti Karou[5] ,Nicolas Barro[1].

Personal contributions to publications and manuscripts

Part I Accepted publications

Article 2, 4, 5 and 6 not directly related to the current thesis work.

To these research articles, the first published article 6, I contribute by performing animal sampling in cattles, sheep and goat and I did part of the specific ELISA experiment. In article 5, I was involved in the diagnosis of the SARS-CoV 2 patient by quantitative PCR and data reporting and curation. In article 4 and 2, I contributed to the field studies by chosen site and villages, and I did part of the pig sampling and material storage that was thereafter used in the article related to this thesis.

Article 1 and 3 related to this current thesis work,

For these articles, I was the main contributor to last published one, a review in which I did literature research and I was writing main parts of the manuscript (Abstract, Introduction, Main text, Conclusion) under the supervision of Noel Tordo and Pierre Roques (Article 1). The research article 3 I made major contributions to the conception of this manuscript and experimental design under the supervision of Drs Pierre Roques and Noel Tordo. I performed all biological assays from sampling, material conditioning and serological assays , I set up the molecular detection and sequencing procedure and analysis as well as statistical analysis and I wrote the first version of the article then contributed to all the stage of the publication procedure.

Part II : Articles ready for submission

Article 7 directly related to the current thesis work.

For this article , I was mainly involved in the conception of the review and contributed by doing literature research and writing main parts of the manuscript (Abstract, Introduction, Main text, Conclusion) under the supervision of Noel Tordo and Pierre Roques. In cooperation with Pierre ROQUES, I was involved in manuscript editing and Figure design.

Article 8 not directly related to the current thesis work.

To these research article, I contributed by performing correction of the manuscript and designing figure.

PART I: INTRODUCTION.

General Introduction

Global context of hepatitis E

Hepatitis E is an infection caused by the Hepatitis E virus (HEV), which is a virus belonging to the Hepeviridae family. The virus was considered self-limiting disease, but today it is rightly recognized as a major public health problem, especially in countries with limited resources (1). In human, several genotypes have been identified worldwide, with genotypes 1 and 2 infecting only humans and associated with large waterborne outbreaks in low-resource settings, whereas genotypes 3 and 4 are zoonotic and circulate among pigs and other animals, posing a risk for foodborne transmission(2). Limited access to drinking water and sanitation infrastructure facilitates the transmission of the disease in our countries with low health coverage. According to the World Health Organization (WHO), an estimated 20 million HEV infections occur annually, leading to over 3.3 million symptomatic cases and approximately 44,000 deaths, mainly in low-income countries(3)

The distinctive feature of HEV is that it can cause acute hepatitis in vulnerable populations, even though it appears to be asymptomatic and self-limiting in most of the population(4). In this vulnerable population, pregnant women are greatly affected, with a high risk of maternal and foetal complications, including a mortality rate of up to 25%(5).

Other risk groups include individuals with chronic liver disease, immunosuppressed patients, and transplant recipients, where HEV infection can evolve into chronic hepatitis with severe outcomes(4)

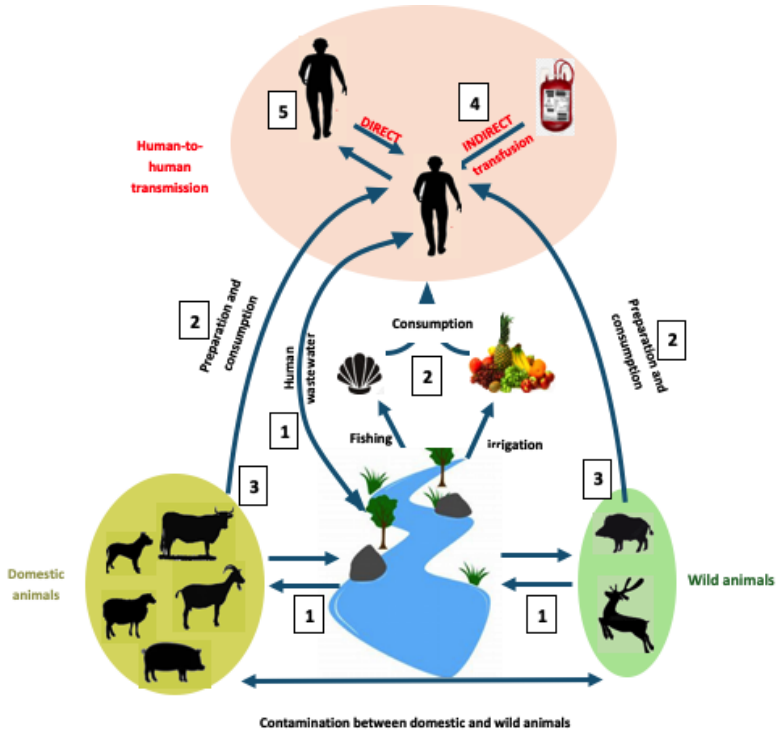


Figure 1: Transmission pathways of Hepatitis E(6)

(1) Waterborne transmission via fecal contamination of drinking water or reverse contamination from humans; (2) Foodborne transmission through consumption of raw or undercooked meat from HEV reservoir animals (pigs, wild boars, deer) or shellfish; (3) Direct or indirect contact with reservoir animals (contact transmission); (4) Transmission via transfusion or organ transplantation; (5) Person-to-person transmission, including mother-to-child.

Most outbreaks in sub-Saharan Africa are waterborne, especially given our inadequate sanitation systems(7). Countries in the West African sub-region are no exception to this trend . In the Republic of Guinea, we have little perspective on the epidemiology of the disease due to the lack of an HEV surveillance system and the lack of research on the subject. The widespread precariousness of sanitation infrastructure, the frequent proximity between humans and animals in rural and peri-urban areas and limited diagnostic capabilities suggest that the virus is circulating silently or is underestimated. Occasional detections of anti-HEV antibodies, particularly among hospital staff, have been reported in a few local studies, reinforcing the

hypothesis of endemic circulation(8) and the need for a coordinated, multidisciplinary approach to better understand the epidemiology of the virus.

This highlights the relevance of the One Health approach, as understanding HEV transmission requires integrating human, animal, and environmental health data to design effective surveillance and control strategies.

Definition of the One Health framework and relevance to HEV

The 'One Health' concept is a multidisciplinary approach that recognizes the interdependence between human, animal and environmental health. Its objective is to promote synergy between the public health, veterinary medicine and environmental science sectors to specifically address health issues at the interface between humans, animals and ecosystems(9).

This concept has become increasingly important in the fight against emerging diseases, more than 60% of which are zoonotic in origin. It is particularly important in the case of hepatitis E, a pathogen that affects a wide range of hosts and has multiple transmission routes, involving both animal reservoirs and environmental sources(10).

The dual epidemiology of HEV perfectly illustrates the need for a 'One Health' approach: HEV 1 and HEV 2 genotypes are transmitted via the fecal-oral route, through contaminated water, highlighting shortcomings in sanitation and access to drinking water. Zoonotic genotypes such as HEV 3, HEV 4 and HEV 7 circulate in various animal species (pigs, wild boars, rabbits, etc.) and can be transmitted to humans through the consumption of contaminated animal products or direct contact with animal excretions. Environmental research has revealed the presence of HEV in wastewater confirming the role of the environment in viral transmission(11).

The 'One Health' approach to hepatitis E detection provides an understanding of the ecology and transmission dynamics of the virus. This strategy is based on: Human and Animal surveillance, to detect emerging strains with zoonotic potential together with environmental surveillance through wastewater monitoring.

Genomic sequencing of strains from human, animal, and environmental sources is essential to assess the link between the different strains and monitor viral evolution, detect potential recombination events, and anticipate the emergence of highly pathogenic variants.

Serological, epidemiological and molecular investigations in humans, targeting at-risk populations such as pregnant women and immunocompromised patients, in addition to genomic and phylogenetic analyses, enable the links between animal, environmental and human strains

to be traced (5,12). Thus, the One Health approach provides a strategic framework for risk assessment and the implementation of effective public health policies. It supports the development of regulations on food safety, biosecurity in livestock farming, water management and vaccination strategies where they exist(13).

Virology of HEV

Taxonomic classification

HEV is a virus belonging to the Hepeviridae family. It comprises quasi-enveloped, positive sense single stranded RNA viruses with an icosahedral capsid measuring 27 to 34 nm in diameter. The viral genome is 7.2 kilobases long and comprises three open reading frames (ORF1, ORF2 and ORF3)(14). ORF1 encodes a non-structural polyprotein comprising several functional enzyme domains (methyltransferase, protease, macro domain, helicase and RNA-dependent RNA polymerase), ORF1 also contains a hypervariable region (HVR) whose variability is thought to contribute to adaptation to different hosts, underlining the zoonotic potential of certain genotypes(15). ORF2 encodes the capsid protein, which is essential for the formation of the viral particle. Finally, ORF3 encodes a small phosphoprotein involved in the assembly and release of virions.

According to the ICTV (International Committee on Taxonomy of Viruses) classification updated in 2024 (https://ictv.global/files/proposals/approved), the Hepeviridae family is divided into two subfamilies: *Orthohepevirinae*, which includes viruses that infect mammals and birds, and *Parahepevirinae*, which includes viruses that infect fish. Within the *Orthohepevirinae* subfamily, the *Paslahepevirus* genus includes zoonotic and human viruses classified under the species *Paslahepevirus balayani*. This species comprises eight main genotypes:

HEV-1 and HEV-2 strictly human, responsible for waterborne epidemics, particularly in low-income countries. HEV-3 and HEV-4 zoonotic transmission, circulating in humans but also in several animal species, including domestic pigs, wild boars, deer, rabbits and camels. HEV-5 and HEV-6 found in wild boars. HEV-7 and HEV-8 found in camelids, with at least one documented case of HEV-7 transmission to humans(16). Other genera in the Orthohepevirinae subfamily include:

Avihepevirus, responsible for hepatitis with splenomegaly in poultry, particularly chickens; *Rocahepevirus*, which includes viruses detected in rodents and carnivores(17). This group

includes rat HEV, now considered to have proven zoonotic potential. *Chirohepevirus*, consisting of HEV-related viruses identified in various bat species(16,18)

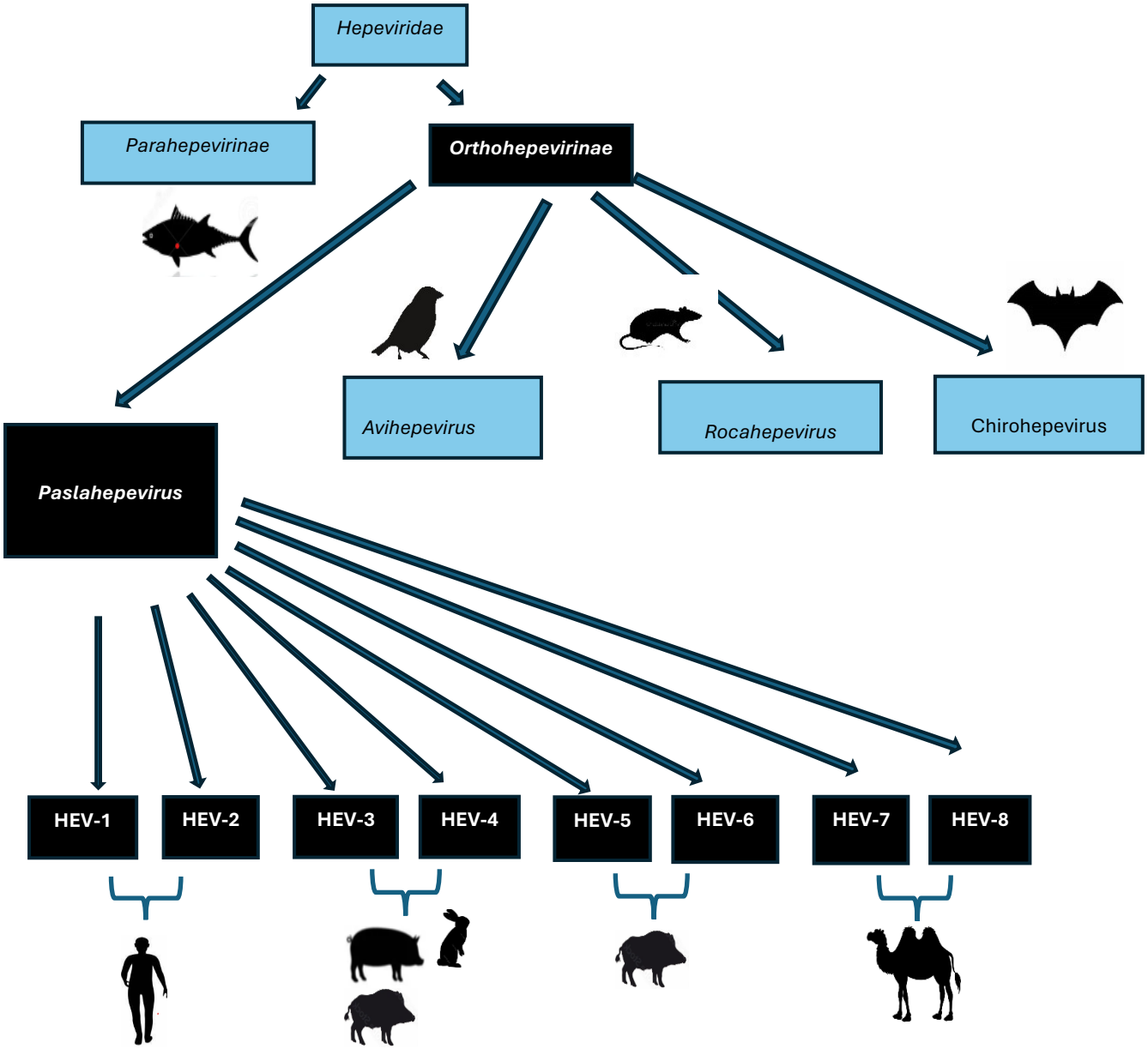


Figure 2: The Hepeviridae family, organized into subfamilies (-virinae), genera (-virus), and the genotypes of the genus Paslahepevirus (hepatitis E virus – HEV) with their associated reservoir species(6).

Viral Biology of Hepatitis E Virus (HEV)

Structure and genomic organization of the VHE

HEV virus is a quasi-enveloped virus with a diameter of between 27 and 34 nm

In cell culture and in blood, HEV can be found associated with host-derived lipid membranes, which confer resistance to neutralizing antibodies and contribute to immune evasion(19), while in bile and feces it appears as a non-enveloped virion as described in the figure 1.

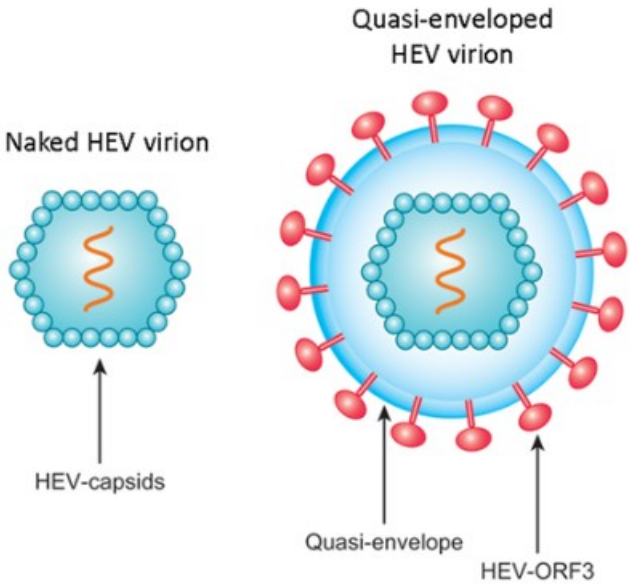


Figure 3: Structural representation of the hepatitis E virus (HEV) in host and environmental contexts(20)
This figure demonstrates the morphology of HEV : a non-enveloped (naked) icosahedral capsid structure, typically found in the environment and feces, and a quasi-enveloped form, observed in the bloodstream of infected hosts, where the virus is cloaked in host-derived membranes.

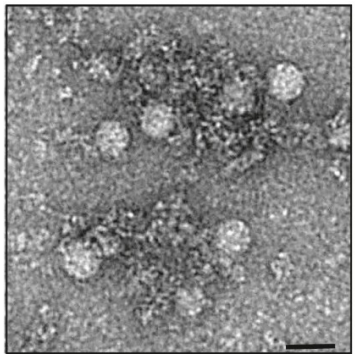


Figure 4: Hepatitis E virions Electronic microscopy imaging.

To visualize Hepatitis E virions, we referred to electron microscopy observations in which were specifically labeled using a mouse monoclonal antibody targeting the capsid protein, followed by a gold-conjugated secondary antibody (Horvatits et al., 2014) Figure 4.

The HEV genome is constituted by a single-stranded positive sense RNA of approximately 7.2 kilobases, with a 5' cap (7-methylguanosine) and a 3' poly A tail (Figure 5). This genomic

organization facilitates direct translation upon entry into the host cell cytoplasm, as the genome functions like an mRNA

It has three open reading frames (ORF1, ORF2 and ORF3) encoding, respectively, a non-structural polyprotein (including polymerase), the capsid protein, and a multifunctional protein (phosphoprotein)(22).

ORF1 mediates viral replication and transcription through its enzymatic domains, while ORF2 and ORF3 are essential for virion assembly and release. The interplay between ORF3 and host cell machinery highlights the complex strategies HEV employs to persist and spread(23).

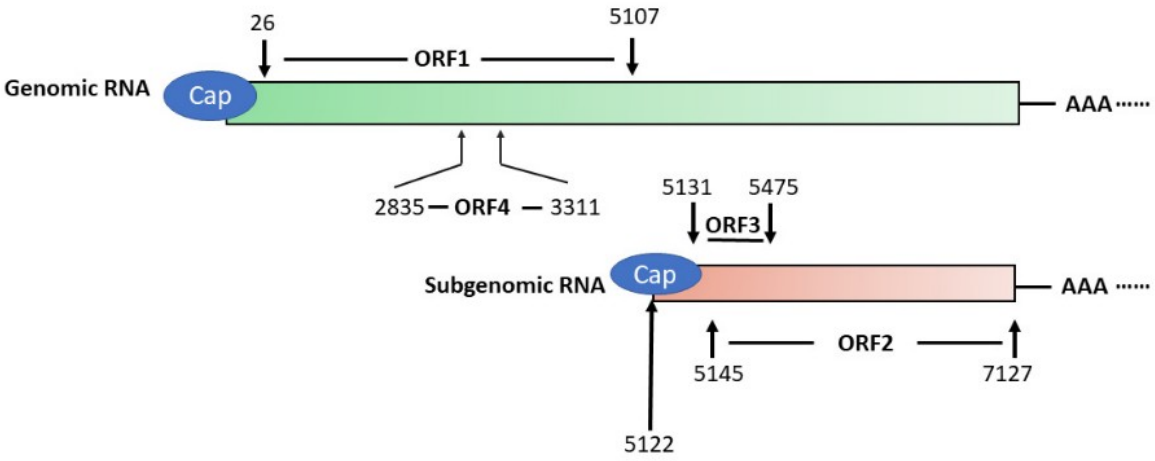


Figure 5: Representation of Hepatitis E Virus (HEV) genome and open reading frames (ORFs): ORF1 (non-structural polyprotein), ORF2 (capsid protein), ORF3 (phosphoprotein), and ORF4 (specific to genotype 1)

As shown in Figure 5, an ORF4 reading frame whose actual function is not well defined exists in HEVs of genotype 1(27). Recent study suggests that ORF4 enhances RdRp activity under endoplasmic reticulum stress conditions, potentially increasing viral replication efficiency during host stress response(27,28).

The ORF1 encodes a large non-structural polyprotein of 1693 amino acids involved in viral replication. Figure 6 highlights its conserved functional regions: Met (methyltransferase) region: RNA capping, Y domain: Membrane association, PCP (papain-like cysteine protease) region: Lipid metabolism and protein processing, HVR (hypervariable) region: Host adaptation, X region: Interferon antagonism and immune evasion, HEL (helicase) region: RNA unwinding and triphosphatase activity, RdRp (RNA-dependent RNA polymerase) region : RNA replication(24). Three (3) of these domains (Met, Hel, RdRp) have been well characterised functionally(25). The highly variable region HVR located within ORF1 exhibits great sequence diversity, even between strains of the same genotype. Some clinical isolates have viral or human insertions within this region, which contributes to the observed genomic diversity(25).

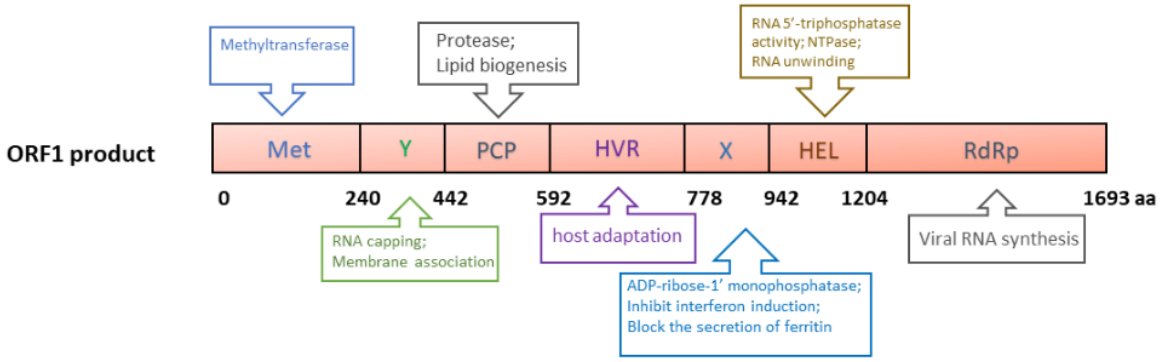


Figure 6: Representation of the ORF1 Polyprotein of HEV and Its Functional Domains

The ORF2 encodes the capsid protein, which is immunogenic and important for viral particle formation; it is used in serological diagnostic tests and in vaccine formulation(20). According to a study, HEV capsid protein ORF2 plays a central role in immune evasion by inhibiting antiviral signaling pathways and protecting viral replication from immune effectors. This function establishes a balance between viral replication and the host antiviral response, thereby enabling persistent infection even in immunocompetent cells. These findings identify ORF2 as a key determinant of HEV persistence and a promising target for novel therapeutic strategies(26).

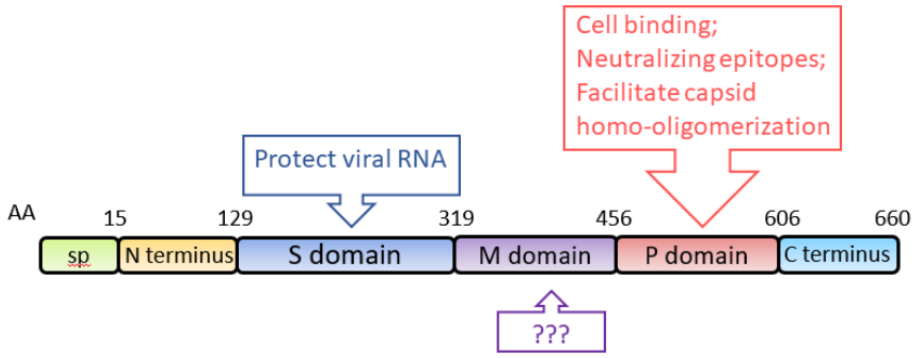


Figure 7 : Schematic representation of the HEV ORF2 protein.

As described in figure 7, the HEV ORF2 capsid protein is organized into three main domains: **S (shell)**, **M (middle)**, and **P (protruding)**. The **S domain** is known to protect the viral RNA, while the **P domain** has been well characterized, being involved in cell binding, neutralizing epitopes, and capsid homo-oligomerization. In contrast, the **M domain (aa 319–456)** remains poorly understood, and its precise role in the viral life cycle or immune interactions has yet to be clearly defined. The question mark ("???") in the figure reflects this current gap in knowledge.

The ORF3 encodes a multifunctional 13 kDa protein that is essential for assembly, release and modulation of the host response. It interacts significantly with the TSG101 protein, an essential component of the ESCRT system involved in virus budding and binds to microtubules to facilitate the release of virions. It also plays a role in suppressing the immune response, and more recently, it has been described as a functional ion channel essential for the secretion of infectious particles(24).

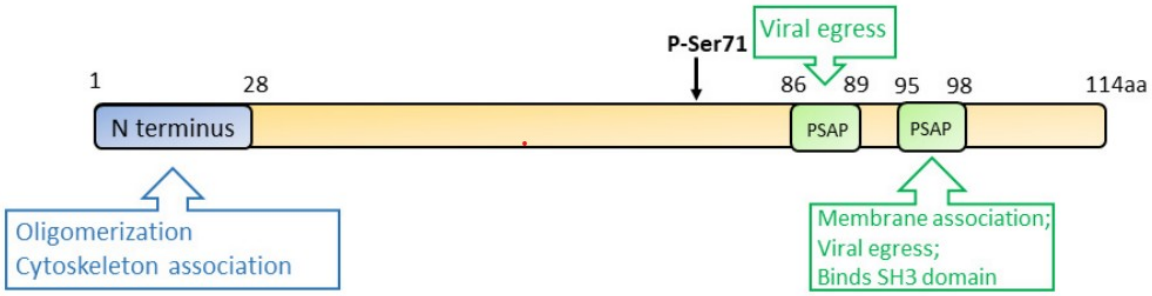


Figure 8: Structural organization of the HEV ORF3 protein.
This figure highlights the functional domains of ORF3 including the hydrophobic region, proline-rich domain (PSAP motif), and phosphorylation sites

Viral cycle and tropism

HEV exists in two forms in the body: first a quasi-enveloped virions, detected in blood and cell cultures, second a naked (unenveloped) virions, found in bile and feces, responsible for fecal-oral transmission.

The quasi-enveloped form provides immune evasion by masking viral epitopes, while the non-enveloped form is highly stable in the environment, facilitating transmission through contaminated water or food.

Although the exact mechanism by which the virus crosses the intestinal barrier remains poorly understood, it is postulated that the virions first infect enterocytes, then pass into the bloodstream in a quasi-enveloped form and then target hepatocytes. During biliary excretion, the lipid envelope is lost, and naked virions, which are more infectious, are released into the environment(19).

Binding to host cells is thought to be mediated by heparan sulphate proteoglycans (HSPGs) and the HSC70 protein, while α3 integrin has recently been proposed as a receptor facilitating dynamin- and clathrin-dependent endocytosis. Rab5 and Rab7 GTPases are also thought to be involved in the internalization of quasi-enveloped virions(29–31).

Once internalised, the lipid envelope (for quasi-enveloped virions) is degraded in lysosomes, allowing the release of genomic RNA into the cytoplasm. This step highlights the dual nature of HEV entry pathways, which share similarities with both enveloped and non-enveloped viruses, making its biology unique among enteric viruses.

The viral RNA is then translated into ORF1 polyprotein. RdRp synthesizes a negative strand, which serves as a template to produce two positive RNAs: a complete genomic RNA and a bicistronic subgenomic RNA (2.2 kb) encoding ORF2 and ORF3.

These RNAs, capped and polyadenylated, are used for the synthesis of viral proteins. The ORF2 and ORF3 proteins and the genomic RNA form a complex in the ER-Golgi intermediate compartment, where new viral particles are assembled. These can be released in an enveloped form (via TSG101) or naked (via bile)(24).

Many aspects of the HEV viral cycle remain poorly understood, including the mechanisms of cell entry, extrahepatic dissemination, and immune escape.

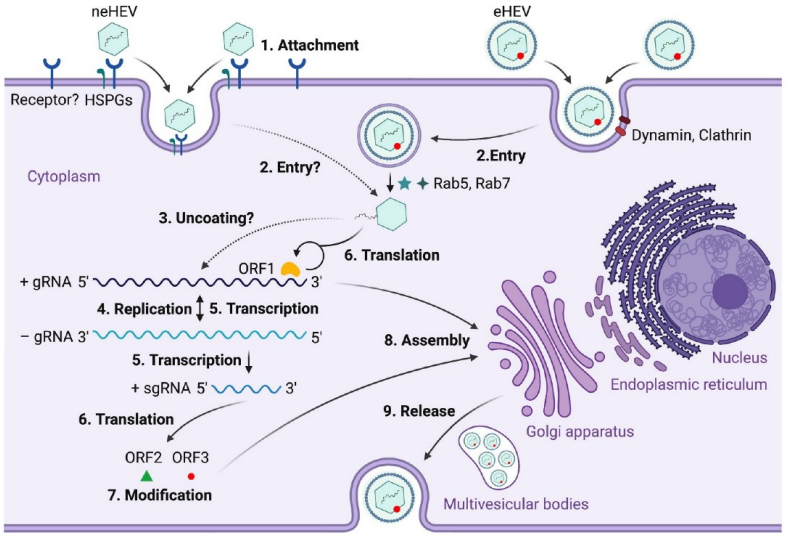


Figure 9: The Hepatitis E Virus (HEV) Replication Cycle and Viral Morphogenesis(20). (Adapted from Wang et al. This figure illustrates the key stages of the HEV life cycle, highlighting the entry pathways of both naked (neHEV) and quasi-enveloped (eHEV) particles, as well as intracellular replication and virion assembly:

1. ***Attachment and Entry****: neHEV virions bind to heparan sulfate proteoglycans (HSPGs) on hepatocyte surfaces, while eHEV particles are internalized via clathrin-mediated, dynamin-dependent endocytosis involving Rab5 and Rab7 GTPases.*
2. ***Uncoating and Translation****: After capsid removal, genomic RNA is released into the cytoplasm, initiating translation of the ORF1 polyprotein.*
3. ***RNA Replication****: A negative-sense RNA intermediate is synthesized to serve as a template for producing both full-length genomic RNA and subgenomic RNAs (sgRNAs).*
4. ***Protein Expression****: ORF2 (capsid) and ORF3 (multifunctional regulatory protein) are translated from sgRNAs and undergo post-translational modifications such as glycosylation, phosphorylation, and palmitoylation.*
5. ***Assembly and Egress****: The capsid protein (ORF2) assembles into virus-like particles that encapsidate newly synthesized genomic RNA, while ORF3 facilitates virion secretion and modulates host pathways to support replication.*

Medical importance of HEV

Clinical manifestation

HEV is generally responsible for asymptomatic, spontaneously resolving infection in immunocompromised individuals, but icteric and fulminant forms are possible, with case fatality rates of 0.5-4%(32).

However, the clinical signs associated with HEV infection are the same in developed and developing countries. In addition to hepatic manifestations, extra-hepatic complications such as neurological disorders (Guillain-Barré syndrome, neuralgic amyotrophy), renal involvement (glomerulonephritis), and hematological abnormalities (thrombocytopenia) have been reported, particularly in immunocompromised patients(33)

After an incubation period of 3 to 5 weeks, the pre-icteric phase, lasting 1 to 27 days, is characterized by a flu-like syndrome with loss of appetite, anorexia, constipation, diarrhea, fatigue, nausea, vomiting, and fever of 38 to 39°C in most cases. In the 10–24-day phase, jaundice is associated with dark urine and discolored stools, hepatomegaly, and even splenomegaly(34).

Curiously, severe forms with fulminant symptoms are mainly observed during epidemic episodes. Their frequency remains at 1% in the general population but can reach 45% in pregnant women(35).

Superinfection with HEV in patients with chronic hepatitis is an aggravating factor in hepatic decompensation, which manifests as ascites and hepatic encephalopathy of varying severity. Almost all chronic HEV cases reported to date have been genotype 3.

Extrahepatic Manifestations

Despite its largely known hepatotropic, HEV was involved in a low but consistent number of neurological diseases from Guillain-Barré syndrome to neuralgic amyotrophy and this as well in developing than in developed countries for up to 5% of the hospitalized HEV patients in a French-English cohort (36).

As for many hepatitis viruses a significant number of acute cases of HEV infection might be also associated to acute pancreatis(36). More importantly, HEV infection due to HEV genotype 3 in the case of transplantation way of contamination seems to be also associated to Cryoglobulinemia, a disease associated to renal disfunction, and recently, it was shown *in vitro* the efficiency of HEV to infect and replicate in renal cells(37).

Innate and Adaptive Immunity, Humoral Response

In healthy individuals, HEV infection leads to innate and adaptive immune responses:

With regard to innate immunity, in patients with acute hepatitis E, a significant and reversible increase in the number of NK cells and T lymphocytes was observed compared to the number observed in healthy controls. This suggests a role in the pathogenesis of HEV infection.

Cytokine profiling has shown elevated levels of interferon-gamma (IFN-γ), TNF-α, and interleukin-10 during acute infection, suggesting a mixed pro-inflammatory and regulatory immune response.

The humoral response follows a classic pattern with the early appearance of IgM at the onset of clinical signs, but these can persist for up to 32 weeks. IgG appears at the peak of transaminases and increases throughout the acute and convalescent phases, persisting for several year(38,39).

Antibodies that appear after symptomatic or asymptomatic infection are protective, but they do not prevent viral replication and excretion.

Diagnosis

HEV cannot be differentiated from other viruses based solely on clinical signs (jaundice or icterus). The first detection test was based on electron immunomicroscopy. Subsequently, laboratory analyses based on the presence of specific anti-HEV antibodies or viral RNA in serum or stool were developed. Serological tests (IgM and IgG) can help distinguish recent from past infection, but IgM may persist for several months, limiting its specificity for acute infection. Therefore, HEV RNA detection by PCR remains the gold standard for confirming active infection. To date, only molecular tests (PCR) that detect viral RNA in serum and stool can detect active infections. Cell culture has not yet been validated as a diagnostic test for HEV(40).

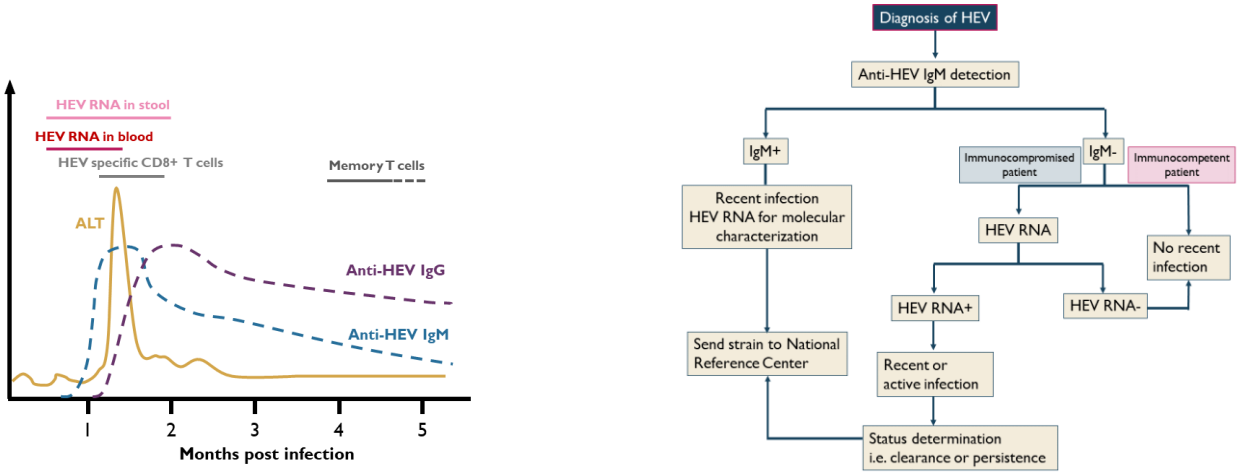


*Figure 10: **A**: kinetic of HEV markers in infected human(41). **B**: Flow diagram of the ideal HEV diagnosis(42)*

Global Epidemiology of HEV

HEV is recognized as one of the main causes of acute viral hepatitis worldwide. According to WHO, approximately 20 million HEV infections occur each year, of which 3.3 million are symptomatic, resulting in nearly 44,000 deaths. However, these figures likely underestimate the real burden due to limited diagnostic capabilities and underreporting, particularly in sub-Saharan Africa and Southeast Asia (https://www.who.int/news-room/fact-sheets/detail/hepatitis).

Epidemiologically in humans, HEV-1 and HEV-2 are mainly transmitted via the fecal-oral route, through contaminated water, and are confined to humans. In contrast, HEV-3 and HEV-4 genotypes are responsible for zoonoses and have a wide range of reservoir hosts. Sporadic cases of human infection with HEV-7 have been reported, suggesting inter-species transmission from camelids. In addition, emerging data indicate that rat HEV, also poses a zoonotic risk (14,43).

Despite this significant burden, hepatitis E remains largely underdiagnosed and underreported, particularly in low-resource countries, where it is often overlooked in overall public health priorities.

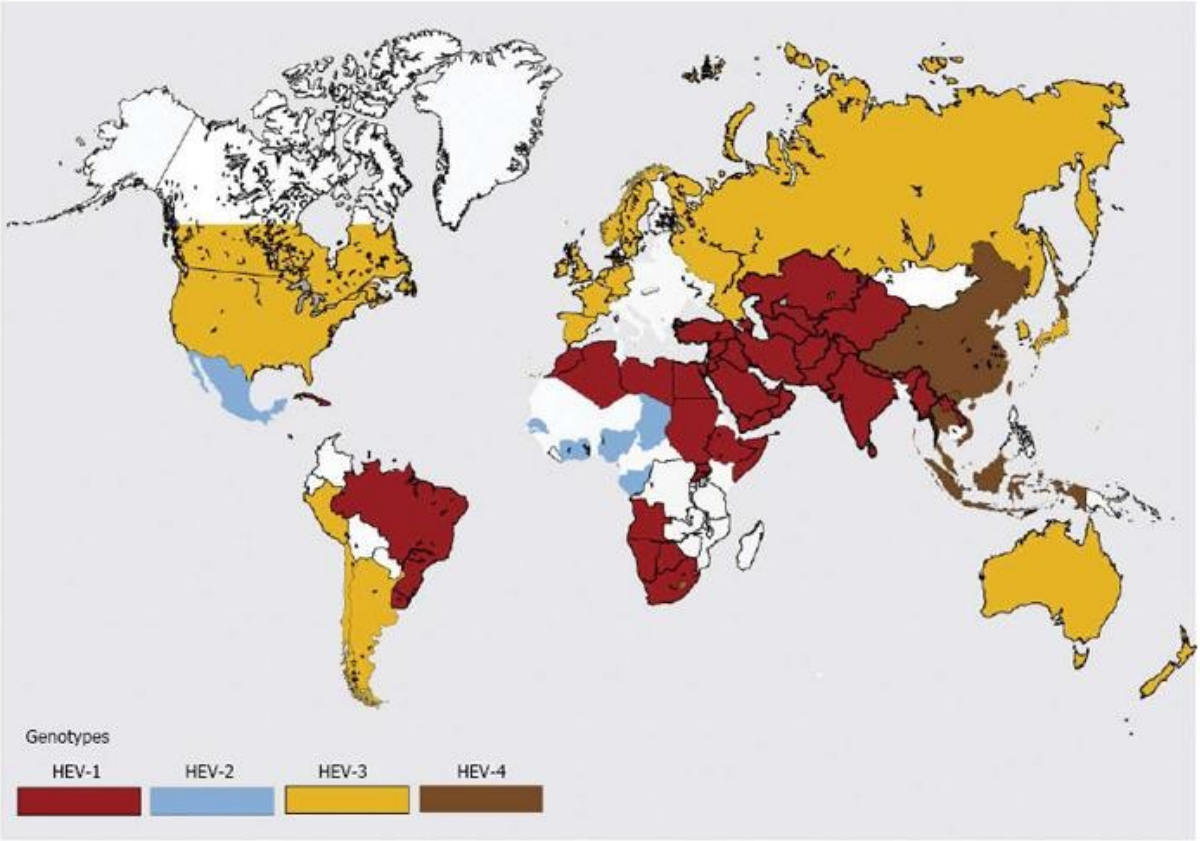


Figure 11: Geographical distribution of major HEV genotypes from Khuroo S et al. 2016

Endemic regions (poor sanitation / HEV 1 and HEV 2)

Genotypes 1 and 2 are primarily found in low-income countries and are transmitted via the fecal–oral route through contaminated water, whereas genotypes 3 and 4 are zoonotic and more prevalent in high-income countries. The geographical distribution of HEV as described in figure 8 (44) is closely linked to the distribution of genotypes. The strictly human genotypes HEV 1 and HEV 2 cause large waterborne epidemics in areas with inadequate sanitation infrastructure, such as South Asia, sub-Saharan Africa and parts of Latin America. These epidemics are often associated with high morbidity and mortality, particularly in pregnant women, in whom infection can lead to fulminant hepatitis with a case fatality rate of up to 25% in the third trimester of pregnancy. HEV is mainly transmitted via the fecal-oral route, particularly through the ingestion of contaminated water in endemic areas (HEV 1 and HEV 2). Clinically, HEV infection manifests itself in a wide range of ways. Most infections, particularly those caused by HEV 3 and HEV 4, remain asymptomatic. Symptomatic forms may present as acute hepatitis, with jaundice, asthenia, nausea, anorexia and abdominal pain(45) . In most cases, the condition resolves spontaneously. However, severe forms can occur, particularly in pregnant women infected with HEV 1 or HEV 2, with a high risk materno foetale mortality.

Low-endemic regions / high-income countries (HEV 3 and HEV 4, zoonotic)

Conversely, the HEV 3 and HEV 4 genotypes, which are of zoonotic origin, are frequently reported in high-income countries, particularly in Europe, North America, China and Japan. Transmission has been caused mainly through the ingestion of undercooked pork or through contact with contaminated water(46) . These infections are often asymptomatic but can progress to a chronic form in immuno-compromised individuals. Chronic infection is mainly observed with HEV 3 and HEV 4 genotypes and is almost exclusively limited to immunocompromised individuals. In the absence of appropriate antiviral treatment, chronic infection can progress to liver fibrosis or cirrhosis. In addition, extrahepatic manifestations, such as neurological (Guillain-Barré syndrome, neuralgic amyotrophy), renal or haematological disorders, have been reported in both acute and chronic forms. Extra-hepatic manifestations such as neurological syndromes (Guillain-Barré syndrome, neuralgic amyotrophy), renal involvement (membranous nephropathy), and hematological disorders (thrombocytopenia, hemolytic anemia) have also been increasingly recognized, particularly with HEV genotypes 3 and 4(47) .

For zoonotic genotypes (HEV 3 and HEV 4), the food route is predominant. Rarer cases of transmission have been reported via parenteral, vertical (mother-to-child) or nosocomial routes. The risk of transmission via blood transfusion is increasingly recognized, particularly in countries where HEV screening is not systematic.

General information / Emerging genotypes

Emerging genotypes, such as HEV 7, identified in camels in the Middle East and Asia, are also causing concern. A case of human infection with HEV 7 has been documented in a liver transplant recipient in the United Arab Emirates following the consumption of raw camel milk. In addition, HEV strains derived from rats have been detected in humans in Hong Kong and

parts of Europe, showing the dynamic evolution of the zoonotic risk associated with hepatitis E(48) .

Despite the growing body of data on HEV, surveillance systems remain inadequate in many regions, particularly in sub-Saharan Africa. Under-diagnosis, lack of routine screening and lack of genomic surveillance limit the overall understanding of the epidemiological impact of the virus. Strengthening diagnostic capabilities and integrating HEV into existing surveillance frameworks are essential levers for better prevention and control(3).

African and West African Epidemiology of HEV

This part is taken from our review in Virology (see annexes)

Detection of the virus in Africa

The first detection of hepatitis E in Africa dates back to 1990 during a cholera outbreak in Sudan (7,49). It marked a turning point in the recognition of hepatitis E as a public health concern on the continent. During this initial outbreak and subsequent ones in various countries, hepatitis E was mainly associated with socio-economic factors such as poverty and inadequate healthcare infrastructure(49). The 2000s were marked by additional outbreaks in Nigeria and Ethiopia, with a clear increase in cases during heavy rainfall and flooding periods, highlighting the impact of environmental factors on the virus transmission dynamics(3).

During the 2010s, surveillance efforts were intensified, providing a clearer picture of virus distribution in Africa, showing that the disease had become endemic in several countries(49) with highly variable prevalence rates. This variability can be attributed to factors such as cultural practices, access to safe drinking water, and sanitary conditions. In particular, populations living in unstable conditions, such as residents of refugee camps, show high anti-HEV IgG rates, as observed in South Sudan (71%) (50) and Niger (38.4%) (51). This clearly highlights the need to target these groups in prevention and awareness programs. With respect to zoonotic aspects, pigs represent the main risk due to their close proximity to humans and their identification as key HEV reservoirs since the first detection in Nigeria (52). Similarly, since the first human case of zoonotic origin in Africa (53) it has been clearly shown that human seroprevalence depends on farming practices and meat consumption.

Circulation of HEV in humans in Africa

HEV circulation in the general population

Epidemic outbreaks of HEV have been reported, such as the large urban outbreak detected in Chad in 2017, characterized by high IgM (7.7%) and IgG (59.6%) seroprevalence, but fortunately associated with limited morbidity and mortality (49). Similarly, between 2012 and 2014 in Senegal, an HEV outbreak occurred among workers in the Kédougou gold mines, with 64% seropositive (1047/1617), underscoring the vulnerability of populations engaged in mining activities (54).

Outside of epidemics, blood donors provide precise information on virus circulation in the general population. In Algeria, 20–22% of outpatients and blood donors tested in hospitals had anti-HEV antibodies, suggesting HEV-1 circulation (55). Seroprevalence among blood donors is 10% IgG and 1.9% IgM in Burkina Faso (56) and even lower in Tunisia (5.4%) with no anti-HEV IgM, indicating limited virus circulation (57). Another Tunisian study confirmed this low seroprevalence among blood donors (4.2%) but reported significantly higher rates in hemophilic patients (7.5%) and hemodialysis patients (10.2%), highlighting an increased risk of parenteral transmission (47). Organ transplantation is another transmission route: in South Africa, a case of HEV-3 transmission via organ graft emphasizes the importance of monitoring immunocompromised individuals (58). Other studies show that HEV can circulate in low-risk populations. In 2021, in Nigeria, anti-HEV IgG was detected in 2.2% of rural adolescents at a secondary school, showing that even young individuals outside high-risk professions are not spared (59).

Pregnant women, a vulnerable population to monitor

HEV-1 is well known to induce severe, and sometimes fatal, disease in pregnant women, particularly in Asia and East Africa (49). IgG seroprevalence, reflecting past HEV circulation, varies widely across African countries: 31.4% in Ethiopia (60) 16.19% in Benin, 12% in both Tunisia (61) and Ghana (62), 7.4% in Senegal (63) and only 3.1% in South Africa (64). However, some studies in Benin reported 1.44% anti-HEV IgM, with samples testing positive simultaneously for IgG, IgM, and even HEV RNA, indicating active circulation (65). Collectively, these findings highlight the importance of targeted surveillance and prevention strategies in this vulnerable population.

Beyond prevalence estimates, it is noteworthy that there are few, if any, published data on the pathogenicity of HEV infection in pregnant women in Africa. In regions where HEV-3 is endemic, virtually no cases of exacerbated disease have been reported in this group. It remains unclear whether this reflects an actual epidemiological reality or a lack of case

investigation. These observations emphasize the need for studies specifically focused on pregnant women to guide the implementation of tailored preventive measures.

Regular medical follow-up is therefore recommended to prevent and detect HEV infection and associated risks. Preventive measures such as proper hand hygiene, safe drinking water, and thorough cooking of food are essential to reduce transmission. In cases of infection, appropriate treatment and monitoring are required to protect both maternal and fetal health. Collaboration among healthcare professionals is critical to ensure optimal management.

At-risk occupations

Intensive pig farming under suboptimal hygienic conditions also facilitates the spread of HEV. In Burkina Faso, studies revealed very high seroprevalence among butchers (76%) compared to the already high seroprevalence in the general population (47.8%) (66). This higher rate is likely linked to the handling of pork products. In Uganda and Madagascar, lower seroprevalence was observed among slaughterhouse workers, at 13.3% and 14.1%, respectively (67,68). In Ghana, where seroprevalence rates were relatively close between pig farmers (15.2%) and the surrounding community (12.4%), HEV circulation was detected in 2.9% and 0.7% of these groups, respectively (69). In Nigeria, one study reported IgG seroprevalence of 1.3% among villagers living near pig farms, 14.9% IgG and 1.3% IgM among pig farmers, and 31.1% IgG and 2.2% IgM among butchers. These results clearly demonstrate that occupations related to pig farming, particularly butchery, are high-risk, with IgM positivity indicating ongoing infection (70).

In Guinea, where epidemic crises such as Ebola (2014–16) and the Covid-19 pandemic (2020–21) overshadowed HEV surveillance, a study on the circulation of HIV and hepatitis viruses reported HEV serological traces among healthcare workers, with low IgG (2.7%) and IgM (1.35%) prevalence. Although viral RNA was not detected by PCR, this study underscores the importance of maintaining broad-spectrum infectious disease surveillance, even during health emergencies, particularly for healthcare personnel at higher risk of infection (8).

Diagnostic confusion or co-infections with other viruses: hepatitis viruses, human immunodeficiency virus (HIV), or yellow fever virus (YFV)

Several viruses can cause hepatitis, and HEV can act alone or in co-infection with other pathogens, complicating both diagnosis and treatment. In Egypt, 72% of patients positive for hepatitis C virus (HCV) also had anti-HEV IgG (71). Conversely, in South Africa's Free State Province, 60% of patients with acute hepatitis tested negative for hepatitis A (HAV), B (HBV),

and C (HCV) viruses, but were positive for anti-HEV IgG, and 0.3% for anti-HEV IgM (72). In northern Uganda, surveillance showed that 42% of acute jaundice syndrome cases were attributable to HEV (73). Results can sometimes be contradictory when comparing the general population with patients suffering from hepatitis. For instance, in Tunisia, one study detected no HEV infection markers either in blood donors or in patients with acute hepatitis (57) while another reported a 19.5% anti-HEV IgG prevalence among acute hepatitis patients (61). A similar comparison in Rwanda found that 11.9% of adult blood donors tested positive for anti-HEV antibodies and 0.5% carried low viral RNA loads, with no significant difference between healthy individuals and those with liver disease (74). By contrast, in Kenya, higher seroprevalence was observed among patients with acute febrile illnesses compared to the general population, reaching 25.7% for IgM and 37.8% for IgG, although no virus was detected by PCR (75). These differing, and even conflicting results, clearly underscore the need to include HEV testing in patients presenting with acute hepatitis.

With respect to HIV/HEV co-infection, in Uganda (2008–09), people living with HIV exhibited a similar anti-HEV serological profile (46% IgG) to that of HIV negative individuals (48%). Given that HEV may cause more severe or prolonged hepatitis in immunocompromised patients, including those living with HIV, genotype HEV-3 was identified in one individual who also tested positive for anti-HEV IgM (76). In Tanzania, a study revealed that 8% of HIV-positive pregnant women carried anti-HEV IgG, indicating past exposure. Although these data do not provide estimates of symptomatic infection, they highlight the heightened vulnerability of pregnant women to potential active HEV infection (77).

In sub-Saharan Africa, jaundice, a clinical marker of liver injury, can also result from malaria or yellow fever virus (YFV) infection. However, many suspected cases may actually be attributable to HEV. In the Democratic Republic of Congo (DRC), 10.4% of suspected cases were positive for anti-HEV IgM (78). In the Central African Republic (CAR), among 3,181 suspected cases sampled between 2008 and 2009 and testing negative for YFV, 48.9% were positive for anti-HEV IgM, with HEV RNA detected in 2.5%, revealing viruses closely related to HEV-1 as well as one HEV-2b strain (79). A follow-up survey conducted about a decade later in the same patient group revealed anti-HEV seroprevalence of 40.3% IgM and 49.7% IgG, again with circulation of genotype HEV-1e(80). In Burkina Faso (2013–15), among 900 patients with febrile jaundice who tested negative for YFV, 18% and 2.6% were positive for anti-HEV IgG and IgM, respectively, and genotyping confirmed circulation of HEV-2b (81). In Cameroon in 2024, among individuals clinically suspected of yellow fever but testing negative

in the laboratory, 8.1% were positive only for anti-HEV IgM, 5.9% only for anti-HEV IgG, and 8.3% for both. Furthermore, viral RNA was identified in 15.2% of sera, confirming circulation of HEV-1e, HEV-3f, and HEV-4b. These findings demonstrate that HEV is likely responsible for a substantial proportion of acute febrile jaundice cases among patients enrolled in Cameroon's yellow fever surveillance program (82). This other study conducted in Nigeria revealed a high prevalence of hepatitis B virus (HBV) among patients with liver disease (50%), with 8% presenting anti-HEV antibodies and 9% HEV RNA, including a rare case of genotype 3. Although HEV prevalence is low, the detection of HBV/HEV co-infections (7%) highlights the need for systematic HEV screening and reinforcement of HBV vaccination in at-risk populations(83).

Circulation of HEV in animals

High prevalence in pigs and/or pork products

Several studies report high HEV seroprevalence in pigs across various African countries: 80% in Burkina Faso (84), 71.2% in Madagascar (68), 66% in Nigeria where genotype HEV-3 is circulating, and 47.7% in Zambia, also affected by genotype HEV-3e (85). In Guinea, overall seroprevalence was lower (22%), though peaks of up to 50% were observed in farms located in the Forest Region, where part of the population is Christian and raises and consumes pork contaminated with genotype HEV-3c (86). In Cameroon, pig seroprevalence is also significant (43.2%) (87) and a study conducted in 2017–18 in slaughterhouses across three cities reported 21% anti-HEV IgM and 17.7% anti-HEV IgG positivity, with HEV closely related to genotype HEV-3 detected in 5.9% of fecal samples from seropositive animals (88). Variable levels of HEV detection in pig feces have been described elsewhere in Africa: lower in Lagos, Nigeria (2.9%) (89) , higher in Ethiopia (12%) (90) and particularly elevated in the Central African Republic (CAR, 36.1%), where genotype HEV-3h circulates in five districts of Bangui (91).

Pork products represent another potential source of contamination. Genotype HEV-3 was detected in 0.8% of pig livers sampled in three regions of Cameroon (92), in 1–1.2% of pig livers in Burkina Faso (93) and Madagascar (94) and in 3% of meat and 22% of pig livers sold at the Saint-Louis market in Senegal (95). In South Africa, several studies identified HEV in pork products, with genetic sequences closely related to those found in humans (96), suggesting a potential zoonotic transmission risk (97), although direct evidence remains limited .

Taken together, these findings demonstrate that, in addition to human contamination of water sources, zoonotic transmission of HEV plays an important role in West Africa (66). They

highlight the endemicity of genotype HEV-3 in many African countries, including those with low seroprevalence such as Sierra Leone (4%) (98). They further emphasize the importance of the "One Health" approach, with pig surveillance being essential given the clear zoonotic risk posed by this reservoir, as illustrated by the heterogeneity of HEV genotypes circulating among humans and pigs in Africa. Moreover, the variability of results between countries and regions indicates the need to adapt surveillance and control strategies to local contexts. Additional studies are required to better understand the dynamics of HEV transmission from pigs to humans.

HEV prevalence in other domestic animals

Beyond pigs, HEV has also been detected in several domestic animal species such as rabbits, dromedaries, cattle, sheep, and goats. In Egypt, HEV-3 was found in 15.2% of rabbit fecal samples, while 12.8% tested positive for anti-HEV IgG (93). In Burkina Faso, seroprevalence reached 52% in hares and 60% in rabbits, and was also observed in other livestock species such as cattle (26.4%), sheep (12%), and goats (28.4%) (56). Phylogenetic analysis showed no relationship between HEV-3 strains in rabbits and those found in pigs or humans. Proximity to pig farms has been identified as a risk factor for HEV exposure in livestock and for wider disease spread. For instance, while the average seroprevalence in cattle is 5.1%, it can reach 32.4% in farms located near pig holdings, where 80.7% of pigs carry anti-HEV antibodies. This has raised concerns among health authorities in Burkina Faso regarding the risk of HEV transmission through dairy products originating from mixed cattle–pig farming systems (84). Across the border from Burkina Faso, Ghana reported HEV circulation in 2011 among five animal species (dromedaries, cattle, goats, sheep, and pigs) in the Kumasi region, with HEV-3 identified in pigs and HEV-7 in dromedaries (99). The virus has also been detected in 2.2% of dromedaries in Ethiopia. In Egypt, HEV-3 RNA was identified in cow's milk sold in rural communities, raising potential public health concerns even though no transmission to humans has yet been demonstrated (100). However, a confirmed case of HEV-7 transmission via camel milk was documented in the United Arab Emirates in 2016 (101). The avian genotype (genus *Avihepevirus*), which has not yet been associated with human disease, was detected in Nigeria in 12.5% of serum and 9.1% of fecal samples from healthy laying hens (102) . Its presence was further confirmed in 2.9% of poultry sampled across three districts of Lagos between 2017 and 2019 (89).

Overall, it appears that several domestic animals in close contact with humans may serve as potential HEV reservoirs. This underscores the need for expanded surveillance across different

animal species and region-specific approaches to better understand infection dynamics and reduce human transmission risk. Farming practices and sanitary conditions play a major role in shaping HEV prevalence among animals and may facilitate viral spread. Co-infection or superinfection with other viruses further increases carriage rates and prolongs the duration of viral shedding (103,104) .

Detection in wastewater :

Due to its fecal–oral transmission route, it is not surprising that HEV is detected in both urban and rural wastewater. Wastewater surveillance is indeed a key tool for tracking active virus circulation in human populations and the environment, with waste management practices also influencing viral dissemination. A study carried out in wastewater treatment plants, rivers, and lavatory facilities across seven regions of South Africa reported circulation of HEV genotypes 3c, 3f, and 4b (105). In Tunisia, both HEV-1 and HEV-3 were detected in wastewater. While the presence of HEV-1, a strictly human genotype, is expected, the detection of HEV-3 mainly transmitted through pigs is surprising in a predominantly Muslim country where pork consumption is low. Further studies are needed to clarify the diversity of HEV strains circulating in humans and animals, as well as the dynamics of their transmission (106).

Presentation of Guinea

Guinea situation in Africa

The Republic of Guinea is in West Africa. It is bordered by Guinea-Bissau to the north-west, Senegal and Mali to the north, Côte d'Ivoire and Mali to the east, Liberia and Sierra Leone to the south, and the Atlantic Ocean to the west. The country has 300 kilometers (km) of coastline and stretches 800 km from east to west and 500 km from north to south. Its total area is 245,857 km2.

Figure 12: Map of Africa showing the different countries that make up the continent, as well as Guinea in the western part of the continent (in green).

Guinea comprises four natural regions based on natural criteria: Lower Guinea, Middle Guinea, Upper Guinea and Forest Guinea (figure 13). Lower Guinea is a region of coastal plains covering 18% of the national territory, characterised climatically by high rainfall ranging from 3,000 to 4,000 mm3 per year. Middle Guinea is a region of mountain ranges covering 22% of the national territory, with annual rainfall levels varying between 1,500 and 2,000 mm3. Upper Guinea is a region of plateaus and wooded savannahs covering 40% of the national territory, where rainfall varies between 1,000 and 1,500 mm3 per year. Forest Guinea is a region of mountain ranges covering 20% of the national territory with rainfall varying between 2,000 and 3,000 mm3 per year.

These natural regions are divided into eight administrative regions: i) the natural region of Lower Guinea (comprising the administrative regions of Kindia, the capital of the region, Boké and Conakry); ii) the natural region of Middle Guinea (the administrative regions of Mamou and Labé, the capital of the region); iii) the natural region of Upper Guinea (the regions of Faranah and Kankan, the capital of the region); iv) the natural region of Forest Guinea (the region of Nzérékoré, the capital of the region) as shown in figure 10.

Figure 13 : Map of Guinea with administrative regions

Guinea has a tropical climate with alternating rainy and dry seasons lasting approximately six months each. This climate exposes the country to a constant risk of flooding and fires during the dry season, particularly in Upper Guinea.

Demographic Situation

According to the World Bank in 2016, Guinea's population was estimated at 12.4 million, with a density of around 50 inhabitants per square kilometer. Women account for 52% of the population. Most of the population is young (44% of the population is under 15 years of age) and live in rural areas (70%), almost exclusively from agriculture and livestock farming. The population is distributed evenly between the special zone of Conakry and the other regions of the country: 20.4% in Lower Guinea, 22.9% in Middle Guinea, 19.7% in Upper Guinea, 21.7% in Forest Guinea and 15.3% in the special zone of Conakry, respectively. Only 4% of Guineans are over 65 years old and the average household size is 6 people (Ministry of Health, 2017).

The crude birth rate was 34% in 2012, with a total fertility rate of 5.1.

Socio-economic situation

In Guinea, agriculture and livestock farming are the main sectors of activity for nearly 80% of the population. Crop production, which is a dominant part of agriculture, accounts for 57% of rural activities, while 30% of rural dwellers derive their income from livestock farming (Ministry of Agriculture, 2018).

Livestock farming is the second most important activity in rural areas after agriculture. Livestock plays an important economic role. The national livestock population consists of cattle, sheep, goats, pigs and poultry. The livestock farming system is traditional, with cattle being the main source of income. Nearly 210,000 families (30% of the population) live off livestock farming (Ministry of Agriculture, 2018). Pigs are mainly concentrated in forest Guinea and low Guinea.

Pigs raised in Guinea are divided into local breeds, improved breeds and crossbreeds.

The local breeds are called ‘running pigs. They are raised in a traditional environment and represent more than 90% of the national livestock population. There has been no detailed study of pig farming in rural areas. This breed is characterized by its morphology: thicker skin than modern breeds, weight after 2 years of 50 to 80 kg, and 4 to 6 piglets per litter.

Exotic breeds: these have been introduced into Guinea over the last 40 years. They are not currently bred in pure form; their uncontrolled crossbreeding with the local breed has produced species whose genotype is difficult to determine. Their characterization is still

incomplete, but the following can be noted: birth weight of 0.75-1.25 kg (slightly larger than the local breed), weight at 6 months of 30-60 kg, which is a sign of precocity. These parameters vary according to the degree of growth and breeding conditions (FAO, 2003).

Animal health is poorly monitored, with nearly 500 veterinarians and other paraprofessionals practicing in the field.

What else about epidemiology of HEV in Guinea

As reported by our previous review, HEV is present in many countries with limited resources, where it is responsible for major epidemics, usually waterborne. The WHO estimates that approximately 20 million HEV infections are reported each year worldwide, leading to nearly 3.3 million symptomatic cases and approximately 70,000 deaths (WHO, 2023). The burden of disease is particularly high in regions where access to safe drinking water, sanitation and health services is limited.

On the African continent, several outbreaks have been documented, notably in Chad, Sudan and the Central African Republic.

Humanitarian crises and the failure of drinking water distribution infrastructure are at the root of these epidemics. All these factors contribute to the rapid spread of the virus, with a marked impact on vulnerable groups, particularly pregnant women.

Hepatitis E represents a major public health challenge in Africa. The genetic diversity of the virus and the multiplicity of its reservoirs highlight the urgent need for in-depth research to better understand and control the disease and its transmission dynamics. Beyond the strictly human HEV-1 and HEV-2 genotypes, pigs act as major reservoirs for zoonotic HEV-3 and HEV-4, facilitating their spread. Recurrent detection of HEV-3 across the continent and HEV-4 in Central Africa is coupled with high seroprevalence in certain populations. The recent identification of HEV-7 in dromedaries in Madagascar and HEV-4b in Southern Africa further broadens the range of zoonotic genotypes with transmission potential.

According to the WHO, hepatitis E is endemic in many developing countries, with epidemic outbreaks exacerbated by poor sanitation. In Africa, both human-to-human and zoonotic transmission are intensified by inadequate sanitation infrastructure and the socioeconomic importance of pig farming. The high prevalence of HEV in pigs and in human populations

either in close contact with pigs or consuming undercooked pork products underlines the need for greater public awareness and improved hygiene practices. Moreover, other domestic animals including rabbits, goats, and cattle may act as intermediate hosts between pigs and humans, either directly or via derived products such as meat or milk. Although rabbit-to-human transmission of HEV-3 has not yet been demonstrated, the potential exists, just as with HEV-7, which has been reported in Asia. The detection of HEV-4, originally identified in Japan, in pigs from Cameroon further illustrates the risks posed by international travel and trade, although a direct link with commercial pork transport has not been established to date.

Given these multiple risks, a multidisciplinary approach rooted in the “One Health” framework is essential to address the persistence of hepatitis E, improve hygiene and farming practices, raise community awareness of transmission risks, and strengthen human infection prevention and management. In particular, close monitoring of at-risk populations is critical most notably pregnant women, who face severe complications and significantly higher mortality rates than the general population. Regular medical surveillance, preventive hygiene measures, and coordinated action between health professionals are crucial to protect this vulnerable group.

From a research perspective, the application of next-generation sequencing technologies could help map HEV genetic diversity across animal and human populations, while also identifying mutations linked to virulence or resistance to treatment. Integration of geospatial and environmental data with animal and human surveillance would allow a better understanding of interactions between animal reservoirs and human populations. Finally, training modules should be developed to strengthen the clinical management of hepatitis E, from recognition of atypical symptoms which may mimic other diseases to the development of specific diagnostic tools capable of distinguishing HEV cases from other causes of jaundice or acute hepatitis such as malaria or yellow fever.

Such research will contribute to a better understanding of HEV transmission dynamics in Africa, help identify vulnerable populations, guide the development of tailored prevention and control strategies, and ultimately support the design of effective public health policies for the continent.

In Guinea, there is insufficient data on the epidemiology of HEV despite a context that combines all the elements conducive to silent circulation of the virus, namely seasonal flooding, poor

waste management, urban overcrowding, and close proximity between humans and livestock. To date, HEV remains an under-recognised public health problem in Guinea due to a lack of molecular surveillance data. Additionally, no national registry exists for HEV cases, and laboratory diagnostic capacity is very limited, which contributes to underreporting and a lack of understanding of the true disease burden**.** Specific studies are therefore needed to better assess the epidemiological situation and guide prevention strategies.

It is in this context that this study was conducted in Guinea in areas selected for their epidemiological profile and the presence of pig farming, potential reservoirs of HEV. It should be noted, however, that the results obtained cannot be generalised to the entire country due to the limited geographical coverage.

The study focused on analysing the detection of hepatitis E virus in humans, in pig populations, and in the environment (wastewater and surfaces). This does not address the issue of assessing individual clinical aspects or the medical management of identified cases. Furthermore, this research integrates molecular, serological, and environmental approaches to provide a comprehensive assessment of HEV circulation. Data collection took place over a period of 36 months, between 2022 and 2025, and involved different levels of sampling and analysis.

This study is an essential first contribution to understanding the circulation of HEV in Guinea from an integrated One Health perspective.

One Health approach to HEV

The hepatitis E virus (HEV) is currently one of the leading causes of acute viral hepatitis worldwide. In addition to strictly human forms (HEV 1 and HEV 2), several zoonotic genotypes (notably HEV 3, HEV 4 and HEV 7) have been identified. Pigs, which are asymptomatic, are the main reservoir of the virus. The process of transmission of the disease to humans includes direct contact with infected animals, the fecal-oral route, and the consumption of undercooked pork or pork products.

Several studies have shown a link between viral strains isolated in humans and those found in animals, confirming the zoonotic nature of the virus.

In Guinea, the proximity of human and animal populations, combined with poor hygiene conditions, limited access to drinking water and poor waste management, creates an environment conducive to the emergence and circulation of multiple transmission pathogens.

The epidemiology of HEV is therefore influenced by a complex set of factors such as dietary practices, the level of hygiene and immune status of populations, surface water contamination, seasonal flooding and the lack of effective sanitation systems, and the intensification of pig farming, which is often not supervised by structured veterinary services.

In response to this problem, the One Health approach provides an essential conceptual framework for better understanding the interactions between humans, animals and the environment that cause HEV transmission. It emphasizes transdisciplinary collaboration between human, animal and environmental health stakeholders to develop more effective prevention, early detection and surveillance strategies.

This includes systematic environmental monitoring, routine serological surveys in at-risk human populations, and genetic characterization of viral strains across species to trace transmission pathways. One Health concept in Guinea including HEV would offer a unique opportunity to generate multisectoral scientific data, while strengthening national capacities for integrated epidemiological surveillance. From a global health perspective, this is a strategic approach, not only to better combat hepatitis E, but also to prevent future zoonotic health crises.

Prevention

In endemic countries where HEV infection spreads mainly through the fecal-oral route, the most important interventions are maintaining quality standards for public water supplies, establishing adequate sanitation and wastewater disposal systems. At the individual level, hygiene measures such as hand washing with clean water and washing food should be observed. Reduce the risk of exposure to the zoonotic potential of the disease by using gloves and other protective equipment to reduce the risk of exposure for those who come into contact with animals, particularly pigs. Inform not only consumers of pork but also at-risk groups (pregnant women, immunocompromised individuals) about the proper cooking of pork, and inform the food industry about the biosecurity measures to be adopted (https://www.healthdata.org/research-analysis/diseases-injuries-risks/factsheets/2021-acute-hepatitis-e-level-4-disease).

Given the zoonotic potential of HEV genotypes 3 and 4, implementing a One Health strategy that integrates human, animal, and environmental surveillance is essential to prevent cross-species transmission and outbreaks.

Treatment and Vaccination

HEV causes a spontaneously resolving disease in immunocompetent individuals, with symptomatic treatment if necessary. In immunocompromised individuals, monotherapy with interferon alpha or ribavirin has shown encouraging results in some cases, although it is

contraindicated in transplant patients because it induces serious adverse effects. Currently, liver transplantation is the only treatment for fulminant acute hepatitis(107).

To date, only one hepatitis E vaccine has been developed and authorized for human use: Hecolin or HEV239, a recombinant vaccine based on the capsid protein of genotype 1 of the virus(9). This vaccine was developed in China and received national approval in 2011 and in Pakistan in 2020 , but it has not yet been prequalified by the World Health Organization (WHO), which limits its global distribution, several large-scale studies involving thousands of vaccinated subjects are currently underway. They indicate sustained vaccine efficacy for more than ten years, with specific antibodies persisting for an estimated eight and a half years.(108). WHO currently recommends vaccination in high-risk groups and outbreak settings where Hecolin is available, but further clinical trials are needed for broader international approval(39).

Additional studies are underway to assess its safety and efficacy in these populations(9). The global availability of a validated vaccine could be a major lever in the prevention of hepatitis E, particularly in endemic areas or areas with high health vulnerability.

PART II: OBJECTIVES

Problem Statement, Research Questions

HEV, an emerging disease transmitted mainly via the fecal-oral route, poses a growing public health challenge, particularly in low-income countries. Environmental issues and the lack of robust epidemiological surveillance systems contribute to the rapid and sustained presence of the virus in these regions.

In Guinea, although official data on the actual prevalence of infection are scarce, several structural and contextual factors strongly suggest active circulation of the virus. The frequent proximity between humans and livestock, particularly pigs recognised as reservoirs of zoonotic genotypes of the virus (especially genotypes 3 and 4) is a key factor in transmission. Pigs are often raised in poor sanitary conditions in certain regions of Guinea. In addition, environmental issues and difficulties in supplying drinking water create conditions conducive to waterborne transmission of the virus.

Moreover, the absence of routine HEV screening in clinical and veterinary settings further limits the detection of both endemic and sporadic cases.

In this regard, a public health strategy focused solely on the human aspect appears insufficient to address all the mechanisms involved in the transmission of hepatitis E.

Thus, given the situation in Guinea, which is characterized by a high degree of interdependence between the three components of One Health, it is imperative to explore all the epidemiological determinants related to the circulation of HEV. This reflection raises two major questions that guide the present study:

> What could be the epidemiological situation of HEV in Guinea?
> How could a One Health approach improve the understanding, surveillance and prevention of this disease?

These two questions form the foundation of the research. The overall objective and specific objectives were structured to address them through a multidisciplinary and integrated approach.

Objectives

This work aims to analyze the potential presence of HEV in humans, animals and the environment from a One Health perspective, to propose concrete avenues for an effective and integrated health response.

General objective

To assess the presence of the hepatitis E virus in animals, environment and humans in Guinea using a One Health approach.

This general objective directly responds to both research questions by combining epidemiological investigation and integrated health analysis.

Specific objectives

To achieve this general objective, the work was divided into several complementary components designed to answer each aspect of the main questions:

1. Identify the presence of the virus in pigs (or other livestock) in the same area.
2. Analyze the environmental samples and conditions (water, sanitation, waste management) that promote transmission.
3. Estimate the prevalence of hepatitis E in human populations in a targeted area.
4. Evaluate the potential link between human and animal HEV strains through molecular and phylogenetic analyses

5. Propose an integrated surveillance strategy according to the One Health model.

Hypothesis

- The proximity between human and animal populations appears to be positively linked to the circulation of the hepatitis E virus in Guinea.
- The absence or inadequacy of basic sanitation infrastructure, such as improved latrines and wastewater management systems, appears to be correlated with the probable presence of the hepatitis E virus in the Lower Guinea and Forest regions.
- The circulation of HEV in Guinea is strongly influenced by mixed factors, highlighting the relevance of an integrated 'One Health' approach to better understand its epidemiological dynamics.

- The lack of effective synergy between the human, animal and environmental health sectors is a major constraint to the development and implementation of effective hepatitis E prevention and control strategies in Guinea.

- Weak intersectoral collaboration limits effective prevention and control of HEV.

Structure of the research

The research plan was therefore organized into three main parts : human, animal, and environmental components. Each designed to contribute specific data and insights. This organization ensures a comprehensive response to the two main research questions and strengthens the integration of findings within the One Health approach.

Significance and Contribution of the Study

This study has a scientific, health, economic and societal contribution, in a situation where the lack of data on hepatitis E is really noted. From a scientific point of view, it will allow us to assemble recent epidemiological data on the circulation of HEV, which will help fill this bibliographic gap in Guinea.

It also provides a foundation for future genomic and molecular studies, enabling the tracking of emerging or zoonotic strains.

In terms of health, the results obtained will make it possible to refine prevention, awareness-raising and surveillance strategies, by identifying vulnerable groups, particularly pregnant women. On the economic and political level, this study could make it possible to act on the need to strengthen drinking water, sanitation and veterinary control infrastructures, while promoting a one health approach.

Finally, through the One Health approach, this work will highlight the importance of collaboration between the human, animal and environmental health sectors, to effectively fight against emerging and re-emerging diseases such as hepatitis E.

It also demonstrates the feasibility of integrated surveillance systems that can serve as a model for other zoonotic and waterborne infections in the region.

Part III: RESULTS

Article 1: Seroprevalence and Phylogenetic Characterization of Hepatitis E Virus (Paslahepevirus balayani) in Guinean Pig Population.

Doukouré B., Le Pennec Y., Troupin C., Grayo S., Eiden M., Groschup M. H., Tordo N., Roques P. (2024). Seroprevalence and Phylogenetic Characterization of Hepatitis E Virus (Paslahepevirus balayani) in Guinean Pig Population. Vector-Borne and Zoonotic Diseases 24(8):540-545. doi:10.1089/vbz.2023.0104

Article pdf

Seroprevalence and Phylogenetic Characterization of Hepatitis E Virus (*Paslahepevirus balayani*) in Guinean Pig Population

Bakary Doukouré,[1,2] Yann Le Pennec,[1] Cécile Troupin,[1,*] Solène Grayo,[1] Martin Eiden,[3] Martin H. Groschup,[3] Noël Tordo,[1] and Pierre Roques[1]

Abstract

Background: Hepatitis E virus (HEV) is transmitted by the fecal route, usually through contaminated water in humans and/or infected animals, especially pigs. The objective of this study was to evaluate the level of anti-HEV antibodies in a panel of pig sera and to identify HEV in pig feces in farms.
Methodology: The presence of HEV antibodies was tested by an in-house ELISA and a commercial ELISA IDvet. HEV genome was assessed by nested RT-PCR, and then, genotype was identified by sequencing (MinION Nanopore technology).
Results: In 2017–2019, the 43% seroprevalence found in Forest Guinea was significantly higher than the 7% found in the Lower region ($p < 0.01$). Presence of HEV genotype 3c was demonstrated during a secondary study in the Lower region (Conakry) in 2022.
Conclusion: The presence of HEV-3c in pigs calls for an evaluation of seroprevalence in human populations and for a HEV genotype human circulation check.
Contribution Heading: This study is the first report, to our knowledge, of seroprevalence and characterization of HEV infection in pigs in Guinea.

Keywords: HEV, pig, antibodies, feces, genotype 3c

Introduction

Hepatitis E virus (HEV) is one of the five known human hepatitis viruses. HEV infection is often mild but can lead to severe, acute liver disease in humans and sometimes progress to fulminant hepatic failure. In pregnant women, it may also lead to severe complications resulting in fetal and/or maternal mortality. Out of more than 20 million yearly global infections, 44,000 result in death (https://www.who.int/news-room/fact-sheets/detail/hepatitis-e), mostly in low-income countries in Asia, Africa, and Latin America (Aubry 2013; Motoya et al. 2019). HEV was originally described to be transmitted primarily by the fecal–oral route but for the past 20 years, HEV has also been considered as a zoonotic pathogen that can be transmitted by domestic or wild animals mainly from swine lineages (Khuroo et al. 2016).

HEV is a quasi-enveloped, positive-sense single-stranded RNA virus within the *Hepeviridae* family, *Paslahepevirus* genus, *Paslahepevirus balayani* species that comprises eight genotypes: genotypes 1–4 are involved in human infection, whereas genotypes 5 and 6 are restricted to wild boars (de paula et al. 2013; Smith et al. 2020), and genotypes 7 and 8 have been described to be able to infect camels, with rare cases in humans (Takahashi et al. 2020). Despite the existence of many genotypes, there is only one serotype (Kamar et al.

[1]Institut Pasteur de Guinée, Conakry, Guinea.
[2]Institute for Tropical Medicine, University of Tübingen, Tübingen, Germany.
[3]Friedrich Loeffler Institute, Greifswald, Germany.
*Current address: Institut Pasteur du Laos, Vientiane, Lao PDR.

2012). Over the past 10 years, new members of the *Hepeviridae* family have been found in rodents, bats, birds, and fishes, sometimes referred to as emerging infectious agents (Purdy et al. 2022).

The recognition of the zoonotic status of HEV genotypes 3 and 4 led to a reassessment of the HEV prevalence worldwide to evaluate the related health burden in humans. Until now, several studies in African countries such as Burkina Faso, Ghana, and Cameroon have shown a high HEV seroprevalence and HEV virus detection in pigs (Cooper et al. 2005; Traoré et al. 2015; El-Duah et al. 2020). A recent epidemic was also described in southeastern Senegal close to the northern Guinea border (Sadio et al. 2022). The assessment of HEV seroprevalence among pigs, known as a reservoir of HEV virus, informs the need for a HEV surveillance system to prevent human cases. Following our first-step survey performed on animals sampled in 2018, an evaluation of hepatitis viruses was recently done in a group of 74 health care workers in Guinea after the COVID epidemic peak using HEV IgM commercial ELISA (Ostankova et al. 2023). In this study, three cases of HEV infection were detected, demonstrating the circulation of HEV in the country, but no identification of the involved genotype was provided. The prevention strategies to avoid HEV infection will depend on the involved virus: from human to human in genotypes 1 and 2, whereas genotypes 3 and 4 may be maintained by an animal reservoir. Thus, the identification of the involved genotype and the animal reservoir should be a very first step to any human health intervention.

The aim of this study was to determine the circulation of HEV among pigs in Guinea as a surveillance tool to prevent human cases. HEV infection was determined indirectly by testing for specific antibodies using ELISA or directly by PCR to detect the virus and characterize the genotype by sequencing.

Materials and Methods

Study population

There was no sample size calculation, as our study was derived from a program in which the main objective was the research of an Ebola reservoir. Because of the relative population sensitivity, we chose to sample the larger number of animals in the farms that accepted the study.

The studied population were pigs from Guinean farms in the two main regions developing pig farming in Guinea, mainly Lower and Forest Guinea. Currently, Guinea Agricultural Ministry services estimated a total of 147,000 pigs in Guinea, with 98% in the two main regions. In total, 20 piggeries in 11 prefectures were surveyed. We collected 886 samples from pigs, all of which were reared and sampled in pens. Sample were collected between 2017 and 2019 in the two main regions (Lower and Forest Guinea) and then in 2022 in Conakry (105 feces samples). The pig farming system is described in Supplementary Table S1. Age, sex, place, city, and day of collection were recorded for each sample.

Sampling blood and feces. Whole blood was collected from the jugular vein in a dry tube of 5 mL. Tubes were centrifuged on site (3000 × *g*, 10 min) to collect serum and aliquoted per 0.5 mL. Sera were stored at −20°C on the field and at −80°C back in the laboratory until use in the biobank of Institut Pasteur de Guinée. During the 2022 study, feces were sampled from the rectum from each individual animal. Pigs sampled were at least 1 month of age and not gestating sows. The feces samples were taken by rectal examination, with a finger inserted into the animal's rectum to remove a small portion with sterile gloves (around 1 g). Each animal was gently restrained by three people to collect the feces (at the same time for blood collection), which was placed in a tube containing 1× PBS (2 µL), sent to the laboratory in a −20°C cooler, and then stored in the laboratory at −80°C before handling.

Serological assay. Because the exposure of pigs to pathogens (parasites and others) and the weather conditions in Africa are largely different from those in Europe or the United States (where most of the ELISA kits were developed), the current procedure is to test animals' sera with different kits.

Two immunoserological ELISA techniques were used. In a first approach, an in-house ELISA set up at Friedrich Loeffler Institute (FLI) for the detection of HEV antibodies in swine serum was used. The HEV-specific bait was a recombinant, bacterially expressed p239 from the open reading frame 2 (ORF2) of HEV genotype 3 comprising amino acid sequence 368–606 of the ORF2 capsid protein. Briefly, in Conakry, we sensitized the ELISA plates (Nunc MaxiSorp 96) with the recombinant protein (1 µg/mL in 0.05 M carbonate buffer, 4°C overnight) and blocked with milk (Difco skimmed milk in 1× PBS, 1 h, 37°C) to limit nonspecific protein interactions and then washed three times with PBS/0.1% Tween 20. We placed test samples (diluted sera 1:25 with PBS/2% skimmed milk) and controls in wells after incubation, and after three washing steps, we added the antibody conjugate (Protein G horseradish peroxidase): this resulted in the formation of the antigen–antibody-conjugate–peroxidase complex.

In a second approach, sera were tested with the indirect commercial ELISA kit ID Screen Hepatitis E Indirect Multispecies (IDvet, Paris, France) with a sensitivity and specificity of 99.5% and 100%, respectively, that also used a recombinant genotype 3 HEV capsid.

In Germany, the in-house ELISA was validated against European pig sera that were pretested with two commercial ELISA kits—PrioCHECK™ HEV Antibody ELISA (ThermoFisher, Dreieich, Germany) and ID Screen® Hepatitis E Indirect Multispecies ELISA (IDvet, Montpellier, France).

Molecular detection. Total RNA was extracted from the fecal samples from pigs collected in Conakry using the QIAamp viral RNA extraction mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. A nested RT-PCR was performed to check for the HEV sequence: the first round of RT-PCR (GoTaq® 1-Step RT-qPCR System [Promega, New York, USA]) generated a 748-base-pair (bp) segment in the ORF2 with primers 3156N (position 5711–5732 along the HEV genome) and 3157N (position 6419–6441), whereas the second round of PCR generated a 325-bp segment at the 5′ end of the ORF2 amplified fragment using primers 3158N (position 5996–6017) and 3159N (position 6322–6343) (Cooper et al. 2005). All steps were done with the GoTaq® Probe 1-Step RT-qPCR System (Promega, New York, USA). The amplification products were separated by electrophoresis on a 1% agarose gel

for 45 min at 100 V stained with SYBR Safe Dye (Invitrogen, Carlsbad, USA).

PCR and sequencing. For sequencing, new DNA fragments were obtained from extracted RNA by optimization using Superscript IV reverse transcriptase (Invitrogen, Vilnius, Lithuania) and diluted 1:10 in nuclease-free water and then PCR was run with Q5 enzyme (NEB, Ipswich, MA, USA) to obtain a 748 partial segment of the capsid gene and a 338 base segment of the polymerase gene using two sets of primers: first HEV-F4228 (position 4279–4307)–HEV-R4598 (position 4627–4649) and the second HEV-F4228–HEV-R4565 (position 4591–4616) (Cooper et al. 2005; Drexler et al. 2012). Hepatitis E Virus Genotyping Tool version 1.0 was used for initial genotyping (Vennema et al. in press).

Phylogenetic analysis. Phylogenetic tree was constructed with Mega 7 software with the maximum likelihood method using 16 HEV references sequences retrieved from GenBank. Sequences were submitted to EMBL GenBank (AC number OR283252–OR283253).

Results

Comparison of the FLI in-house kit with the IDvet

ROC analysis performed in Germany with two different commercial kits as references gave a sensitivity of 96.1% and a specificity of 96.8% for German pigs. In our laboratory assay, we performed a complementary ROC analysis of the in-house assay using the Guinean pigs' pretested sera with the single commercial ID Vet kit and found a sensitivity of 90.1% and specificity of 82.1% for the in-house test (XLSTAT statistical and data analysis solution: www.xlstat.com). Using the in-house ELISA, developed by the FLI team, an overall seroprevalence of 22% (IC95%: 19.0–25%) (193/886) was found to signify circulation of HEV in pigs. Among the 510 samples double tested with the two kits, we obtained 176 positives with the in-house kit. Thus, 80.11% of the positive sera from in-house ELISA (141/176) were found to be positive according to the commercial kit "IDvet" in Supplementary Table S2. However, a Kappa comparison test of the two systems using the data of the 510 double-tested sera indicated an almost perfect accordance between the two kits (kappa = 0.84; Table 1; https://www.graphpad.com/quickcalcs/kappa1/?K=3).

Table 1. Comparison of the Two ELISA Kits (In-House FLI versus Commercial IDvet) on 510 Samples Selected by Chance within the 886 Pig Samples

	Elisa IDvet			
Elisa FLI	*Positive*	*Doubtful*	*Negative*	*Total*
Positive	141	8	27	176
Doubtful	0	0	0	0
Negative	0	0	334	334
Total	141	8	361	510

Analysis of the HEV seroprevalence

The pig breeder sites are found mainly in two regions: the Lower region and Forest Guinea (Fig. 1). Farms in Forest Guinea are home to 89% of the registered Guinean pig population, and most of them keep the animals in enclosures. In Lower Guinea (8.4% of the pig population), on some farms, the pigs also feed freely in the bushes around the houses. We thus performed separate analysis of the two regions.

Presence of anti-HEV IgG among pigs. Figure 1 and Table 2 show the distribution of positive samples obtained using the in-house ELISA in the two regions and the various prefectures of interest. After a multivariate logistic regression with ELISA in-house results as the outcome, results in-house are significantly related to region where the samples were collected. The seroprevalence in Forest Guinea prefectures (43% [IC95% 38–48%]) was significantly higher than the one in Lower Guinea (7% [IC95% 5–9%]) (Table 2).

With a 5% risk, adjusting by region, sex, and HEV in-house result, we have not been able to show any statistically significant relationship between results in-house and age (month). All these statistics tests are reported in Supplementary Data S1.

Genotype 3c HEV was found in pigs

Following the results from the serological survey on pig farms, we decided to assess the presence of HEV in pig feces where the virus is naturally excreted. This has been done on 105 feces samples from free-range pigs in the Conakry suburbs, different from those tested serologically, all of them between 6 and 12 months of age. Two of these 105 porcine feces samples were positive for HEV-RNA (two animals around 6 months of age) using nested PCR targeting ORF2 (Cooper et al. 2005).

Sequencing and phylogenetic analysis. Two PCR products targeting the capsid and RNA polymerase coding region were sequenced using the Nanopore MinION technology. Only one of the two positive samples (sample 76) provided a sufficient sequencing depth (>30×) to obtain the consensus for capsid and polymerase partial sequences. A phylogenetic analysis of the two combined sequences was compared with reference sequence strains from GenBank (Smith et al. 2016). This analysis identified HEV from genotype 3c (Fig. 2).

Discussion

In Guinea, pork meat is consumed mainly in the Lower and Forest regions and not in the two other regions (Middle and Upper regions). We thus checked for the presence of HEV in the two larger pig production regions with ELISA and found 43% (IC95% [38–48%]) positive in the Forest region and 7% (IC95% [5–9%]) in the Lower region sera as a proof of HEV circulation. A rough comparison of the HEV seroprevalence with surrounding countries indicated higher seroprevalence in Ghana, where it reached 62.4% in 2011 (Bagulo et al. 2022) but close to the one found in Cameroon (43.2% in 2017–2018) (Modiyinji et al 2020).

Interestingly, the seroprevalence in Lower Guinea was substantially lower (7%) than in Forest Guinea (43%). Many Ebola, Lassa, and other human cases have recently been diagnosed in the Forest region, indicating that the climate, wildlife,

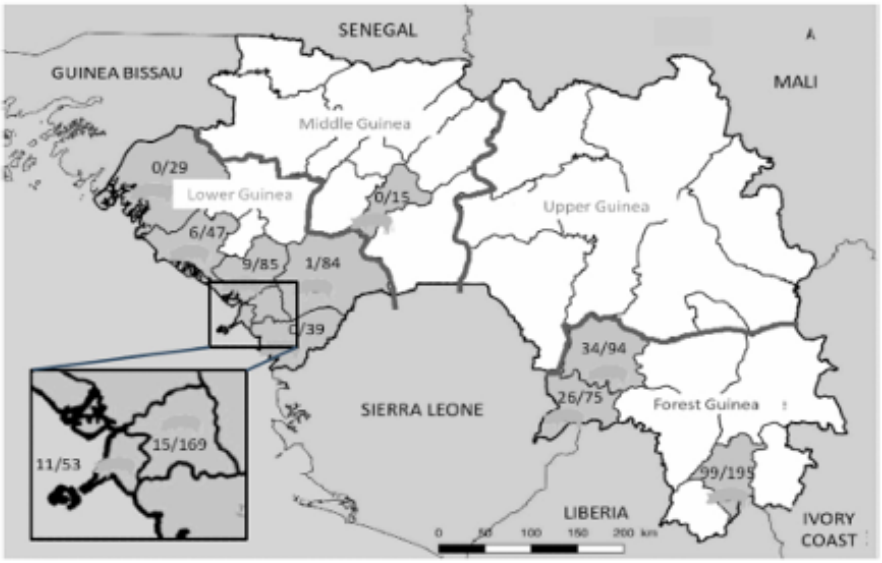


FIG. 1. Spatial distribution of positives samples by ELISA using the FLI kit. The 886 pig sera that were collected in 11 prefectures are indicated in light gray. In each prefecture, the number of seropositives out of the number of animals tested is noted (xx/xx). Conakry (11/53) and Coyah (15/169) are so small, so a zoomed image (left corner) was created to identify these two prefectures in the black square indicated on the map.

and environmental conditions are favorable for zoonotic transmission. Animal husbandry systems include intensive and semi-intensive forms, where the spatial proximity of the animals promotes virus transmission (Forest Guinea), and extensive or free-range farming (Lower Guinea), where pigs are allowed to roam freely for food. However, although in 2022, in Conakry, we found mainly semi- or free-range farming, during 2017–2019, most of the farms visited in Lower Guinea were totally enclosed. Interestingly, the seroprevalence in pigs sampled in Conakry was higher than in the surrounding prefecture.

Therefore, in Conakry, the virus is present in pig farms, as shown by the two positive samples detected by RT-PCR. Upon sequencing of the gene fragments and phylogenetic analysis, the circulating HEV virus belonged to the genotype 3c. This first result in the West African coast reveals the very same genotype previously identified in Cameroonian and Ghanaian pig sera as well as in pig's feces from Madagascar (El-Duah et al. 2020; Modiyinji et al. 2020). No data are available from nearby countries such as Senegal and Mali, where pig breeding is very low. In the Ivory Coast, consumption of pork meat is more common, but there are no data about the animal population or the human one. Recently in Guinea, a study described some human HEV-positive cases but did not provide any information about the sampling date or genotype and only sporadic information about the detailed risk factor. However, this highlights the interest of the HEV studies in Guinea (Ostankova et al. 2023). In the absence of a recognized gold standard for the HEV ELISA specific to pigs, the use of two diagnostic kits (in-house validated in Germany and a commercial IDvet kit) that were not very different in terms of sensitivity and specificity (ROC curve and the Kappa test) strongly supports our conclusion and the results we obtained However, such tests are never perfect, and this also calls for new studies to clearly identify the involved viruses either in the animals or in the environment within the two regions of pig breeding.

TABLE 2. RESULTS OF THE ELISA FLI KIT BY PREFECTURES AND PREVALENCE WITH CONFIDENCE INTERVAL

Population	*Tested population*	*Case number*	*Prevalence*	*Confidence interval 95%*	
Total	886	193	22%	19%	25%
Boffa	47	6	13%	3%	22%
Boke	29	0	—	—	—
Conakry	53	11	21%	10%	32%
Coyah	169	9	5%	2%	9%
Dalaba	15	0	—	—	—
Dubreka	86	9	10%	4%	17%
Forecariah	40	0	—	—	—
Kindia	84	1	1%	−1%	4%
Total Coast Guinea	523	36	7%	5%	9%
Kissidougou	94	34	36%	26%	46%
Nzerekoré	195	97	50%	43%	57%
Guékédou	74	26	35%	24%	46%
Total Forest Guinea	363	157	43%	38%	48%

Some limitations were noted in this study, as the limited number of sampling sites may not be representative of the entire pig population. However, it provided a first snapshot of the situation. Hence, it was previously shown that the HEV seroprevalence may vary largely from site to site, but in our case, this is only significant when we compare the two regions. Within the Forest region, the sites where sampling was done by chance always reported a relatively high seroprevalence, whereas in the Lower region, we only found a few positive pigs or none in some sites. All these results remained within the confidence interval of the computed percentage.

Conclusion

Widespread HEV infections were demonstrated in pigs in Guinea by the seroprevalence study. The molecular study indicates that the HEV genotype 3c is circulating in the Guinean pig population. Further studies will address the reasons for the discrepancy in seropositivity between the Lower and

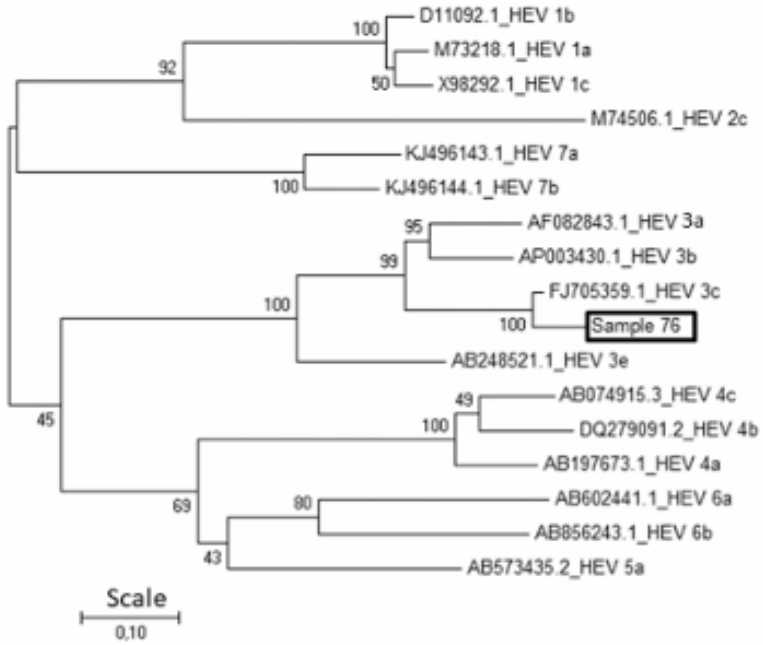


FIG. 2. Phylogenetic analysis of the sequences obtained from the feces samples (polymerase and capsid combined). Sample 76 sequences as indicated by a square were aligned with 16 reference sequences retrieved from GeneBank using MEGA7 software. The evolutionary history was inferred by using the maximum likelihood method based on the general time reversible model. The tree with the highest log likelihood (−5757, 6931) is shown. The robustness of the branching order was assessed by the bootstrap method (1000 replicates), as shown next to the branches (values below 50% are not shown). Initial tree(s) for the heuristic search were obtained automatically by applying the neighbor-join and BioNJ algorithms to a matrix of pairwise distances estimated using the maximum composite likelihood approach and then selecting the topology with superior log likelihood value. A discrete gamma distribution (+G) and portion of invariable sites (+I) to model evolutionary rate differences among sites was used (five categories; $+G = 1, 1356$; $+I = 53, 3484\%$ sites). All positions containing gaps and missing data were eliminated, leaving 762 positions in the final dataset.

Forest regions. Additional sequencing of HEV will need to be carried out to determine the HEV diversity in the pig population. It is also important to conduct a similar study in humans to identify the risk factors for HEV infection.

Acknowledgments

The authors thank the Guinean authorities and are grateful to the Guinean breeders and veterinarians for their expert collaborations during the sampling process, particularly Dr. Traore.

Ethical Consideration

The study was validated by the National Ethics Committee for Health Research (CNERS) from Guinea (ref 040/CNERS/17, June 1, 2017). All procedures performed in studies involving animals followed all international, national, and/or institutional guidelines for the care and use of animals.

Authors' Contributions

P.R., N.T., and C.T.: Conceptualization of study; B.D., S.G., and P.R.: Data curation; B.D. and P.R.: Formal analysis; M.E. and M.H.G.: Resources; B.D., C.T., N.T., and Y.L.P.: Investigation; B.D., P.R., and N.T.: Writing. All authors read and approved the final version.

Author Disclosure Statement

No conflicting financial interests exist.

Funding Information

The following programs contributed to fund the current research: EBOLA FORESIGHT project financed by the German Federal Ministry of Food and Agriculture (File Ref. 323-06.01-03-2815FSEBOL) and the European Union grant EBOSURSY N° FOOD/2016/379-660. Sequencing was supported by Agence Française de Développement through the AFROSCREEN project (grant agreement CZZ3209), coordinated by ANRS | Maladies infectieuses émergentes in partnership with Institut Pasteur and IRD. The authors would additionally like to thank members from the AFROSCREEN Consortium (https://www.afroscreen.org/en/network/) for their work and support on genomic surveillance in Africa. Formerly receiving stipend from IPGui, B.D. is currently funded by a PhD grant from CADEIRA Project DAAD fund.

Data Availability

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

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

Supplementary Material

Supplementary Table S1
Supplementary Table S2
Supplementary Data S1

References

Aubry P. Hépatites virales en zones tropicales. Actualités 2012. Médecine Trop 2013;1–8.

Bagulo H, Majekodunmi AO, Welburn SC, et al. Hepatitis E seroprevalence and risk factors in humans and pig in Ghana. BMC Infect Dis 2022;22(1):132–112; doi: 10.1186/s12879-022-07127-6

Cooper K, Huang FF, Batista L, et al. Identification of genotype 3 hepatitis E virus (HEV) in serum and fecal samples from pigs in Thailand and Mexico, where genotype 1 and 2 HEV strains are prevalent in the respective human populations. J Clin Microbiol 2005;43(4):1684–1688.

De Paula S, Wiele M, Mbunkah AH, et al. Hepatitis E virus genotype 3 strains in domestic pigs, Cameroon. Emerg Infect Dis 2013;19(4):666–668; doi: 10.3201/eid1904.121634

Drexler JF, Seelen A, Corman VM, et al. Bats worldwide carry hepatitis E virus-related viruses that form a putative novel genus within the family Hepeviridae. J Virol 2012;86(17): 9134–9147.

El-Duah P, Dei D, Binger T, et al. Detection and genomic characterization of hepatitis E virus genotype 3 from pigs in Ghana, Africa. One Health Outlook 2020;2:10; doi: 10.1186/s42522-020-00018-3

Kamar N, Bendall R, Legrand-Abravanel F, et al. Hepatitis E. Lancet 2012;379(9835):2477–2488.

Khuroo MS, Khuroo MS, Khuroo NS. Transmission of hepatitis E virus in developing countries. Viruses 2016;8(9):253; doi: 10.3390/v8090253

Modiyinji AF, Sanding GMAM, Atsama MA, et al. Serological and molecular investigation of hepatitis E virus in pigs reservoirs from Cameroon reveals elevated seroprevalence and presence of genotype 3. PLoS One 2020;15(2):e0229073–9.

Motoya T, Umezawa M, Goto K, et al. High prevalence of hepatitis E virus infection among domestic pigs in Ibaraki Prefecture, Japan. BMC Vet Res 2019;15(1):87–88.

Purdy MA, Drexler JF, Meng X-J, et al. ICTV virus taxonomy profile: Hepeviridae. ICTV virus taxonomy profile: Hepeviridae 2022. J GenVirol 2022;103(9); doi: 10.1099/jgv.0.001778

Sadio BD, Faye M, Kaiser M, et al. First hepatitis E outbreak in southeastern Senegal. Sci Rep 2022;12(1):17878; doi: 10.1038/s41598-022-22491-8

Smith DB, Izopet J, Nicot F, et al. Update: Proposed reference sequences for subtypes of hepatitis E virus (species Orthohepevirus A). J Gen Virol 2020;101(7):692–698.

Smith DB, Simmonds P, Izopet J, et al. Proposed reference sequences for hepatitis E virus subtypes. J Gen Virol 2016; 97(3):537–542; doi: 10.1099/jgv.0.000393

Takahashi M, Nishizawa T, Sato Y, et al. Prevalence and genotype/subtype distribution of hepatitis E virus (HEV) among wild boars in Japan: Identification of a genotype 5 HEV strain. Virus Res 2020;287:198106; doi: 10.1016/j.viruses.2020.198106.;287

Traoré KA, Bienvenue Ouoba J, Huot N, et al. Hepatitis E virus exposure is increased in pork butchers from Burkina Faso. Am J Trop Med Hyg 2015;93(6):1356–1359.

Vennema H, Kroneman A. Emweb bvba from the RIVM (National Institute for Public Health and the Environment NL). Hepatitis E Virus Genotyping Tool (v1.11) 2024. Available from: https://www.genomedetective.com/app/typingtool/hev/ Manuscript in preparation.

Ostankova YV, Shchemelev AN, Boumbaly S, et al. Prevalence of HIV and viral hepatitis markers among healthcare workers in the Republic of Guinea. Diagnostics (Basel) 2023;13(3): 378; doi: 10.3390/diagnostics13030378Academic

Address correspondence to:
Bakary Doukouré
Unité de Virologie
Institut Pasteur de Guinée
Route de Donka
Quartier Landreah
Commune de Dixinn
BP 4416
Conakry
Guinea

E-mail: bakary.doukoure@pasteur-guinee.org

Supplementary files:

Supplementary file S1: A- Number and pig farming description for serological screening

« Préfecture »	Sampling sites	Site number	Tested population/sites	Farming system and description
BOFFA	Urban area	3	47	animals are in pens, City centre
BOKE	Kamsar	1	29	animals are in pens, Rural area on a farm
CONAKRY	Dabompa	1	30	City centre livestock market
	Simbaya Gare	1	23	
COYAH	Doumbouyah	1	169	animals are in pens, Rural area, under fruit trees with bats in circulation
DALABA	Tangama, Bouberet, Pethel	1	15	animals are in pens, City centre
DUBREKA	Kenindé	1	86	animals are in pens, Rural area under mango trees
FORECARIAH	Maferinyah	1	40	animals are in pens, Rural area
KINDIA	Friguiagbe	1	84	animals are in pens, Rural area
Total coast		**11**	**523**	
GUECKEDOU	Suburb area	2	74	animals are in pens, Rural area
KISSIDOUGOU	Urban area	4	94	animals are in pens, 1 site at city centre and 3 péri-centre area
N'ZEREKORE	Urban area	2	153	animals are in pens, 1 site in the city centre and close suburb
	S/P Koulé	1	42	animals are in pens, Rural, peri-forest area
Total Forest		**9**	**363**	
Total study		**20**	**886**	

Supplementary file S1 B-Number and pig farming description for molecular screening

Préfecture	Sampling sites	Number of tested animals	Farming system and description
Conakry	Boussoura	30	Free roaming along the shoreline
	Coleah Imprimerie	30	Locked in a large vacant lot crossed by a creek
	Coleah Badè	30	Free roaming along the shoreline
	Dixinn Bora	15	Free roaming along the creek

Supplementary file S2: Results of the ELISA ID Vet kit by prefectures and prevalence with confidence interval

Population	Tested Population	Case Number	Prevalence	Confidence Interval 95%	
Total	**510**	**142**	**28%**	**24%**	**32%**
Boffa	44	8	18%	7%	30%
Boke	28	0	-	-	-
Conakry	39	4	10%	1%	0%
Coyah	70	1	1%	-1%	4%
Dalaba	6	0	-	-	-
Dubreka	35	1	3%	-3%	8%
Forecariah	11	0	-	-	-
Kindia	39	1	3%	-2%	8%
Total Lower Guinea	**272**	**15**	**6%**	**3%**	**8%**
Kissidougou	64	31	48%	6%	36%	61%
Nzerekoré	133	75	56%	4%	48%	65%
Guékédou	41	21	51%	8%	36%	67%
Total Forest Guinea	**238**	**127**	**53%**	**3%**	**47%**	**60%**

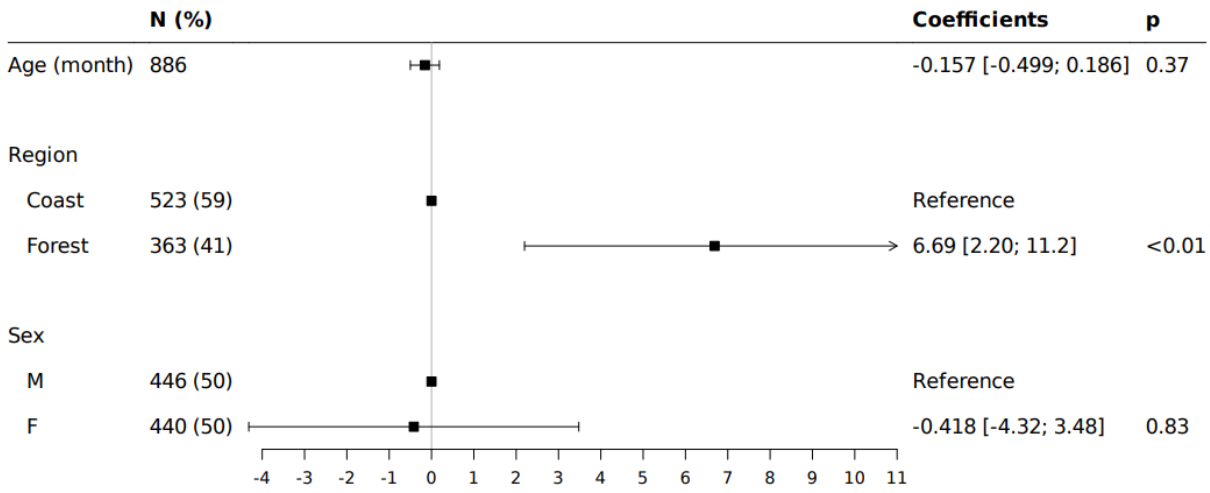
Supplementary file S3: Multivariate logistic regression with the ELISA in house results according to region, age, sex

Multivariable Analysis

With a 5% risk, by adjusting for Region, Sex and HEV in house result, we have not been able to show any statistically significant relationship between Results in house and Age (month).Results in house is significantly related to Region.

- Results in house of the group Region Forest is on average superior of 6.69 to Results in house of the group Region Coast (p = **<0.01**)

		Coefficients	**p**
Age (month)		-0.157 [-0.489; 0.102]	**0.4**
Region	Forest vs Coast	6.69 [3.63; 10.5]	**<0.01**
Sex	**F vs M**	**-0.418 [-4.78; 3.24]**	**0.82**



Serological and molecular detection of HEV in pigs population in Guinea 2023 (Complementary study of the latest article)

After this initial study, which effectively demonstrated the presence of HEV serological traces in the Republic of Guinea, the next step was to confirm these serological results. This necessarily involved further sampling campaigns in roughly the same prefectures, but this time, in addition to serum, it was necessary to collect pig feces samples to detect the virus in these regions of Guinea, as feces had only been collected in Conakry, the capital. The prefectures chosen where Boffa and Nzerekore/Yomou (Figure 11).

Using the methodology from our first article *Seroprevalence and Phylogenetic Characterization of Hepatitis E Virus (Paslahepevirus balayani) in Guinean Pig Population* for the treatment of feces and sera, we thus assessed the presence of hepatitis E virus (HEV) RNA in pigs in the two main areas of Guinea where pigs were bred in 2023, using the same molecular detection methods and Elisa assays.

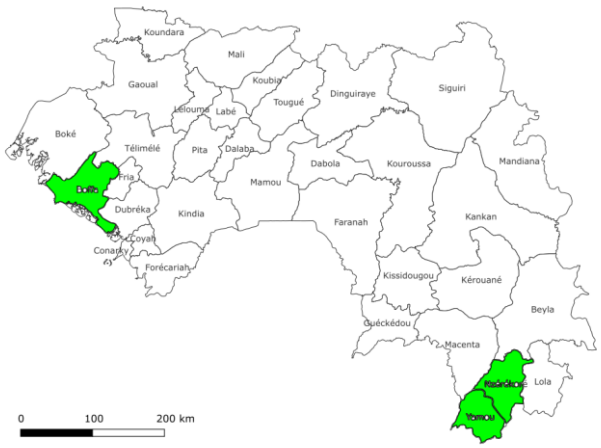


Figure 14: Map of the Republic of Guinea showing the geographical distribution of HEV sampling sites in humans, pigs, and wastewater across different regions involved in the study

Results and discussion

In this complementary study, we have summarized the main results obtained during the study and their discussions.

Description of pig farms and pigs.

Sampling missions for pigs focused on several sub-prefectures (12 in total) in Forest Guinea and Lower Guinea, namely: Dieke Centre, Yalenzou, Bignamou, Goueke, Koule, Pela, Palé, Womey (Forest Zone) and Boffa Centre, Douprou, Tougnifily, Mankountan (Lower Guinea).

Most of the pig farms surveyed (68%) are in rural areas, which is consistent with the traditional geographical distribution of pig farming in Guinea. Rural areas often offer more space for pens but also have fewer veterinary and health facilities and more informal practices. Urban areas, which account for nearly a third of pig farms (32%), pose specific challenges in terms of public health: proximity to homes, manure management, risks of environmental contamination (wastewater, markets), and increased human exposure to zoonotic agents such as HEV.308 pigs were sampled, divided into males and females, aged between 6 and 12 months.

HEV Serological Detection in Pigs

Table 1: *Seroprevalence of Anti-HEV Antibodies in Pigs from Nzerekoré (Forest Guinea) and Boffa (Lower Guinea): Local-Level Data with 95% Confidence Intervals*

Population	**Tested Population**	**Case Number**	**Prevalence**	**Confidence Interval 95%**	
Total	**308**	**108**	**35%**	**30%**	**40%**
Dieke centre	27	6	22%	7%	38%
Yalenzou	25	10	40%	21%	59%
Bignamou	16	1	6%	0%	18%
Goueke	17	14	82%	64%	100%
Koule	27	8	30%	12%	47%
Pela	24	13	54%	34%	74%
Pale	41	12	29%	15%	43%
Womey	23	22	96%	87%	100%
Total Nzerekoré	**200**	**86**	**43%**	**36%**	**50%**
Boffa centre	34	22	65%	49%	81%
Douprou	4	0	0%	0%	0%
Tougnifily	52	0	0%	0%	0%
Mankountan	18	0	0%	0%	0%
Total Boffa	**108**	**22**	**20%**	**13%**	**28%**

Geographic Variations in Anti-HEV Seroprevalence among Pig Populations

The seroprevalence of anti-HEV antibodies varied markedly between the two study regions, with higher levels observed in Nzerekoré compared to Boffa. This difference may reflect more intense or prolonged viral circulation in Nzerekoré, or distinct environmental and behavioral exposures between the regions.

At the local scale, the range of seroprevalence values was considerable (from 6% to 96%), suggesting heterogeneous exposure among pig populations. Localities such as Womey and Goueke in Nzerekoré, and central Boffa in Lower Guinea, emerged as potential hotspots of HEV transmission. The elevated level of IgG observed in these localities could result from recurrent exposure to the virus, possibly driven by environmental contamination or specific husbandry practices.

Conversely, localities such as Bignamou, Douprou, and Tougnifily exhibited very low or null seroprevalence, which may indicate either an absence of viral introduction, lower animal mobility, or geographic isolation limiting transmission. These spatial contrasts point to a patchy circulation pattern of HEV, consistent with localized exposure events rather than widespread endemicity.

Such variability underscores the importance of considering ecological and management-related factors such as herd density, animal movement, and proximity to human settlements or water sources in understanding HEV dynamics in swine populations.

In the following section, we explore how environmental conditions and farming practices may have shaped these spatial disparities in HEV exposure across the study sites (Table 1).

Molecular detection of HEV in pig feces

In this study, 308 pigs fecal samples were analyzed for the presence of HEV RNA using three molecular approaches targeting different regions of the viral genome.RT-qPCR targeting the phosphoprotein (ORF3) and RT-PCR targeting the viral capsid (ORF2) did not detect any positive samples.Signals were observed in some samples using RT-PCR targeting the polymerase (ORF1), with bands close to the expected sizes; however, confirmation by the Friedrich Loeffler Institute (FLI) did not validate these signals, indicating non-specific amplifications. To conclude, none of the 308 fecal samples tested contained detectable HEV RNA.These results suggest that, in the sampled regions (Forest and Lower Guinea), active HEV circulation in pigs is likely low or absent at the time of the study. It is important to note that pigs shed the virus transiently, typically for a short period following infection (2–4

weeks). Therefore, single time point sampling may not coincide with viral shedding periods, potentially yielding negative results despite prior exposure.Furthermore, the absence of detection despite using multiple primer sets targeting distinct genomic regions strengthens the reliability of the negative findings.

Other studies conducted in West Africa, showed that HEV prevalence in pigs varies considerably across regions and farms, the absence of HEV RNA in our samples may reflect limited viral circulation. These results also suggest that the zoonotic risk associated with porcine feces in the studied areas is low, although ongoing environmental and serological surveillance remains necessary to assess overall risk.

Finally, several limitations should be considered: single-time-point sampling does not capture the full annual dynamics of the virus, the viral shedding window is short, and primer sensitivity could be affected by the possible presence of divergent local variants. Nevertheless, the absence of active HEV RNA in porcine feces represents an important indicator for understanding viral circulation and zoonotic risk in Guinea.

Conclusion

The study on the serology and phylogenetic characterisation of the hepatitis E virus (Paslahepevirus balayani) in Guinea's pig population (latest article) and the complementary study conducted in 2023 on pigs provide us with an integrated and evolving picture of the epidemiological situation of HEV within Guinea's pig populations.

The first study, based on pig samples collected between 2017 and 2019 and some pig feces from Conakry in 2022, was a fundamental step in documenting, for the first time, the presence of HEV in pigs in Guinea. It revealed a high seroprevalence of anti-HEV antibodies in both the Lower Guinea and Forest Guinea regions, indicating significant previous exposure of the pig population to the virus. In addition, molecular detection and partial phylogenetic characterization provided the first genetic evidence of HEV circulation in pigs in the country, linking local strains to those already identified in West Africa. These results provided a solid basis for zoonotic risk assessment and justified the implementation of a complementary study. The complementary study, conducted in 2023, aimed to confirm and further investigate these results through a geographical extension of sampling and the inclusion of more pig fecal samples in order to search for the active virus. Although serological analyses confirmed the

persistence of traces of exposure, no detection of viral RNA was observed in the 308 samples tested. This absence can be explained by the brief period of virus excretion, the timing of sampling not coinciding with the viraemia phase, or by low or discontinuous viral circulation at the time of the study.

Joint analysis of the two studies thus reveals a transition from significant previous exposure to low current viral activity in pigs in Guinea. This finding suggests either an endemic but fluctuating viral dynamic, or a reduction in transmission linked to ecological, environmental or farming practice changes, given the differences between sampling years. From a One Health perspective, these results highlight the need to establish integrated longitudinal surveillance combining animal, human and environmental compartments in order to better understand the dynamics of HEV circulation and anticipate zoonotic risks. These two complementary studies lay the foundations for an epidemiological surveillance system for HEV in Guinea and pave the way for broader research integrating other zoonotic pathogens circulating at the human-animal-environment interface.

These data underscore the need for longitudinal surveillance combining serological, molecular, and environmental approaches to better characterize HEV circulation dynamics in the swine production chain and its potential link to human contamination.

Article 2: First detection of Rocahepevirus in urban wastewater from Guinea: a One Health alert.

Introduction

Hepatitis E virus (HEV) is an emerging zoonotic pathogen of global public health concern. While human infections with genotypes 1 and 2 are typically associated with contaminated water in endemic regions, genotypes 3 and 4 are zoonotic and are commonly found in domestic pigs, wild boars, and environmental reservoirs. Transmission to humans often occurs via the consumption of undercooked pork or through exposure to contaminated water.

Pigs are the principal animal reservoir for HEV genotype 3. Many studies worldwide have confirmed widespread HEV circulation in pig populations, with viral RNA detected in feces, liver tissue, and wastewater from slaughterhouses and farms. Wastewater systems, particularly in low-income urban settings, represent another important but underexplored route for HEV transmission, especially when untreated waste is discharged into surface water used for drinking or farming.

In Guinea, data on HEV circulation in animals and the environment are extremely limited. Knowing the importance of pig farming in peri-urban and rural areas, and the widespread use of surface water sources, the risk of zoonotic and environmental transmission of HEV is high. This study aimed to assess the presence of HEV in pig feces and wastewater in selected regions of Guinea.

This dynamic study was conducted in 2023, pig serum and feces samples were collected in Nzerekoré and Boffa prefectures of Guinea, selected for their high density of pig farms and informal slaughter sites.

Objective

Given that hepatitis E is primarily transmitted through contaminated water, we focused our sampling on urban areas near wastewater treatment plants, sewage effluents, and coastal discharge zones, to assess whether, as reported elsewhere, the virus was circulating in wastewater and coastal effluent waters of the capital.

The main objective of this study was to investigate the presence of hepatitis E virus (HEV) RNA in wastewater in Guinea during 2023.

Specific objectives included:

1. To detect HEV RNA in environmental samples from wastewater and effluents.
2. To genotype HEV strains from wastewater using molecular methods.
3. To contribute to understanding potential zoonotic and environmental transmission routes.

Article ready for submission

Abstract

Rocahepevirus ratti (HEV-C1 genotype) has been detected in 35 out of 180 urban wastewater samples in Conakry, Guinea. Phylogenetic analysis of partial HEV ORF1 genome segments reveals clustering with African rodents HEV-C1 strains highlighting environmental contamination and potential zoonotic risk for human population in proximity.

Full text

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 emerge 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 in feces (1). Although limited data exist on environmental HEV circulation in Africa (2), *Rocahepevirus ratti* (HEV-C1), primarily circulating in rodents, has been recently detected (3). It is occasionally associated with human infections (4,5). This study aimed to investigate the presence of HEV in urban wastewater of the Conakry area, as part of a One Health surveillance program of the Institut Pasteur de Guinée for the ANSS (Agence National de Sécurité Sanitaire).

Methods

Between December and April 2025, 180 wastewater samples were collected from 5 communes in Conakry. Into each commune, 2 sites were sampled once per week. Samples were concentrated by polyethylene glycol (PEG 8000) precipitation, RNA was extracted using the Qiagen Viral RNA kit, and HEV RNA was detected using : (i) Paslahepevirus specific RT-qPCR (ORF3) (6) confirmed by nested RT-PCR (ORF1 and 2) (7); (ii) Rocahepevirus specific nested RT-PCR (ORF1) (8). ORF1 sequencing of the positive samples was realized by primers specific to Rocahepevirus or Paslahepevirus using the Oxford Nanopore Technologies MinION platform. Raw signal data were demultiplexed through barcode assignment and subjected to basecalling. The resulting FASTA files were processed with a workflow adapted from the ARTIC protocol to generate consensus sequences,

Results

Of 180 water-samples, 135 (74%) were positive using ORF3 Paslahepevirus specific RT-qPCR. The 10 tested sites had regular positive signal which confirms the continuous circulation of HEV in Conakry wastewater. One distinguished sites with active transmission (sites 3 and 4: Ct < 32) and other with weak but persistent signals (sites 2 and 14: Ct >32). Figure 1 illustrates clearly the spatial distribution of these sampling sites and the frequency of HEV detection across the 18-week monitoring period.

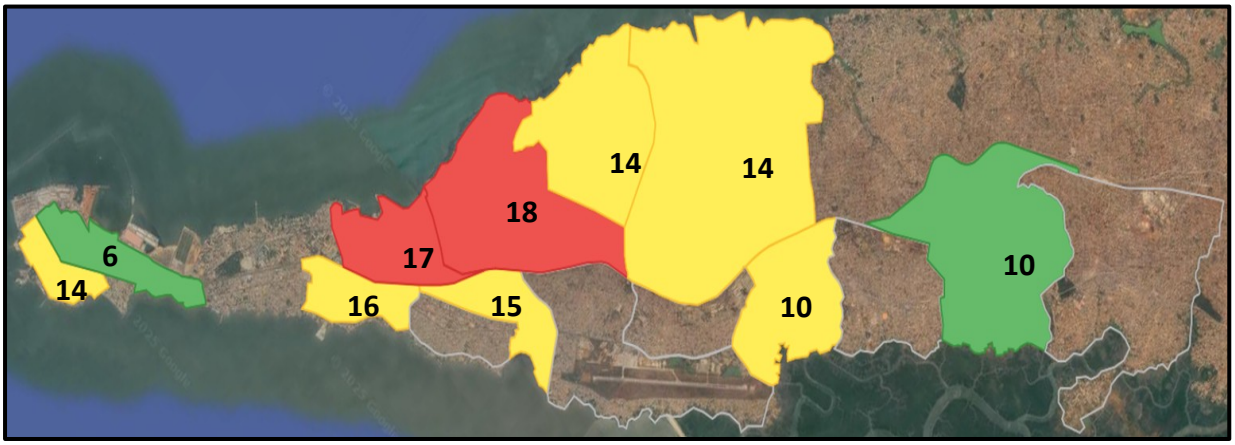


Figure 1. Spatial distribution of HEV detection sites in Conakry wastewater. For each site, the number of weeks (out of 18) during which HEV RNA was detected is indicated. The color code highlights the sites where high viral load was detected frequently (about 11 weeks) in **red**; moderately (1–4 weeks)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

Thirty-five of the 135 positive samples were confirmed using ORF1 nested RT-PCR, none using ORF2. As ORF1 encodes the conserved RNA polymerase, this suggested that these 35 samples may correspond to another *Hepevirus* genus. Indeed, most of them were also positive using ORF1 RT-PCR specific for *Rocahepevirus ratti*, 14 were sequenced and 12 generated 900 pb consensus sequences. Figure 2 shows the phylogenetic analysis using the rat HEV strain V-105 genome for reference (GenBank accession no. JX120573). The Conakry's sequences correspond to the sub-genotype HEV-C1 of *Rocahepevirus ratti*. They cluster with HEV rodent sequences from Cameroon (GenBank PP764563.1– PP764564.1) (3). (figure 2).

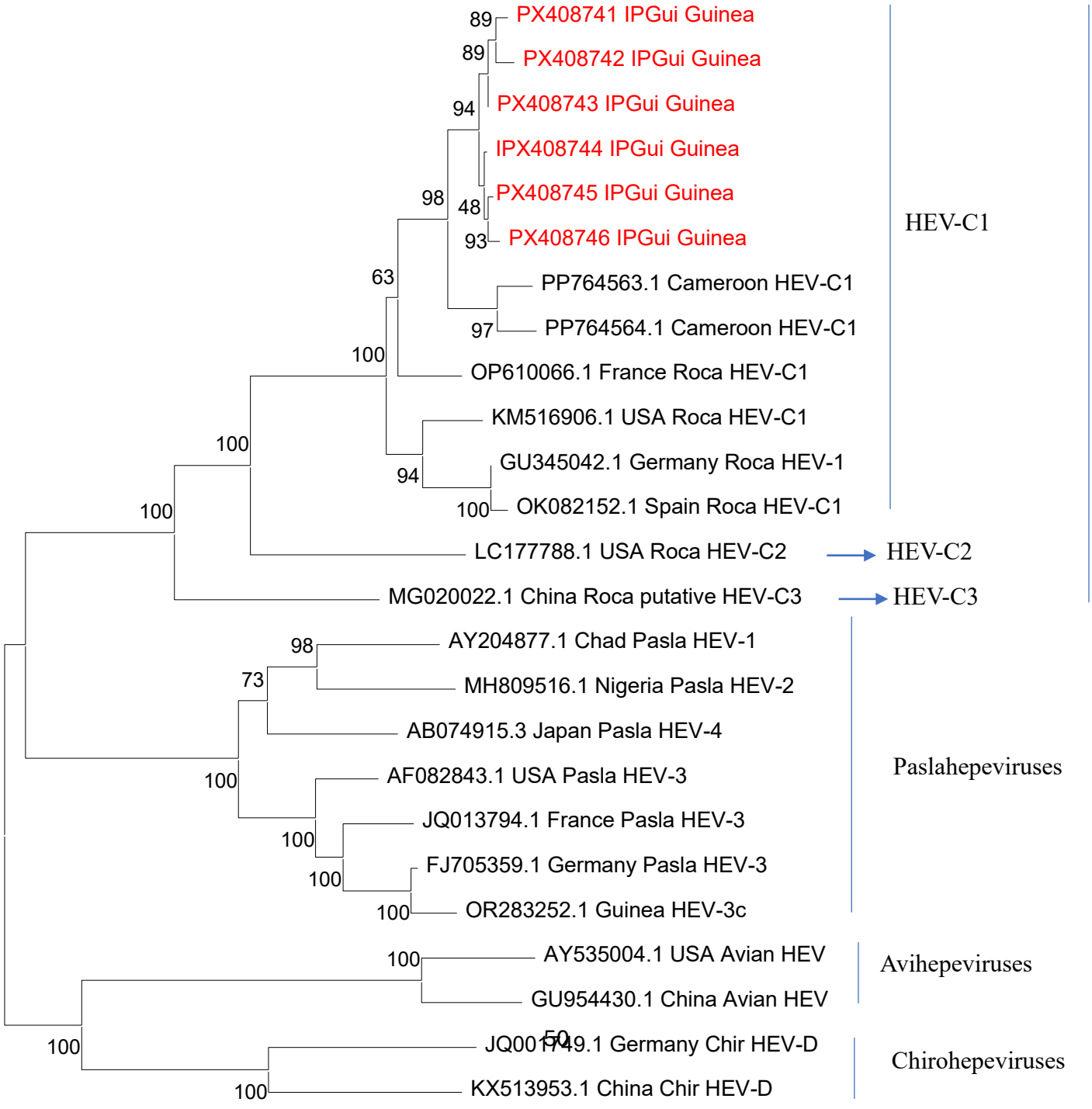


Figure 2: Phylogenetic tree of the Hepeviridae family based on a 900 nt of the ORF1 gene, constructed using the Neighbor-Joining method with 500 bootstrap replicates in MEGA 12. . HEV sequences in Guinean wastewater are in red (6). Numbers correspond to the bootstrap percentage supporting each node.

Discussion / Conclusion

This is the first report of *Rocahepevirus ratti* RNA (HEV-C1) circulation in Conakry wastewater. As *paslahepevirus* of genotype 3 (HEV-3) was detected in pigs feces and few effluent streams in 2023 in Conakry (1), we first tested wastewater samples with *paslahepevirus* specific RT=PCR and confirmed 74% (135/180) of positive samples. 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 HEV-C1 strain. This result suggests that *rocahepevirus ratti* is circulating in a diffuse and prolonged way with hot spots suggesting local concentration of rat population.

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 aa well as related human exposure seems mandatory in West Africa and particularly in Guinea.

Acknowledgments

We gratefully acknowledge the support of the Institut Pasteur de Guinée for fieldwork and sample collection. 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). We also thank the National Reference Center for Hepatitis E (CNR-HEV) in France for their technical guidance and valuable discussions.

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.

Author Bio : **Bakary Doukouré** is a PhD candidate in virology at Tubingen university, hosted at the Institut Pasteur de Guinée. His doctoral research focuses on the epidemiology of hepatitis E virus in Guinea, in a One Health approach integrating human, animal, and environmental data. His interests include zoonotic transmission pathways, molecular detection of emerging viruses, and strategies to strengthen surveillance systems for viral hepatitis in West Africa.

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
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.
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.
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.
5. Gherlan GS. Rocahepevirus ratti: an underrecognised cause of acute hepatitis. *World J Hepatol*. 2024;16:1084–90.
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.
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.
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.
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.
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.

Article 3: Seroprevalence and Molecular Detection of Hepatitis E Virus in Pregnant Women

Manuscript in preparation

Introduction

Hepatitis E virus (HEV) is a main cause of acute viral hepatitis , with significant health implications in developing countries. It is an enterically transmitted virus, primarily through fecal-oral routes, often associated with poor sanitation, contaminated water, and zoonotic reservoirs such as pigs. Among the different genotypes, HEV-1 and HEV-2 are restricted to humans and are responsible for large outbreaks in endemic regions, while HEV-3 and HEV-4 are zoonotic . Pregnant women represent a high-risk group for HEV infection, particularly in the third trimester, where infection has been associated with severe complications such as fulminant hepatitis, maternal mortality, and adverse pregnancy outcomes (e.g., miscarriage, stillbirth, and preterm delivery).In sub-Saharan Africa, including Guinea, the burden of HEV in pregnant women remains insufficiently documented despite the presence of environmental and socio-sanitary conditions that favor transmission. Conakry, the capital city of Guinea, presents a typical urban setting with diverse water and sanitation challenges, high population density, and significant public health vulnerabilities.

Objective

The main objective of this study was to determine the prevalence and molecular evidence of hepatitis E virus (HEV) infection among pregnant women in Conakry in 2023.

Specifically, the study aimed to:

1. Detect serological markers of HEV (anti-HEV IgG and IgM antibodies) in pregnant women.
2. Identify active HEV infection through molecular detection of HEV RNA using RT-PCR.
3. Describe the epidemiological profile (age, trimester of pregnancy, HIV status, history of transfusion, etc.) of the positive cases.

4. Explore potential environmental and behavioral risk factors associated with HEV exposure in this population.

Materials and Methods

Type and Design of Study

This was an analytical study conducted from October to December 2023 across the health center of Koulewondy in Conakry, Guinea.

Study Population and Inclusion Criteria

The study included pregnant women of all trimesters who attended antenatal consultations or were hospitalized during the study period. The Inclusion criteria were informed consent given and pregnant women in a good health situation for sampling

Sampling and Sample Size

A convenience sample of 206 pregnant women was recruited across one of the most frequented health center in Conakry (Centre de santé de Koulewondy). The sample size was calculated to estimate a presumed seroprevalence of 10–15% with a 95% confidence interval and a precision of 5%.

Data Collection

A structured questionnaire was used to collect sociodemographic and clinical data, including Age, HIV status, history of blood transfusion.

Biological Sample Collection and Processing

Human samples

Serum samples were collected from pregnant women at the Koulewondy health centre, near Ignace Deen Hospital, between October and December 2023.

The samples were transported at +4°C and then stored at -80°C until analysis.

Blood samples (5 mL) were collected from each participant under aseptic conditions. Sera were separated and stored at –20°C until analysis.

Laboratory Analyses

1. **Serology**: Detection of anti-HEV IgG and IgM antibodies was performed using commercial ELISA kits (IgM ELISA kits), following the manufacturer's instructions (Figure 12).

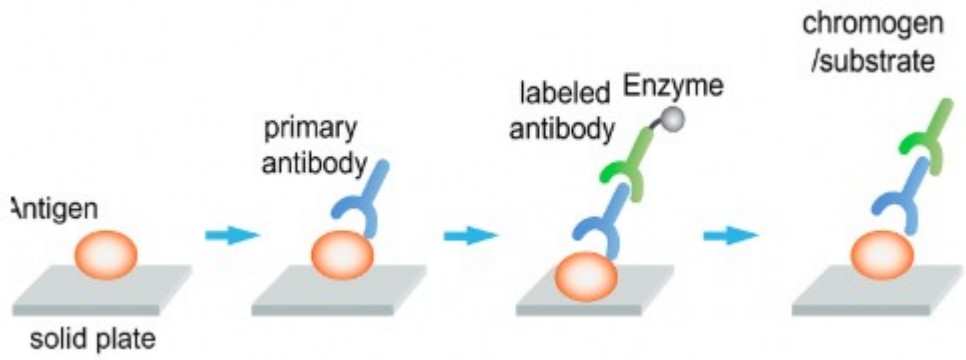


Figure 15: Principle of the Indirect ELISA Technique:
antigens are first adsorbed onto a solid plate. A primary antibody specific to the antigen is then added and binds to it. After washing, a secondary antibody (anti-species antibody) conjugated with an enzyme is added; this antibody recognizes and binds to the primary antibody. When a chromogenic substrate is introduced, the enzyme catalyzes a color-producing reaction, the intensity of which is proportional to the amount of specific antibody present in the sample.

2. **Molecular Diagnosis**:

HEV RNA was extracted from serum samples using QIAamp Viral RNA Mini Kit (Qiagen).

RT-PCR was conducted targeting **ORF1 (HEV-38/39 and 37/27)** and **ORF2 (HEV-34/36 and 35/29)** regions with primers validated for genotype 1,2,3 and 4 detections (see appendix).

Amplification products were visualized on agarose gel, and positive samples were confirmed by sequencing.

Data Analysis

Data were entered and cleaned and analyzed using Excel.

Seroprevalence was expressed with 95% confidence intervals.

Ethical Considerations

The study protocol was approved by the National Ethics Committee for Health Research of Guinea. All participants provided written informed consent before inclusion. Data confidentiality and participant privacy were strictly maintained.

Results

1. Sociodemographic characteristics of the population

Categories	Population /Pourcentage
Age	
]20	18 (9)
[20-35]	177 (86)
]35	11 (6)
Step of pregnancy	
1st Quarter	10 (5)
2nd Quarter	147 (72)
3rd Quarter	22 (11)
Not determined	27 (14)
Statut HIV	
Positives	2 (1)
Negatives	204 (99)
Transfusion history	
Non	173 (84)
Oui	33 (16)
Provenance	
Kaloum	75 (37)
Externe	131 (64)
History of birth	
Primiparous	154 (75)
/ultiparous	52 (26)
History of Stillbirth	
Yes	45 (22)
No	161 (79)

Table 2*: Sociodemographic, obstetric and medical history characteristics of pregnant women included in the hepatitis E study at Koulewondy Health Center (Conakry, Guinea)*

Among the 206 pregnant women included in the study (Table 2), the average age was 29.28 years (±0.88), with a predominance of women aged 20 to 35 years (86%). The majority were in the second trimester (86%) of pregnancy.

Almost all the pregnant women were HIV negative (204 out of 206), or approximately 99%. This rate indicates a low prevalence of HIV in this group of pregnant women. This could be important to consider in an analysis of co-infections or associated risk factors.

Eighty-four percent of pregnant women had no history of blood transfusion. Among those who did (n = 33), 60% reported having donated blood and 40% reported having received blood. Although rare, transfusion-transmitted HEV infection has been reported in some regions, suggesting that blood transfusion may represent a potential route of exposure.

Approximately 75% of pregnant women are primiparous (this is their first pregnancy). Approximately 25% have already had at least one pregnancy (non-primiparous). This high proportion of primiparous women may reflect a young average age in this population, which corresponds to the age data previously provided (the majority are between 20 and 35 years old).

Seventy-eight percent 78% of pregnant women have no history of obstetric complications, which can be explained in part by the high proportion of primiparous (75%). Approximately 22% of women have already experienced at least one obstetric complication. A few rare cases (3 women) have a significant history (≥4 complications), which warrants enhanced obstetric monitoring.

3. Serological results

The seroprevalence of anti-HEV IgG antibodies in pregnant women included in our study is very low (2.4%), suggesting limited previous exposure to the virus. This population therefore appears to be predominantly immunologically naive to HEV, which could constitute a vulnerability factor in the event of active circulation of the virus.

No IgM was detected in the panel of 206 pregnant women.

4. Molecular results (RT-qPCR and RT-PCR)

All RNA from the serum samples of the 206 pregnant women was extracted and subjected to molecular testing. No HEV RNA was detected, indicating an absence of the virus in this population of pregnant women.

Discussion

1. Low seroprevalence of anti-HEV IgG antibodies (2.4%)

The low seroprevalence of anti-HEV IgG antibodies (2.4%) observed among pregnant women suggests limited past exposure to hepatitis E virus within this population. This finding implies that most individuals remain immunologically naïve, and therefore potentially susceptible to infection in the event of viral circulation.

At the global level, HEV seroprevalence shows substantial heterogeneity, largely influenced by sanitation conditions, access to safe water, and the distribution of viral genotypes. In industrialized countries, the prevalence is generally below 10%(109), whereas in South and Southeast Asia, particularly in India, rates can exceed 30–40%, reflecting higher levels of endemic transmission through the fecal–oral route(110).

In West Africa, available data indicate higher seroprevalence levels, typically ranging from 5% to 15% among pregnant women, as reported in countries such as Senegal(63) and Nigeria(111). The markedly lower prevalence found in Conakry may therefore reflect either a genuinely limited circulation of the virus, demographic characteristics specific to the study population, or improvements in sanitation and water infrastructure that reduce exposure risk.

2. Absence of anti-HEV IgM antibodies

The complete absence of anti-HEV IgM antibodies further supports the lack of recent or ongoing infections in the study cohort. Since IgM antibodies represent markers of acute or recent infection, these results indicate that no epidemic or active transmission event was occurring at the time of sampling, the same result had been found in Vietnam(112) and in Ethiopia(60). Combined with the low IgG seroprevalence, this pattern suggests that HEV circulation in the area is weak, sporadic, or possibly seasonal.

3. Negative molecular results (RT-qPCR and RT-PCR)

Molecular assays performed on serum samples confirmed the absence of detectable HEV RNA among the 206 pregnant women tested. This molecular negativity reinforces the serological evidence of limited viral activity and suggests a stable epidemiological situation during the study period. Similar findings have been reported globally, where HEV RNA detection in asymptomatic individuals, especially in pregnant women outside epidemic contexts, remains uncommon. In contrast, during outbreaks documented in Ethiopia, Egypt, and Nigeria, viral RNA was consistently identified in a significant proportion of symptomatic cases(5). The

present findings thus indicate an epidemiological context free from active transmission at the time of investigation.

4. Implications in the context of pregnancy

The absence of active infection and the low exposure rate observed in this cohort are encouraging from a public health perspective, given that HEV infection particularly with genotype 1 can lead to severe and sometimes fatal outcomes in pregnant women, including fulminant hepatitis and maternal-fetal complications. Nevertheless, the immunological naïveté of this population may represent a vulnerability in the face of future outbreaks. Maintaining vigilance through regular surveillance, health education, and improved hygiene practices is therefore critical to prevent epidemic resurgence.

Conclusion

In summary, the findings of this study indicate that hepatitis E virus currently circulates at a very low level among pregnant women in Conakry, with limited prior exposure and no evidence of ongoing infection. This epidemiological profile contrasts with reports from several West and North African settings where higher prevalence rates and recurrent outbreaks have been documented. Differences in environmental conditions, population characteristics, or timing of data collection may partially explain these variations.

Although the present results suggest a favorable situation, continued vigilance remains warranted. Strengthening preventive measures such as improving access to safe drinking water, integrating HEV screening into routine antenatal care, and enhancing awareness among healthcare providers and patients would help reduce potential risks. In addition, sustained molecular and environmental surveillance is essential to enable early detection of new cases and to better understand possible sources of infection, including animals, water, and blood transfusion pathways. Adopting a One Health perspective that integrates human, animal, and environmental data will be fundamental to preventing and controlling future hepatitis E outbreaks in Guinea.

PART IV: GENERAL DISCUSSION

Summary of Key Findings (One Health Integration)

The combined serological, molecular and environmental data indicate that hepatitis E virus circulation in Guinea follows a multi-host, multi-source pattern typical of tropical settings with intense human–animal–environment interactions. The findings reveal a complex HEV ecology in Guinea, involving distinct animal and environmental reservoirs.

Despite the lack of recorded HEV epidemic in Guinea and specifically both in the lower coast and the forest area, HEV circulation can be assessed in Guinea. Thus in 2017-2019, pigs anti-HEV seroprevalence was 22% at the national level with marked regional disparities (43% in Forest Guinea vs. 7% in Lower Guinea), confirmed by a complementary study in 2023 in the same regions: 35% nationally (43% in Forest Guinea vs. 20% in Lower Guinea).

In additional sampling in Conakry performed in 2022 (105 pig feces), virus were detected in 2/105 samples and were sequenced as HEV-3c. However, during the 2023 campaigns in Nzerekoré/Boffa, we did not detect HEV within 308 fecal samples that were tested by RT-PCR/RT-qPCR.

The apparent decline in detectable HEV-3 RNA in pigs between 2019 and 2023 may reflect seasonal or temporal variations in viral shedding, highlighting the need for longitudinal surveillance rather than single cross-sectional assessments.

To assess the risk of HEV circulation and environment contamination of human through the wastewater we performed an environmental survey around the pigs tenure in Conakry wastewater. We thus found a single water sample close to a pig enclosure containing trace of Paslahepevirus genotype 3c as found previously but a substantial proportion of samples (35/135, ≈26%) were positive by specific RT-PCR and confirmed by NGS as *Rocahepevirus ratti* (HEV-C1), with no concurrent detection of Paslahepevirus HEV-3 in the same effluents.

Finally, to assess the risk of circulation in Humans by targeting a population of pregnant women in Conakry representative of the general population, we found a low level of specific IgG seroprevalence (2.4%), absence of IgM, and no detectable HEV RNA in the 206 sera analyzed.

Integrated Interpretation and Critical Discussion

Pig infection data versus Environmental Data: Two Coexisting Reservoirs

The data indicated a coexistence of porcine serological signals (past exposure to HEV-3) and environmental contamination predominantly by HEV-C1 (rodents) within the same geographic area. This pattern suggested that multiple parallel epidemiological cycles may coexist:

> Pig / Paslahepevirus (HEV-3c) cycle: evidenced by the presence of antibodies and occasional molecular detections (HEV-3c) in feces and wastewater.

Rodent → environment (Rocahepevirus HEV-C1) cycle: documented by frequent HEV-C1 RNA detection in urban wastewater.

These two cycles do not preclude interactions (cross-contamination of environments, co-contamination of water sources), but they have distinct primary reservoirs, implying different potential intervention strategies. Although these two cycles appear distinct, environmental overlap (wastewater sites, drainage systems) could facilitate indirect exchanges of viral particles between reservoirs. This dual circulation suggests that Guinea might represent a natural interface between zoonotic (HEV-3) and rodent-associated (HEV-C1) *hepeviruses*.

Methodological Implications and Diagnostic Limitations

Molecular target and interpretation of negative porcine samples: The absence of *Paslahepevirus* RNA in 308 fecal samples may reflect truly low circulation at the time of sampling, a short viral shedding window. Concordant results across laboratories (FLI confirmation) support the validity of the negatives, but caution is warranted regarding temporal representativeness.

Antigenic divergence between genera: Commercial serological tests, commonly labeled as "anti-HEV," are mostly based on *Paslahepevirus* antigens (ORF2). They may not efficiently detect antibodies against *Rocahepevirus* (HEV-C)(113). Consequently, the absence of IgG/IgM "HEV" in humans does not exclude exposure to HEV-C1. This has major implications: current human serological surveillance may underestimate exposure to type C hepeviruses.

Zoonotic Risk and Potential Spill-over of Rocahepevirus

HEV-C1 (rodents) has a documented zoonotic potential in the literature, sporadic human cases reported in Asia(114) and Europe(115). The detection of HEV-C1 in ≈26% of urban wastewater samples in Conakry suggests widespread environmental contamination linked to the rodent population and potentially to hotspots where rat density and human contact are high.

Although human HEV-C infections remain rare and likely underdiagnosed, the significant presence of environmental RNA warrants risk assessment for workers in wastewater treatment plants, sanitation personnel, people in contact with sewage, and populations living near landfills and markets.

The detection of HEV-C1 without concurrent HEV-3 in these effluents reinforces the hypothesis of a dominant rodent reservoir for urban environmental contamination, rather than porcine-origin contamination in this specific setting.

Implications for Human Epidemiology and Blood Safety

Although HEV was not detected in humans, this does not imply absence of exposure, particularly to HEV-C1 which may escape standard assays. However, environmental HEV-C1 circulation may go undetected by standard assays, and atypical or undiagnosed human infections could be missed.

Regarding blood safety, most policies focus on *Paslahepevirus* (HEV-3); the risk related to HEV-C1 remains poorly understood but warrants evaluation, including the rare possibility of transmission through blood products.

These findings underline that the absence of HEV detection in humans does not imply the absence of risk. Integrating HEV-C1 into environmental and blood safety surveillance would strengthen Guinea's preparedness for emerging zoonoses.

Comparison with African and International Studies

Africa: Several West African countries report HEV-3 in pigs with high porcine seroprevalence in Ghana(99) and Cameroon(88).

In West Africa, where HEV surveillance remains fragmented, the Guinean findings provide one of the few integrative datasets linking animal, environmental and human data under a One Health framework. The detection of HEV-3c in Guinea aligns with these regional observations. Environmental detection of *Hepeviruses* (including HEV-3) has also been reported sporadically in Egypt(116), but the predominance of HEV-C1 in urban environments is less commonly documented in African literature, only found and genotyped in Cameroun recently(12), making the Guinean data notable.

Global context: In Europe and Asia, HEV-3 presence in pigs and wastewater is well documented(117); case series of human infections linked to HEV-C1 (rat HEV) have also been described, confirming the possible zoonotic nature of the *Rocahepevirus* genus(118).

Operational Recommendations (One Health Priorities)

Expanded and Targeted Molecular Surveillance

Integrate PCR panels specific for both *Paslahepevirus* and *Rocahepevirus* (HEV-C) in veterinary, human, and environmental surveillance. Implement longitudinal monitoring of wastewater (fixed sites) to track the temporal dynamics of HEV-C1 and establish correlations with public health events.

Rodent Surveillance

surveillance in these hotspots to confirm reservoirs can help us to estimate animal prevalence, and sequence genomes of HEV to assess phylogenetic proximity to known human strains. Furthermore, given that rodents are known reservoirs for several other zoonotic pathogens such as Hantaviruses, Lassa virus, and Leptospira spp., integrated surveillance would provide valuable insights into broader One Health risks associated with rodent populations.

In Guinea, multiple studies have demonstrated that the same peri-domestic rodent species, particularly Mastomys natalensis, Rattus rattus, and Mus musculus, can simultaneously carry a

variety of zoonotic agents. These include arenaviruses (e.g., Lassa)(119), bunyaviruses (e.g., Hantavirus)(120), and bacterial pathogens like Leptospira interrogans(121). The circulation of these microorganisms in shared ecological origin increases the risk of multiple pathogen shedding to humans, especially in urban town such as Conakry.

Thus, broadening the investigation beyond hepatitis E to include molecular screening for Hantavirus , Arenavirus and Leptospira would enhance understanding of the pathogen diversity circulating in rodent reservoirs. All this could help identify potential co-infection dynamics and help to prevent future zoonotic outbreaks.

Adaptation of Serological Diagnostics

Evaluate cross-reactivity of standard anti-HEV ELISAs against HEV-C antibodies; develop or apply HEV-C-specific serological tests if necessary (for human and occupational prevalence studies).

Prevention and Protection Measures

Strengthen protection for workers exposed to wastewater and waste management (PPE, vaccination if available in the future). Urban hygiene actions: integrated rodent control, improved sanitation, and wastewater treatment.

Research and Communication

Prioritize full-genome sequencing of local HEV-C1 and HEV-3 strains to monitor evolution and search for possible recombination or adaptation events. Inform public health authorities and veterinary services about the detection of HEV-C1 in the environment to structure a coordinated One Health response.

These studies provide the first integrated evidence of HEV circulation in Guinea, combining zoonotic (HEV-3c) and rodent-associated (HEV-C1) cycles. These results highlight the need for continuous, multi-sectoral surveillance encompassing humans, animals, and the environment, in line with the One Health principles. Strengthening diagnostic capacities and genomic monitoring will be essential to anticipate and mitigate future hepatitis E outbreaks in the region

PART V: General Conclusion of Thesis

This thesis makes a significant contribution to understanding the epidemiology of Hepatitis E Virus (HEV) in Guinea through an integrated One Health approach linking animal, environmental, and human compartments. The results reveal a complex, multi-level epidemiological situation characterized by silent viral circulation in the environment, past porcine exposure without active infection, and the absence of recent human infection among the studied populations.

In pigs, observed seroprevalence indicates substantial prior exposure, although molecular detection revealed low or intermittent viral activity. These findings suggest that the pig cycle, dominated by the zoonotic HEV-3 genotype, exists but is minimally active at the time of the study. Concurrently, the detection of HEV-3c RNA in a few pig samples demonstrates viral circulation in specific localities, confirming the presence of a local animal reservoir.

In the environmental compartment, the widespread detection of *Rocahepevirus ratti* (HEV-C1) in Conakry wastewater indicates rodent-derived fecal contamination, previously undocumented in Guinea. This finding highlights the importance of rodents as emerging reservoirs of HEV-related viruses and suggests the coexistence of parallel ecological cycles between *Paslahepevirus* and *Rocahepevirus*. The identification of zoonotic-potential HEV-C1 justifies the implementation of targeted molecular surveillance and integrated urban rodent control measures.

Among pregnant women, low IgG seroprevalence (2.4%) and the absence of IgM or viral RNA indicate limited exposure and no active infection at the time of the study. These data contrast with observations from other West African regions, where higher prevalence and outbreaks have been reported. Nevertheless, the high proportion of immunologically naive individuals suggests potential vulnerability in the event of viral resurgence.

The integration of these findings demonstrates that Guinea is currently in a phase of low-level viral circulation, characterized by persistent environmental contamination without marked clinical expression or substantial animal transmission. However, the co-occurrence of HEV-3c and HEV-C1 underscores the need to strengthen epidemiological surveillance and research on inter-reservoir interactions.

This thesis advocates strengthening national capacities in three key areas:

1. Integrated molecular and serological surveillance of human, porcine, rodent, and environmental populations, including diagnostic tools specific to different *Hepevirus* genera.
2. Improvement of sanitation infrastructure and rodent control to limit contamination of wastewater and food products.
3. Incorporation of HEV screening in prenatal care and blood transfusion policies as part of a proactive public health strategy.

In conclusion, the results of this research demonstrate that HEV circulation in Guinea is both silent and multifaceted, involving multiple reservoirs and potential transmission routes. The One Health approach applied here provides a solid scientific framework for establishing sustainable surveillance, improving understanding of eco-epidemiological determinants, and effectively preventing emerging viral zoonoses in the country.

PART VI : Bibliography

1. Amar N, Bendall R, Legrand-Abravanel F, Xia N, Shao S, Ijaz S, Izopet J, et al. Hepatitis E. *Lancet*. 2012;379(9835):2477–88.
2. Uchida T. Hepatitis E. *Ryōikibetsu shōkōgun shirīzu*. 1995:17–9. https://www.who.int/news-room/fact-sheets/detail/hepatitis-e
3. Bagulo H, Majekodunmi AO, Welburn SC. Hepatitis E in sub-Saharan Africa – a significant emerging disease. *One Health*. 2020;11:100186.
4. Pilly IECN. Hépatites virales. *Revue médicale*. 2020;(163):1–10.
5. Dagnew M, Belachew A, Tiruneh M, Moges F. Hepatitis E virus infection among pregnant women in Africa: systematic review and meta-analysis. *BMC Infect Dis*. 2019;19(1):1–14.
6. Doukoure B. Epidemiological situation of hepatitis E in Africa. Virologie 2025;29(5):1–14. doi: 10.1684/vir.2025.1112
7. Vernier L, Lenglet A, Hogema BM, Moussa AM, Ariti C, Vollmer S, et al. Seroprevalence and risk factors of recent infection with hepatitis E virus during an acute outbreak in Chad, 2017. *BMC Infect Dis*. 2018;18(1):1–10.
8. Ostankova YV, Shchemelev AN, Boumbaly S, Baldé TAL, Zueva EB, Valutite DE, et al. Prevalence of HIV and viral hepatitis markers among healthcare workers in Guinea. *Diagnostics (Basel)*. 2023;13(4):1–11.
9. Velavan TP, Pallerla SR, Johne R, Todt D, Steinmann E, Schemmerer M, et al. Hepatitis E: an update on One Health and clinical medicine. *Liver Int*. 2021;41(7):1462–73.
10. Lebov J, Grieger K, Womack D, Zaccaro D, Whitehead N, Kowalcyk B, et al. A framework for One Health research. *One Health*. 2017;3:44–50.
11. Casares-Jimenez M, Garcia-Garcia T, Suarez-Cardenas JM, Perez-Jimenez AB, Martin MA, Caballero-Gomez J, et al. Correlation of hepatitis E and rat hepatitis E viruses in urban wastewater monitoring and clinical cases. *Sci Total Environ*. 2024;908:167912.
12. 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(8):e0322765.
13. Tene SD, Diouara AAM, Sané S, Coundoul S. Hepatitis E virus infection in the context of the One Health approach: a systematic review. *Pathogens*. 2025;14(7):1–26.
14. Purdy MA, Harrison TJ, Jameel S, Meng XJ, Okamoto H, Van der Poel WHM, et al. ICTV virus taxonomy profile: Hepeviridae. *J Gen Virol*. 2017;98(11):2645–6.
15. Kenney SP, Meng XJ. Hepatitis E virus genome structure and replication strategy. *Cold Spring Harb Perspect Med*. 2019;9(1):a031815.

16. Primadharsini PP, Nagashima S, Okamoto H. Genetic variability and evolution of hepatitis E virus. *Viruses*. 2019;11(5):456.

17. Sridhar S, Yip CCY, Wu S, Cai J, Zhang AJX, Leung KH, et al. Rat hepatitis E virus as cause of persistent hepatitis after liver transplant. *Emerg Infect Dis*. 2018;24(12):2241–50.

18. De Paula VS, Wiele M, Mbunkah AH, Daniel AM, Kingsley MT, Schmidt-Chanasit J. Hepatitis E virus genotype 3 strains in domestic pigs, Cameroon. *Emerg Infect Dis*. 2013;19(4):666–8.

19. Nagashima S, Takahashi M, Kobayashi T, Nishizawa T, Nishiyama T, Primadharsini PP, et al. Characterization of the quasi-enveloped hepatitis E virus particles released by the exosomal pathway. *J Virol*. 2017;91(22):e00822–17.

20. Wang B, Meng XJ. Structural and molecular biology of hepatitis E virus. *Comput Struct Biotechnol J*. 2021;19:1907–16.

21. Horvatits T, Wißmann JE, Johne R, Groschup MH, Gadicherla AK, Schulze zur Wiesch J, et al. Hepatitis E virus persists in the ejaculate of chronically infected men. *J Hepatol*. 2021;75(1):55–63.

22. Panda SK, Varma SPK. Hepatitis E: molecular virology and pathogenesis. *J Clin Exp Hepatol*. 2013;3(2):114–24.

23. Pallerla SR, Harms D, Johne R, Todt D, Steinmann E, Schemmerer M, et al. Hepatitis E virus infection: circulation, molecular epidemiology, and impact on global health. *Pathogens*. 2020;9(10):856.

24. Ledesma R, Nimgaonkar I, Ploss A. Hepatitis E virus replication. *Viruses*. 2019;11(8):710.

25. Kenney SP, Meng XJ. Hepatitis E virus genome structure and replication strategy. *Cold Spring Harb Perspect Med*. 2019;9(1):a031815.

26. Mehnert AK, Stegmaier S, Ramirez C, Magalhães VG, Siebenkotten C, Hu J, et al. The hepatitis E virus capsid protein ORF2 counteracts antiviral responses to enable persistence in hepatocytes. *bioRxiv*. 2025;2025.02.03.636239.

27. Nair VP, Anang S, Subramani C, Madhvi A, Bakshi K, Srivastava A, et al. Endoplasmic reticulum stress induced synthesis of a novel viral factor mediates efficient replication of genotype-1 hepatitis E virus. *PLoS Pathog*. 2016;12(6):e1005713.

28. Yadav KK, Boley PA, Fritts Z, Kenney SP. Ectopic expression of genotype 1 hepatitis E virus ORF4 increases genotype 3 HEV viral replication in cell culture. *Viruses*. 2021;13(1):80.

29. Fabrizio C, Bavaro DF, Scudeller L, Lepore L, Balena F, Lagioia A, et al. Variability of HIV-1 V2 env domain for integrin binding: clinical correlates. *Virology*. 2019;535:266–71.

30. Wissing MH, Brüggemann Y, Steinmann E, Todt D. Virus–host cell interplay during hepatitis E virus infection. *Trends Microbiol*. 2021;29(4):309–19.

31. Fu R, Jordan P, Engels Z, Weihs JA, Mürle J, Chi H, et al. Integrin beta 1 facilitates non-enveloped hepatitis E virus cell entry through the recycling endosome. *Nat Commun*. 2025;16(1):1–18.

32. Feng X. Hepatitis C infection: a review. *Lippincotts Prim Care Pract*. 1999;3(3):345–53.

33. Damiris K, Meybodi MA, Niazi M, Pyrsopoulos N. Hepatitis E in immunocompromised individuals. *World J Hepatol*. 2022;14(3):482–94.

34. Rutherford AE. Overview of acute viral hepatitis. *Merck Manual Prof Ed*. 2015. Available from: https://www.merckmanuals.com/professional/hepatic-and-biliary-disorders/hepatitis/overview-of-acute-viral-hepatitis

35. Chaudhry SA, Verma N, Koren G. Hepatitis E infection during pregnancy. *Can Fam Physician*. 2015;61(7):607–8.

36. Pischke S, Hartl J, Pas SD, Lohse AW, Jacobs BC, Van der Eijk AA. Hepatitis E virus infection beyond the liver. *J Hepatol*. 2016;65(5):899–902.

37. Wahid A, Meyer N, Wundes C, Hüffner L, Janshoff S, Frericks N. Extrahepatic replication and genomic signatures of the hepatitis E virus in the kidney. *Front Microbiol*. 2025;16(2):1–12.

38. López-López P, Frias M, Camacho A, Machuca I, Caballero-Gómez J, Risalde MA, et al. Seroreversion of IgG anti-HEV in HIV cirrhotic patients: a long-term longitudinal study. *Transbound Emerg Dis*. 2022;69(5):e1541–8.

39. Huang X, Lu J, Liao M, Huang Y, Wu T, Xia N. Progress and challenges to hepatitis E vaccine development and deployment. *Vaccines*. 2024;12(7):773.

40. Diagnosis of Hepatitis E Virus Infection: Current Capabilities and Shortcomings. :1–17.

41. Lhomme S, Marion O, Abravanel F, Chapuy-Regaud S, Kamar N, Izopet J. Diagnosis of hepatitis E virus infection: current capabilities and shortcomings. *Viruses*. 2024;16(1):1–17.

41. Lhomme S, Marion O, Abravanel F, Chapuy-Regaud S, Kamar N, Izopet J. Hepatitis E pathogenesis. *Viruses*. 2016;8(8):1–18.

42. Kamar N, Izopet J, Pavio N, Aggarwal R, Labrique A, Wedemeyer H, et al. Hepatitis E virus infection. *Nat Rev Dis Primers*. 2017;3:17086.

43. Smith DB, Simmonds P, Izopet J, Oliveira-Filho EF, Ulrich RG, Johne R, et al. Proposed reference sequences for hepatitis E virus subtypes. *J Gen Virol*.

2016;97(3):537–42.

44. Khuroo MS, Khuroo MS, Khuroo NS. Hepatitis E: discovery, global impact, control and cure. *World J Gastroenterol*. 2016;22(31):7030–45.

45. StatPearls. Hepatitis E. *NCBI Bookshelf*. 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK459141/

46. Song YJ. Studies of hepatitis E virus genotypes. *Indian J Med Res*. 2010;132:487–8.

47. Bartholomeusz A, Locarnini S. Hepatitis viruses associated with antiviral therapy. *Antivir Ther*. 2006;11(5):567–77.

48. Lee GH, Tan BH, Teo ECY, Lim SG, Dan YY, Wee A, et al. Chronic infection with camelid hepatitis E virus in a liver transplant recipient who regularly consumes camel meat and milk. *Gastroenterology*. 2016;150(2):355–7.

49. Kim JH, Nelson KE, Panzner U, Kasture Y, Labrique AB, Wierzba TF. A systematic review of the epidemiology of hepatitis E virus in Africa. *BMC Infect Dis*. 2014;14(1):308.

50. Azman AS, Bouhenia M, Iyer AS, Rumunu J, Laku RL, Wamala JF, et al. High hepatitis E seroprevalence among displaced persons in South Sudan. *Am J Trop Med Hyg*. 2017;96(6):1296–301.

51. Lagare A, Ibrahim A, Ousmane S, Issaka B, Zaneidou M, Kadadé G, et al. Outbreak of hepatitis E virus infection in displaced persons camps in Diffa region, Niger, 2017. *Am J Trop Med Hyg*. 2018;99(4):1055–7.

52. Owolodun OA, Gerber PF, Giménez-Lirola LG, Kwaga JKP, Opriessnig T. First report of hepatitis E virus circulation in domestic pigs in Nigeria. *Am J Trop Med Hyg*. 2014;91(4):699–704.

53. Bouscaillou J, Komas N, Tricou V, Nakouné E, Sélékon B, Fontanet A, et al. Imported hepatitis E virus, Central African Republic, 2011. *Emerg Infect Dis*. 2013;19(2):335–7.

54. Sadio BD, Faye M, Kaiser M, Diarra M, Balique F, Diagne CT, et al. First hepatitis E outbreak in southeastern Senegal. *Sci Rep*. 2022;12(1):1–10.

55. Behloul N, Zhang M, Meng J. Binding preference of anti-HEV antibodies in sera collected in Algeria for antigens derived from HEV genotype 1. *Hepat Mon*. 2016;16(8):e39263.

56. Ouoba JB, Traore KA, Rouamba H, Setondji KVM, Minoungou GL, Ouoba BL, et al. Prevalence of anti-hepatitis E virus antibodies in domestic animals from Burkina Faso. *Vet Anim Sci*. 2019;7:100059.

57. Houcine N, Jacques R, Salma F, Lyon D, Nord V. Seroprevalence of hepatitis E virus infection in rural and urban populations. *J Med Virol*. 2012;84(9):1483–8.

58. Andersson MI, Stead PA, Maponga T, Van der Plas H, Preiser W. Hepatitis E virus infection: an underdiagnosed infection in transplant patients in Southern Africa? *J Clin Virol*. 2015;70:23–5.

59. Mbachu CNP, Ebenebe JC, Okpara HC, Chukwuka JO, Mbachu II, Elo-Ilo JC, et al. Hepatitis E prevalence, knowledge, and preventive practices among secondary school adolescents in rural Nigeria: a cross-sectional study. *BMC Public Health*. 2021;21(1):1–8.

60. Ababa A, Abebe M, Ali I, Ayele S, Overbo J, Aseffa A, et al. Seroprevalence and risk factors of hepatitis E virus infection among pregnant women in Ethiopia. *PLoS One*. 2017;12(6):e0179026.

61. Hannachi N, Hidar S, Harrabi I, Mhalla S, Marzouk M, Ghzel H, et al. Séroprévalence et facteurs de risque de l'hépatite virale E chez la femme enceinte dans le centre tunisien. *Pathol Biol*. 2011;59(5):e115–8.

62. Obiri-Yeboah D, Awuku YA, Adu J, Pappoe F, Obboh E, Nsiah P, et al. Seroprevalence and risk factors for hepatitis E virus infection among pregnant women in the Cape Coast Metropolis, Ghana. *PLoS One*. 2018;13(1):e0191685.

63. Diouara AAM, Lo S, Nguer CM, Senghor A, Diop Ndiaye H, Manga NM, et al. Hepatitis E virus seroprevalence and associated risk factors in pregnant women attending antenatal consultations in Senegal. *Viruses*. 2022;14(8):1782.

64. Simani OE, Seipone TP, Selabe G, Seheri LM, Mphahlele MJ, Mayaphi SH, et al. Low seroprevalence of hepatitis E virus in pregnant women in an urban area near Pretoria, South Africa. *IJID Reg*. 2022;2:70–3.

65. De Paschale M, Ceriani C, Romanò L, Cerulli T, Cagnin D, Cavallari S, et al. Epidemiology of hepatitis E virus infection during pregnancy in Benin. *Trop Med Int Health*. 2016;21(1):108–13.

66. Traoré KA, Ouoba JB, Huot N, Rogée S, Dumarest M, Traoré AS, et al. Hepatitis E virus exposure is increased in pork butchers from Burkina Faso. *Am J Trop Med Hyg*. 2015;93(6):1356–9.

67. Ukuli AQ, Mugimba KK. Seroprevalence of hepatitis E in swine abattoir workers. *Afr Health Sci*. 2017;17(4):1022–8.

68. Temmam S, Besnard L, Andriamandimby SF, Foray C, Rasamoelina-Andriamanivo H, Héraud JM, et al. High prevalence of hepatitis E in humans and pigs and evidence of genotype 3 virus in swine, Madagascar. *Am J Trop Med Hyg*. 2013;88(2):329–38.

69. Bagulo H, Majekodunmi AO, Welburn SC, Bimi L. Hepatitis E seroprevalence and risk factors in humans and pigs in Ghana. *BMC Infect Dis*. 2022;22(1):1–12.

70. 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 infection in southwestern Nigeria: a prospective cohort study. *Front Microbiol*. 2023;14:1–8.

71. Elhendawy M, Abo-Ali L, Abd-Elsalam S, Hagras MM, Kabbash I, Mansour L, et al. HCV and HEV: two players in an Egyptian village, a study of prevalence, incidence, and co-infection. *Environ Sci Pollut Res*. 2020;27(27):33659–67.

72. Maphumulo SS, Goedhals D, Van Rooyen C, Vawda S. High seroprevalence of hepatitis E virus in the Free State province of South Africa. *S Afr J Infect Dis*. 2024;39(1):1–4.

73. Gerbi GB, Williams R, Bakamutumaho B, Liu S, Downing R, Drobeniuc J, et al. Hepatitis E as a cause of acute jaundice syndrome in northern Uganda, 2010–2012. *Am J Trop Med Hyg*. 2015;92(2):411–4.

74. Twagirumugabe T, Saguti F, Habarurema S, Gahutu JB, Bergström T, Norder H. Hepatitis A and E virus infections have different epidemiological patterns in Rwanda. *Int J Infect Dis*. 2019;86:12–4.

75. al. R et. 乳鼠心肌提取 HHS Public Access. Physiol Behav. 2018;176(5):139–48.

76. Boon D, Redd AD, Laeyendecker O, Engle RE, Nguyen H, Ocama P, et al. Hepatitis E virus seroprevalence and correlates of anti-HEV IgG antibodies in the Rakai District, Uganda. *J Infect Dis*. 2018;217(5):785–9.

77. Harritshøj LH, Theilgaard ZP, Mannheimer E, Midgley SE, Chiduo M, Ullum H, et al. Hepatitis E virus epidemiology among HIV-infected women in an urban area in Tanzania. *Int J Infect Dis*. 2018;73:7–9.

78. Makiala-Mandanda S, Le Gal F, Ngwaka-Matsung N, Ahuka-Mundeke S, Onanga R, Bivigou-Mboumba B, et al. High prevalence and diversity of hepatitis viruses in suspected yellow fever cases in the Democratic Republic of Congo. *J Clin Microbiol*. 2017;55(5):1299–312.

79. Tricou V, Bouscaillou J, Laghoe-Nguembe GL, Béré A, Konamna X, Sélékon B, et al. Hepatitis E virus outbreak associated with rainfall in the Central African Republic, 2008–2009. *BMC Infect Dis*. 2020;20(1):1–7.

80. De Marguerite Nombot-Yazenguet MP, Doté JW, Koyaweda GW, Zemingui-Bembete PA, Selekon B, Vickos U, et al. Hepatitis E outbreak in Bocaranga-Koui, Central African Republic, 2018–2019. *BMC Infect Dis*. 2024;24(1):1–10.

81. Dimeglio C, Kania D, Mantono JM, Kagoné T, Zida S, Tassembedo S, et al. Hepatitis E virus infections among patients with acute febrile jaundice in Burkina Faso. *Viruses*. 2019;11(6):1–8.

82. Modiyinji AF, Tankeu LTA, Monamele CG, Moumbeket MHY, Ngoupo PAT, Simo HT, et al. Hepatitis E virus infections among patients with acute febrile jaundice in two regions of Cameroon: First molecular characterization of hepatitis E virus genotype 4. PLoS One. 2024;19(2):1–12.

83. Akanbi OA, Oluremi AS, Japhet MO, Osundare FA, Klink P, Harms D, et al. Prevalence of Hepatitis E genotype 3 among liver disease patients in Southwestern Nigeria. 2025.

84. Tialla D, Cissé A, Ouédraogo GA, Hübschen JM, Tarnagda Z, Snoeck CJ. Prevalence of hepatitis E virus antibodies in cattle in Burkina Faso associated with swine mixed farming. J Vet Sci. 2022;23(3):1–10.

85. Chambaro HM, Sasaki M, Muleya W, Kajihara M, Shawa M, Mwape KE, et al. Hepatitis E virus infection in pigs: a first report from Zambia. Emerg Microbes Infect. 2021;10(1):2169–72.

86. Doukouré B, Pennec YL, Troupin C, Grayo S, Eiden M, Groschup MH, et al. Seroprevalence and phylogenetic characterization of Hepatitis E Virus (Paslahepevirus balayani) in Guinean pig population. 2024;24(8):540–5.

87. Modiyinji AF, Atsama MA, Monamele GC, Nola M, Njouom R. High seroprevalence of hepatitis E among pigs suggests an animal reservoir in Cameroon. J Infect Dev Ctries. 2018;12(8):676–9.

88. Modiyinji AF, Sanding GMAM, Atsama MA, Monamele CG, Nola M, Njouom R. Serological and molecular investigation of hepatitis E virus in pig reservoirs from Cameroon reveals elevated seroprevalence and presence of genotype 3. PLoS One. 2020;15(2):e0229073.

89. Hospital M, Ikeja A. Molecular detection of hepatitis E virus among swine and poultry birds in Lagos, Nigeria. 2024;25(2):201–9.

90. Bari FD, Wodaje HB, Said U, Waktole H, Sombo M, Leta S, et al. First molecular detection of hepatitis E virus genome in camel and pig faecal samples in Ethiopia. Virol J. 2021;18(1):160.

91. De Marguerite Nombot-Yazenguet MP, Modiyinji AF, Tricou V, Manirakiza A, Njouom R, Komas NPJ. Investigating animal reservoirs for hepatitis E virus in Bangui, Central African Republic. PLoS One. 2024;19(3):1–10.

92. Aubry P. Hépatites virales en zones tropicales. Actualités Médecine Trop. 2013;1–8.

93. El-Adly AM. Serological and genetic diversity of hepatitis E virus among rabbits population in Egypt. Open Vet J. 2023;13(5):515–22.

94. Sayed IM, Hammam ARA, Elfaruk MS, Alsaleem KA, Gaber MA, Ezzat AA, et al. Enhancement of the molecular and serological assessment of hepatitis E virus in milk samples. Microorganisms. 2020;8(8):1–14.

95. Tene SD, Diouara AAM, Kane A, Sané S, Coundoul S, Thiam F, et al. Detection of Hepatitis E Virus (HEV) in pork sold in Saint-Louis, the North of Senegal. Life. 2024;14(4):1–12.

96. Korsman S, Bloemberg J, Brombacher M, Giuricich A, Halley-Stott RP, Kaba M. Hepatitis E in pig-derived food products in Cape Town, South Africa, 2014. S Afr Med J. 2019;109(8):584–6.

97. Chauhan RP, Gordon ML. Characterization of a near full-length Hepatitis E virus genome of subtype 3c generated from naturally infected South African backyard pigs. Pathogens. 2022;11(9).

98. Suluku R, Jabaty J, Fischer K, Diederich S, Groschup MH, Eiden M. Hepatitis E seroprevalence and detection of genotype 3 strains in domestic pigs from Sierra Leone collected in 2016 and 2017. 2024;1–8.

99. El-Duah P, Dei D, Binger T, Sylverken A, Wollny R, Tasiame W, et al. Detection and genomic characterization of hepatitis E virus genotype 3 from pigs in Ghana, Africa. One Heal Outlook. 2020;2(1).

100. Sayed IM, Elkhawaga AA, El-Mokhtar MA. Circulation of hepatitis E virus (HEV) and/or HEV-like agent in non-mixed dairy farms could represent a potential source of infection for Egyptian people. Int J Food Microbiol. 2020;317:108479.

101. Africa E, Emirates UA, Rasche A, Saqib M, Liljander AM, Bornstein S, et al. Hepatitis E virus infection in dromedaries, North and East Africa, United Arab Emirates, and Pakistan, 1983–2015. Emerg Infect Dis. 2016;22(7):1249–52.

102. Osamudiamen FT, Akanbi OA, Zander S, Oluwayelu DO, Bock CT, Klink P. Identification of a putative novel genotype of avian hepatitis E virus from apparently healthy chickens in southwestern Nigeria. Viruses. 2021;13(6).

103. Salines M, Demange A, Stéphant G, Renson P, Bourry O, Andraud M, et al. Persistent viremia and presence of hepatitis E virus RNA in pig muscle meat after experimental co-infection with porcine reproductive and respiratory syndrome virus. Int J Food Microbiol. 2019;292:144–9.

104. Salines M, Dumarest M, Andraud M, Mahé S, Barnaud E, Cineux M, et al. Natural viral co-infections in pig herds affect hepatitis E virus (HEV) infection dynamics and increase the risk of contaminated livers at slaughter. Transbound Emerg Dis. 2019;66(5):1930–45.

105. Salemane K, Coetzee LZ, Pocock G, Genthe B, Taylor MB, Mans J. Water-based epidemiological investigation of hepatitis E virus in South Africa. Food Environ Virol. 2024;(0123456789).

106. Béji-Hamza A, Hassine-Zaafrane M, Khélifi-Gharbi H, Della Libera S, Iaconelli M, Muscillo M, et al. Hepatitis E virus genotypes 1 and 3 in wastewater samples in Tunisia. Arch Virol. 2015;160(1):183–9.

107. Dalton HR, Kamar N, Baylis SA, Moradpour D, Wedemeyer H, Negro F. EASL clinical practice guidelines on hepatitis E virus infection. J Hepatol. 2018;68(6):1256–71.

108. Kim SB, Agampodi S, Saluja T, Sahastrabuddhe S, Kim HS, Kim JH, et al. Hepatitis E vaccine landscape update 2025. Expert Rev Vaccines. 2025;24(1):1–16.

109. Faber MS, Jilg W, Thamm M, Stark K. Hepatitis E virus seroprevalence among adults, Germany. Emerg Infect Dis. 2012;18:0–3.

110. Maity S, Chowdhary S, Swaminathan A, Ashtaputre N, Paul P. The silent spread of hepatitis E in India: from epidemiological insight to public health action. 2025.

111. Ehi Airiohuodion P, Wartel A, Yako AB, Mac PA. Seroprevalence and burden of hepatitis E viral infection among pregnant women in central Nigeria attending antenatal clinic at a federal medical centre. Front Med. 2022;9:1–11.

112. Huy PX, Chung DT, Linh DT, Hang NT, Rachakonda S, Pallerla SR, et al. Low prevalence of HEV infection and no associated risk of HEV transmission from mother to child among pregnant women in Vietnam. Pathogens. 2021;10(10).

113. 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(1):1–8.

114. amaguchi J, Orf GS, Perez LJ, Phoompoung P, Chirakarnjanakorn S, Suputtamongkol Y, et al. First evidence of spillover of Rocahepevirus ratti into humans in Thailand. Transbound Emerg Dis. 2025;2025(1).

115. Panajotov J, Schilling-Loeffler K, Mehl C, Meneghini D, Fickel J, Beyer J, et al. Long-term circulation and molecular evolution of rat hepatitis E virus in wild Norway rat populations from Berlin, Germany. Infect Genet Evol. 2025;135:October.

116. Kamel AH, Ali MA, El-Nady HG, Deraz A, Aho S, Pothier P, et al. Presence of enteric hepatitis viruses in the sewage and population of Greater Cairo. Clin Microbiol Infect. 2011;17(8):1182–5.

117. Wang H, Churqui MP, Taslimi S, Tunovic T, Andius LD, Lagging M, et al. Distinct distribution of HEV-3 subtypes across humans, animals, and environmental waters in Sweden. Emerg Microbes Infect. 2025;14(1):1–9.

118. Jiang HJ, Underwood TC, Bell JG, Ranjan S, Sasselov D. Mimicking lighting-induced electrochemistry on the early Earth. Proc Natl Acad Sci. 2017;120.

119. Karan L, Makenov M, Korneev M, Sacko N, Boumbaly S, Bayandin R, et al. Lassa virus in the host rodent Mastomys natalensis within urban areas of N'zerekore, Guinea. bioRxiv. 2019;616466.

120. Klempa B, Fichet-Calvet E, Lecompte E, Auste B, Aniskin V, Meisel H, et al. Hantavirus in African wood mouse, Guinea. Emerg Infect Dis. 2006;12(5):838–40.

121. Pricemou S, Soropogui B, Bérété F, Beavogui MB, Samoura A, Picardeau M, et al. Diversity of Leptospira species and their rodent reservoirs in the Guinean forest. Microorganisms. 2025;13(4):1–11.

PART VII : Appendix

A- RNA extraction protocol

Description
This procedure describes how to perform RNA extraction using the QIAMP kit from QIAGEN.
Target: serum, plasma, saliva or nasopharyngeal samples in VTM, CSF, acellular body fluids and cell culture supernatants. NOT for whole blood.
Responsibilities
Persons authorised to use the kit and work in L2.
Equipment and consumables
Equipment
PSM Type II HERASAFE KS15, n°série 42199122, Thermo Scientific
Micropipet (1000µL, 200µL, 20µL,10µL)
Pipetboy
Microcentrifuge Sorvall Legend Micro 17, n°série 42216316, Thermo Scientific
Vortex REAX 2000 Heidolph N°54119, n°série 16937
Fisherbrand™ Mini Vortex Mixer, 2800 RPM 115V
Consumables
Personal protective equipment
Sterile filter tips (1000µL, 200µL, 20µL,10µL)
Serological Pipets (25mL, 10mL, 5mL)
Tubes Falcon (50mL, 15mL)
Tubes Eppendorf (2mL, 1,5mL)

Kit contents

QIAamp Viral RNA Mini Kit	(50)	(250)
Catalog no.	52904	52906
No. of preps	50	250
QIAamp Mini Spin Columns	50	250
Collection Tubes (2 ml)	150	750
Buffer AVL*	31 ml	155 ml
Buffer AW1* (concentrate)	19 ml	98 ml
Buffer AW2† (concentrate)	13 ml	66 ml
Buffer AVE†	3 x 2 ml	20 ml
Carrier RNA (poly A)	310 µg	1550 µg

* Contains chaotropic salt, which is an irritant. Not compatible with disinfecting reagents that contain bleach. See page 6 for safety information.
† Contains sodium azide as a preservative.

The kit should be stored at room temperature (15–25°C). All reagents should be stored at room temperature once opened. The kit and all reagents can be stored under these conditions until their expiry date.

Preparation of reagents

Reconstitute the carrier RNA / upon opening the kit

Add **310 µl** of AVE buffer to the tube containing **310 µg** of lyophilised carrier RNA to obtain a solution of **1 µg/µl**.

Dissolve the carrier RNA thoroughly, divide it into aliquots of appropriate size, and store at **-30 to -15°C**. Do not freeze and thaw the carrier RNA aliquots more than 3 times.

Prepare the AVL buffer + RNA carrier + internal control

Reagents	Volume for 1 tube	Volume for X tubes
Buffer AVL	0.56mL	…mL
RNA-carrier	5.6µL	…µL
Internal Control *	4µL	…µL

** The addition of internal control is optional.*

Gently mix by inverting the tube 10 times; do not vortex

Preparation of washing reagents

Buffer AW1
It is concentrated. Before using it for the first time, add the volume of ethanol indicated on the box and in the following table:

Kit Cat No	No preps	AW1 Concentrated	Ethanol	Final volume
52904	50	19ml	25ml	44ml
52904	250	98ml	130ml	228ml

Buffer AW2
It is concentrated. Before using it for the first time, add the volume of ethanol indicated on the box and in the following table:

Kit Cat No	No preps	AW2 Concentrated	Ethanol	Final Volume
52904	50	13ml	30ml	43ml
52904	250	66ml	160ml	226ml

AW1 and AW2 buffers are stable for 1 year at room temperature unless the kit expires.
Aliquot the wash buffers (AW1 and AW2) and ethanol

Reagents	Volume for 1 tube	Volume for x tubes
Buffer (AW1 and AW2)	**500µL**	**…µL (…mL)**
Ethanol	**560µL**	**…µL (…mL)**

Protocol

1. Sample lysis

   a. Transfer 560 µL of AVL Buffer + RNA carrier + internal control to a 2 mL tube, then add 140 µL of sample to be extracted (or water for the extraction control). Homogenise the solution thoroughly by inverting and vortexing for 15 seconds.
   If less than 140 µL, add PBS to bring the sample volume to 140 µL.
   b. Incubate for 10 minutes at room temperature inside the laboratory. Centrifuge the tube briefly before opening.
   c. Add 560 µL of alcohol (96–100% ethanol) to each tube to precipitate the RNA. Vortex vigorously for 15 seconds. This will produce the sample lysate. Centrifuge the tube briefly before opening.

2. Purification of the lysate and RNA fixation

   a. Take a mini-column from the QIAamp™ Viral RNA Mini Kit - Identify the column.
   b. Transfer 630 µL of the sample lysate to the column - Plug - Centrifuge at 8000rpm for 1 minute - Discard the collection tube containing the filtrate - Store the column in a clean collection tube.
   c. Transfer the rest of the sample lysate to the same column - Plug - Centrifuge at 8000rpm for 1 minute - Discard the collection tube containing the filtrate - Store the column in a clean collection tube.
   d. Add 500 µL of AW1 buffer (washing buffer 1) to each column - Cap - Centrifuge at 8000rpm for 1 minute - Discard the collection tube containing the filtrate - Store the column in a clean collection tube.
   e. Add 500 µL of AW2 buffer (washing buffer 2) to each column - Plug - Centrifuge at 14,000 rpm for 3 minutes - Discard the collection tube containing the filtrate - Keep the column.

3. RNA elution

   Place the column in a new collection tube and centrifuge at 14,000 rpm for 1 minute (to dry the membrane and remove any alcohol residue) - Discard the collection tube - Keep the column.
   Place the column in a 1.5 mL Eppendorf microtube - Add 60 µL of AVE buffer (water-containing buffer) – Cap and incubate for 1 minute at room temperature.

   Centrifuge at 8000 rpm for 1 minute to elute the RNA. Discard the column. Keep the microtube.
   Storage
   To prevent degradation, RNAs are stored at -20°C if they are to be processed on the same day or the day after extraction, then at -80°C for long-term storage.
   Viral RNA is stable for over a year when stored between -30 and -15°C or between -90 and -65°C.

B-Protocol of RTqPCR (from Jothikumar et al. 2002)

HEV primer and probe sequences:

Forward Primer JVHEVF: 5'-GGTGGTTTCTGGGGTGAC-3'
Reverse Primer JVHEVR : 5'-AGGGGTTGGTTGGATGAA-3'
Probe : JVHEVP : 5'-TGATTCTCAGCCCTTCGC-3'

Primers and probes are supplied in lyophilised tubes, with each tube containing the targets and being 10 times concentrated. They must be diluted to 1/10 to obtain a working solution.
Preparation of working solutions (10 µM):
These solutions will be used to prepare each mix and stored at –20°C.
Mix preparation (Luna Universal Probe One-Step RT-qPCR Kit)

	One reaction for RT qPCR
One step reaction mix	10 µL
RT enzyme mix	1 µL
Primers FW (10µM)	0.8 µL
Primers Rv (10 µM)	0.8 µL
Probe (10 µM)	0.4 µL
Eau nuclease free	7 µL
Total Volume (µL)	20 µL
Matrice	5 µL RNA

Amplification/ CFx Biorad

Program HEV

Step	Cycle	Temperature	Time
RT	1	55°C	10 minutes
qPCR	1	95°C	1 minute
	40	95°C	10 secondes
		53°C	30 secondes
Cooling	1	4°C	∞

Result

The reading is done in FAM (Fluorophore).

C-Protocol of Nested RTPCR (Capsid)

HEV primer and probe sequences (Li et al)
Outer primers
Forward Primer: HEV-34 5' -CCGACGTCYGTYGAYATGAA- 3'
Reverse Primer HEV-36 5'-TTRTCCTGCTGAGCRTTCTC-3'
Inner primers
Forward Primer: HEV-35 5'- AAGTGAGCGCCTACAYTAYCG -3'
Reverse Primer HEV-29 5'-CTCGCCATTGGCTGAGAC-3'

Primers are supplied in lyophilised tubes, with each tube containing the targets and being 10 times concentrated. They must be diluted to 1/10 to obtain a working solution.
Mix preparation (LunaScript Multiplex One-Step RT-PCR Kit)

	One reaction for RT PCR	**One reaction to Nested PCR**
Eau nuclease free	14 µL	16 µL
Luna mix 5X	5 µL	5 µL
Enzyme	1 µL	1 µL
FW (10µM)	1 µL	1 µL
Rv (10 µM)	1 µL	1 µL
Total Volume (µL)	22 µL	24 µL
matrice	3 µL	1 µL

Amplification

Program

Step	Cycle	Temperature	Time	
RT	1	55°C	10 minutes	Only for the First PCR
PCR	1	98°C	1 minute	
	30	98°C	10 secondes	
		54°C	30 secondes	56°C (For nested one)
		72°C	30 secondes	
	1	72°C	5 minutes	
Cooling	1	4°C	∞	

Results

Amplicon size 306 bp with positive control of the same size and negative control without band

D-Protocol of RT PCR (Polymerase)

Primers and probe sequences for HEV (Li et al.)
Outer primers
Forward Primer HEV-38 5'-GAGGCYATGGTSGAGAARG-3'
Reverse Primer HEV-39 5'-GCCATRTTCCAGACRGTRTTCC-3'
Inner primers
Forward Primer: HEV-37 5'-GGTTYCGYGCYATTGARAARG-3'
Reverse Primer HEV-27 5'-TCRCCAGAGTGYTTCTTCC-3'

Mix preparation (LunaScript Multiplex One-Step RT-PCR Kit)

	One reaction for RT PCR	**One reaction to Nested PCR**
Eau nuclease free	14 µL	16 µL
Luna mix 5X	5 µL	5 µL
Enzyme	1 µL	1 µL
FW (10µM)	1 µL	1 µL
Rv (10 µM)	1 µL	1 µL
Total Volume (µL)	22 µL	24 µL
matrice	3 µL	1 µL

Amplification Program

Step	Cycle	Temperature	Time	
RT	1	55°C	10 minutes	Only for the First PCR
PCR	1	98°C	1 minute	
	30	98°C	10 secondes	
		54°C	30 secondes	56°C (For nested one)
		72°C	30 secondes	
	1	72°C	5 minutes	
Cooling	1	4°C	∞	

Result

Amplicon size 306 bp with positive control of the same size and negative control without band

E- Review Article Epidemiology of Hepatitis E in Africa

Revue

Virologie

Virologie 2025, 29 (5) : 1-14

Epidemiological situation of hepatitis E in Africa

Situation épidémiologique de l'hépatite E en Afrique

Bakary Doukouré[1 2],
Noël Tordo[1],
Pierre Roques[1]

[1] Institut Pasteur de Guinée,
Unité de virologie,
BP 4416 Conakry Guinée

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

Article accepted on 22 September 2025

Abstract. Hepatitis E virus (HEV) belongs to the *Hepeviridae* family, *Orthohepevirinae* subfamily, *Paslahepevirus* genus which includes eight genotypes. HEV genotype 1 (HEV-1) and genotype 2 (HEV-2) are specific to humans, while genotype 3 (HEV-3) and genotype 4 (HEV-4) circulate mainly in pigs, wild boars and deer, but have also a zoonotic potential. HEV genotype 5 (HEV-5) and 6 (HEV-6) viruses circulate in wild boars in Japan and genotype 7 (HEV-7) and 8 (HEV-8) viruses circulate in camelids. The worldwide distribution of HEV is influenced by ecological and socioeconomic factors. In developing countries in Africa, transmission of the virus through fecally contaminated water accounts for a high proportion of epidemics. Direct human-to-human transmission is less frequent, although cases of infection through blood transfusion have been reported in several countries. Thanks to the "One Health" approach, zoonotic transmissions of HEV from pig to human have been more recently observed. These zoonotic infections are mainly due to the handling or consumption of pork meat or contact with pig manure, contaminating the environment. They alert on professions or populations at-risk, such as livestock farmers or butchers. In addition, HEV infection is particularly severe in pregnant women, leading to fetal and maternal death due to acute liver failure. Finally, the development and application of serological or molecular detection tests in Africa indicates that HEV can be incriminated in symptoms without etiology or falsely attributed to other hepatic viruses or to the yellow fever virus. This review updates studies on the epidemiology of HEV in Africa, a crucial step to better understand the virus and develop surveillance strategies to prevent and better control epidemics.

Keywords : hepatitis E virus (HEV), zoonosis, pigs, Africa, One Health approach, pregnant women, epidemiology

Résumé. Le virus de l'hépatite E (HEV), appartient à la famille des *Hepeviridae*, sous-famille *Orthohepevirinae*, genre *Paslahepevirus* qui comprend huit génotypes. Le HEV de génotype 1 (HEV-1) et de génotype 2 (HEV-2) est spécifique aux humains, tandis que celui des génotypes 3 (HEV-3) et 4 (HEV-4) circule principalement chez les porcs, les sangliers et les cerfs mais a aussi un potentiel zoonotique. Le HEV des génotypes 5 (HEV-5) et 6 (HEV-6) circule chez les sangliers au Japon et celui des génotypes 7 (HEV-7) et 8 (HEV-8) chez les camélidés. La répartition mondiale du HEV est influencée par des facteurs écologiques et socio-économiques. Dans les pays en développement d'Afrique, la transmission du virus par l'eau contaminée par des matières fécales est responsable d'une forte proportion des épidémies. La transmission interhumaine directe est plus rare, même si des cas d'infections par transfusion sanguine ont été signalés dans plusieurs pays. L'approche « *One Health* » a permis d'identifier plus récemment la transmission du HEV du porc à l'humain. Cette infection zoonotique est

Correspondance : B. Doukouré
<bakary.doukoure@pasteur-guinee.org>

Pour citer cet article : Doukouré B, Tordo N, Roques P. Epidemiological situation of hepatitis E in Africa. *Virologie* 2025; 29(5) : 1-14 doi:10.1684/vir.2025.1112

principalement due à la manipulation/consommation de viande de porc ou au contact avec du lisier de porc dans l'environnement. Elles alertent sur les professions ou les populations à risque comme les éleveurs ou les bouchers. L'infection par le HEV est particulièrement sévère chez la femme enceinte, entraînant des décès fœtaux et maternels par insuffisance hépatique aiguë. Enfin, le développement et l'application de tests de détection sérologique ou moléculaire en Afrique permet d'incriminer le HEV pour des symptomatologies sans étiologie ou faussement attribuées à d'autres virus hépatiques ou au virus de la fièvre jaune. Cette revue met à jour les études sur l'épidémiologie du HEV en Afrique, une étape cruciale pour mieux comprendre le virus et développer des stratégies de surveillance pour prévenir et mieux contrôler les épidémies.

Mots-clés: virus de l'hépatite E (HEV), zoonose, porcs, Afrique, approche *One Health*, femmes enceintes, épidémiologie

Hepatitis E: transmission and etiologic agents

Hepatitis E, first described in India in 1955 [1], has emerged as a serious public health concern. The hepatitis E virus (HEV) is mainly transmitted via the fecal–oral route, often through contaminated water [2,3]. However, the literature also reports zoonotic transmission mechanisms, in both industrialized and resource-limited settings [4]. The disease is particularly prevalent in regions with poor hygiene, limited access to safe drinking water, and inadequate sanitation facilities [3]. Moreover, conflicts exacerbate the situation by combining food insecurity with the absence of drinking water treatment (*figure 1*).

Most HEV infections resolve spontaneously within 2–6 weeks. However, infection can occasionally lead to fulminant hepatitis with acute liver failure, which may be life-threatening. Furthermore, in immunocompromised populations (HIV/AIDS, other liver diseases, cancer, or immunosuppressive therapy following transplantation), chronic forms of HEV infection and disease have been observed [5]. According to the World Health Organization, approximately 20 million HEV infections occur worldwide each year, resulting in 55,000 deaths in 2023. HEV can cause severe forms of hepatitis in pregnant women, particularly during the third trimester, with a mortality rate of up to 20% [6]. Management of HEV-infected pregnant women is essential, but no specific treatment has yet been developed. Ribavirin and interferon-α have been used successfully, alone or in combination, in a few cases [5], Although ribavirin is contraindicated during pregnancy due to its teratogenicity, therapeutic options for immunocompromised patients remain limited. A vaccine against hepatitis E has been developed, but it is currently available only in China [7].

HEV is a positive-sense single-stranded RNA virus of approximately 7.2 kb, initially classified within the Caliciviridae family. Its taxonomy has evolved considerably over the years with the discovery of novel viruses in animal reservoirs, including domestic pigs, wild boars, rabbits, dromedaries, camels, rodents, birds, bats, and even fish [8, 9]. This diversity has led to the establishment of the Hepeviridae family and two subfamilies, according to the latest classification in 2024 (https://ictv.global/taxonomy/) (*figure 2*). The subfamily Parahepevirinae infects fish (Piscihepevirus). The subfamily Orthohepevirinae includes the genera Avihepevirus (birds), Chirohepevirus (bats), Rocahepevirus (rodents), and Paslahepevirus, which are the most studied and comprise eight genotypes, some of which are pathogenic to humans. Genotypes HEV-1 and HEV-2 are exclusively human, while HEV-3, HEV-4, and HEV-7 are zoonotic [7, 10]. These genotypes are found in both humans and animals, primarily in suidae, but also in certain ruminants such as sheep and goats, although evidence for their role as reservoirs remains insufficient [11]. HEV-5 and HEV-6 have been identified in Japanese wild boars, while HEV-7 and HEV-8 have been found in camelids [8]. HEV genotypes and subtypes vary according to host and geographic location. Some studies have linked HEV-1 to severe disease in pregnant women, whereas HEV-3 infections are often asymptomatic or chronic in immunocompromised individuals [12, 13].

The emergence of zoonotic HEV strains, primarily derived from pigs, represents a major challenge for both the control and epidemiological understanding of the

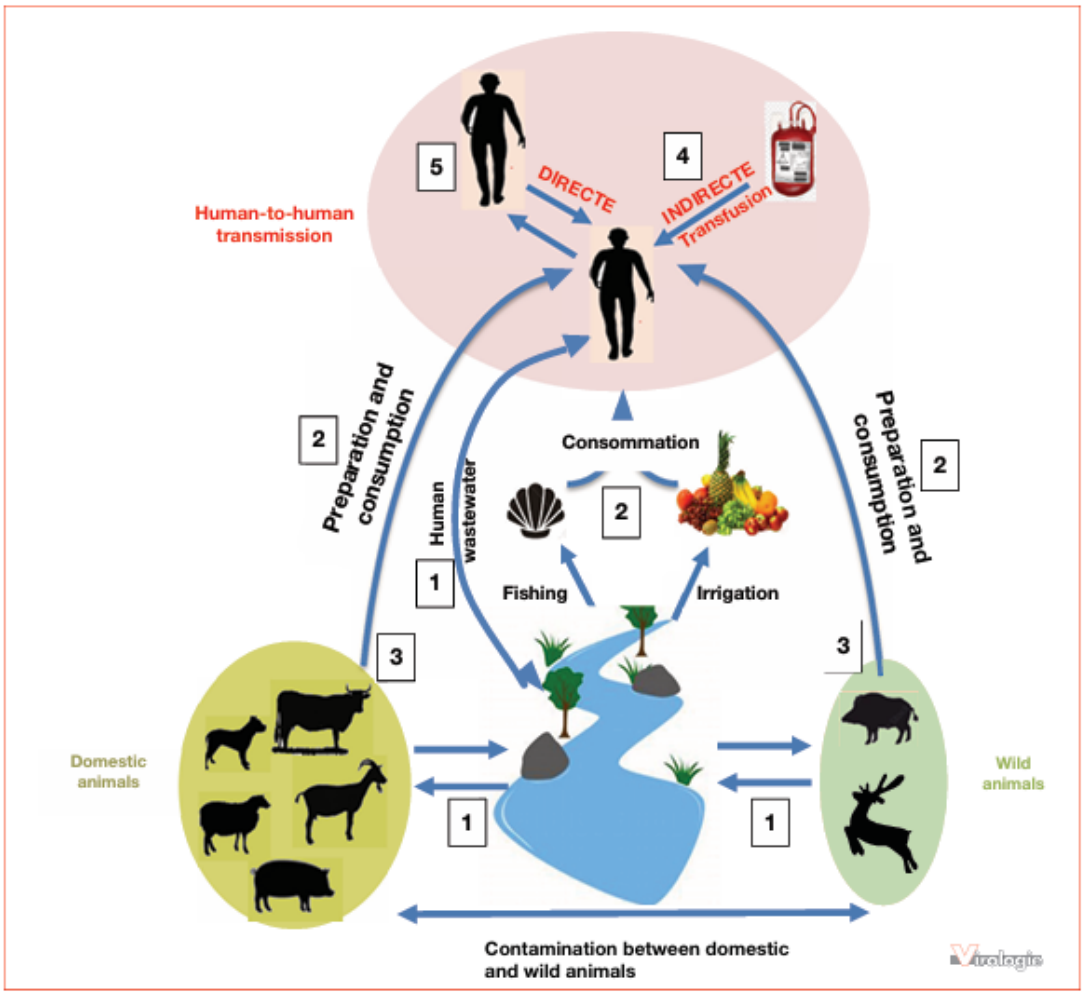


Figure 1. Transmission pathways of Hepatitis E. (1) Waterborne transmission *via* fecal contamination of drinking water or reverse contamination from humans; (2) Foodborne transmission through consumption of raw or undercooked meat from HEV reservoir animals (pigs, wild boars, deer) or shellfish; (3) Direct or indirect contact with reservoir animals (contact transmission); (4) Transmission *via* transfusion or organ transplantation; (5) Person-to-person transmission, including mother-to-child.

infection due to interspecies transmission and the increased genetic diversity it introduces. This evolution complicates prevention and surveillance strategies, particularly where pig farming is closely linked to human activities. HEV-3 was first identified in pigs in the USA [14] and later in Europe and Asia [15, 16]. These strains are transmitted to humans mainly through the consumption of undercooked pork [17]. While human-to-human transmission remains the primary route of HEV infection in Africa, the emergence of zoonotic variants complicates public health efforts, as implementing food safety measures in resource-limited regions is challenging. It is therefore crucial to conduct awareness campaigns targeting rural communities exposed to poor

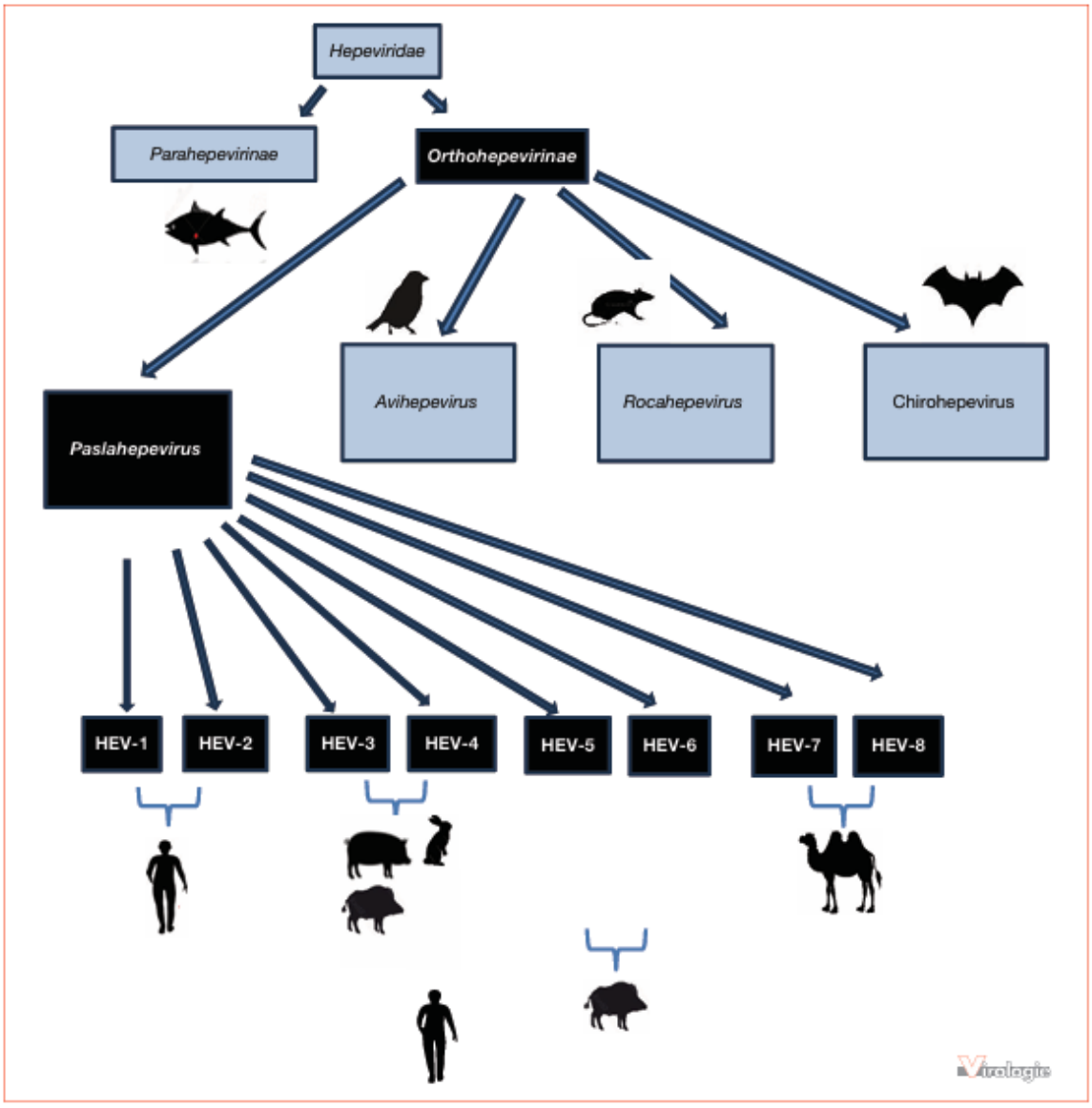


Figure 2. The Hepeviridae family, organized into subfamilies (-virinae), genera (-virus), and the genotypes of the genus Paslahepevirus (hepatitis E virus – HEV) with their associated reservoir species.

environmental conditions, high population density, and limited economic resources, factors that increase the risk of HEV transmission.

This review explores the evolution of knowledge on hepatitis E in Africa, including its historical and geographical context, epidemiology, and current status, as well as the distribution of seroprevalence and viral genotypes across different reservoirs. A literature search (PubMed, Google Scholar) focused on studies published between 2010 and 2025 to collect the most recent data. Informative references were found in 24 (44.44%) of the 54 African countries: West Africa (20), Central Africa (9), East

Africa (10), North Africa (9), and Southern Africa (7). These studies report a wide range of data concerning: seroprevalence in the general population (blood donors, healthy individuals) or at-risk groups (pig handlers, pregnant women); patients with acute hepatitis, occurring either sporadically (isolated cases) or epidemically (clusters linked to the same infection source); surveys of animal reservoirs such as birds (genus Avihepevirus, currently described as non-pathogenic to humans) and mammals (genus Paslahepevirus) including wild boars, rabbits, dromedaries, pigs, and other domestic animals (cattle, sheep, goats) and their derived food products; and human consumption water or water used in daily human activities.

Detection of the virus in Africa

The first detection of hepatitis E in Africa dates back to 1990 during a cholera outbreak in Sudan [18, 19]. It marked a turning point in the recognition of hepatitis E as a public health concern on the continent. During this initial outbreak and subsequent ones in various countries, hepatitis E was mainly associated with socio-economic factors such as poverty and inadequate healthcare infrastructure [19]. The 2000s were marked by additional outbreaks in Nigeria and Ethiopia, with a clear increase in cases during heavy rainfall and flooding periods, highlighting the impact of environmental factors on the virus transmission dynamics [3].

During the 2010s, surveillance efforts were intensified, providing a clearer picture of HEV distribution in Africa (*figure 3*), indicating that the disease had become endemic in several countries [20] with highly variable prevalence rates. This variability can be attributed to factors such as cultural practices, access to safe drinking water, and sanitary conditions. In particular, populations living in unstable conditions, such as residents of refugee camps, show high anti-HEV IgG rates, as observed in South Sudan (71%) [21] and Niger (38.4%) [22]. This clearly highlights the need to target these groups in prevention and awareness programs. With respect to zoonotic aspects, pigs represent the main risk due to their close proximity to humans and their identification as key HEV reservoirs since the first detection in Nigeria [23]. Similarly, since the first human case of zoonotic origin in Africa [24], it has been clearly shown that human seroprevalence depends on farming practices and meat consumption.

Circulation of HEV in humans in Africa

HEV circulation in the general population

Epidemic outbreaks of HEV have been reported, such as the large urban outbreak detected in Chad in 2017, characterized by high IgM (7.7%) and IgG (59.6%) seroprevalence, but fortunately associated with limited morbidity and mortality [19]. Similarly, between 2012 and 2014 in Senegal, an HEV outbreak occurred among workers in the Kédougou gold mines, with 64% seropositive (1047/1617), underscoring the vulnerability of populations engaged in mining activities [25].

Outside of epidemics, blood donors provide precise information on virus circulation in the general population. In Algeria, 20–22% of outpatients and blood donors tested in hospitals had anti-HEV antibodies, suggesting HEV-1 circulation [26]. Seroprevalence among blood donors is 10% IgG and 1.9% IgM in Burkina Faso [27] and even lower in Tunisia (5.4%) with no anti-HEV IgM, indicating limited virus circulation [28]. Another Tunisian study confirmed this low seroprevalence among blood donors (4.2%) but reported significantly higher rates in hemophilic patients (7.5%) and hemodialysis patients (10.2%), highlighting an increased risk of parenteral transmission [29]. Organ transplantation is another transmission route: in South Africa, a case of HEV-3 transmission via organ graft emphasizes the importance of monitoring immunocompromised individuals [30]. Other studies show that HEV can circulate in low-risk populations. In 2021, in Nigeria, anti-HEV IgG was detected in 2.2% of rural adolescents at a secondary school, showing that even young individuals outside high-risk professions are not spared [31]. *Table I* summarizes immunological and/or molecular studies conducted across Africa, targeting human, animal, and environmental reservoirs.

Pregnant women, a vulnerable population to monitor

HEV-1 is well known to induce severe, and sometimes fatal, disease in pregnant women, particularly in Asia and East Africa [20]. IgG seroprevalence, reflecting past HEV circulation, varies widely across African countries: 31.4% in Ethiopia [32] 16.19% in Benin, 12% in both Tunisia [2] and Ghana [33], 7.4% in Senegal [34] and only 3.1% in South Africa [35]. However, some studies in Benin reported 1.44% anti-HEV IgM, with samples testing positive simultaneously for IgG, IgM, and even HEV RNA, indicating active circulation [36]. Collectively, these findings highlight the importance of targeted surveillance and prevention strategies in this vulnerable population.

Figure 3a. Serological data on HEV circulation in African countries (gray) or absence of data (white).

Figure 3b. Available molecular data for HEV-1.

Figure 3c. Available molecular data for HEV-2.

Figure 3d. Available molecular data for HEV-3.

Figure 3e. Available molecular data for HEV-4 (blue) and avian HEV (red)

Figure 3. Cumulative serological and molecular data on HEV in Africa from articles published between 2010 and 2025.

Tableau 1. Serological prevalence and molecular typing data of hepatitis E virus in Africa over the past 15 years.

Author *et al* (reference)	Country	Sample origin	Population type	Genotype	IgM (%)	IgG (%)
Abebe *et al*. 2017 [32]	Ethiopia	Human	Pregnant women		0.5	31.6
Andersson *et al*. 2015 [30]	South Africa	Human	Liver transplant recipients	HEV-3		
Azman *et al*. 2017 [21]	South Sudan	Human	Refugees (camps)		4	71
Bagulo *et al*. 2022 [40]	Ghana	Human/pig	Community and farmers			Human : 11.9
			Livestock farming			pig : 62.4
Bari *et al*. 2021 [60]	Ethiopia	Pig/dromadary	Livestock farming	HEV		
Behloul *et al*. 2016 [26]	Algeria	Human	Community members and blood donors			20.17
Béji-Hamza *et al*. 2014 (73)	Tunisia	Wastewater	Urban areas	HEV-3		
Boon *et al*. 2018 [47]	Uganda	Human	Community members and HIV patients	HEV-3		47
Chambaro *et al*. 2021 [55]	Zambia	Pig	Livestock farming	HEV-3		47.7
Chauhan *et al*. 2022 [64]	South Africa	Pig	Livestock farming	HEV-3c		
De Paschale *et al*. 2016 [36]	Benin	Human	Pregnant women		1.44	16.19
De Paula *et al*. 2013 [9]	Cameroon	Pig	Livestock farming	HEV-3		
Dimeglio *et al*. 2019 [52]	Burkina Faso	Human	Patients with acute jaundice syndrome	HEV-2b	2.6	18.22
Diouara *et al*. 2022 [34]	Senegal	Human	Pregnant women		0.5	7.4
Doukoure *et al*. 2024 [56]	Guinea	Pig	Pivestock farming	HEV-3		22
El-Adly *et al*. 2023 [66]	Egypt	Rabbit	Livestock farming	HEV-3		12.80
El-Duah *et al*. 2020 [67]	Ghana	Pig	Livestock farming	HEV-3		77.5
Elhendawy *et al*. 2020 [42]	Egypt	Human	HCV-positive patients			71.4
Furukawa *et al*. 2016 [46]	Kenya	Human	Patients with acute febrile illness		25.7	37.8
Gerbi *et al*. 2015 [44]	Uganda	Human	Patients with acute jaundice syndrome			42
Hannachi *et al*. 2011 [2]	Tunisia	Human	Pregnant women		1	12
Harritshøj *et al*. 2018 [48]	Tanzania	Human	HIV-positive pregnant women			8.0
Hospital *et al*. 2024 [59]	Nigeria	Poultry/pig	Livestock farming	HEV		
Korsman *et al*. 2019 [63]	South Africa	Human	Patients hospitalized for acute hepatitis		1.6	30
Lagare *et al*. 2018 [22]	Niger	Human	Refugees in camps			75
Mandanda *et al*. 2017 [49]	R.D Congo	Human	Patients with acute febrile jaundice		10.4	
Maphumulo *et al*. 2024 [43]	South Africa	Human	Suspected cases negative for HAV, HBV, and HCV		0.33	60.9
Mbachu *et al*, 2021 [31]	Nigeria	Human	Community			2.2
Modiyinji *et al*, 2018 [57]	Cameroon	pig	Livestock farming			43.2
Modiyinji *et al*. 2020 [58]	Cameroon	pig	Livestock farming	HEV-3	21.0	17.7
Modiyinji *et al*. 2024 [53]	Cameroon	Human	Patients with acute febrile jaundice	HEV-1e, 3f, 4b	8.1	5.9

Author *et al* (reference)	Country	Sample origin	Population type	Genotype	IgM (%)	IgG (%)
Neffati Houcine *et al*. 2012 [28]	Tunisia	Human	Pregnant women and patients with acute hepatitis		3	5.4
N-Yazenguet *et al*. 2024 [51]	Central African Republic	Human	Patients with acute jaundice syndrome	HEV-1e	40.3	49.7
N-Yazenguet *et al*. 2024 [61]	Central African Republic	Domestic animals	Livestock farming	HEV-3h		
Obiri-Yeboah *et al*. 2018 [33]	Ghana	Human	Pregnant women		0.2	12.2
Oluremi *et al*. 2023 [4]	Nigeria	Human	Community		1.3	14.9
Osamudiamen *et al*. 2021 [70]	Nigeria	Poultry	Livestock farming	HEV Avian		
Ostankova *et al*. 2023 [41]	Guinea	Human	Healthcare workers		1.35	2.7
Ouoba *et al*. 2019 [27]	Burkina Faso	Domestic animals	Livestock farming			34.9
Owolodun *et al*. 2014 [23]	Nigeria	Pig	Livestock farming	HEV-3		47.2
Sadio *et al*. 2022 [25]	Senegal	Human/ wastewater/ rodent	Patients with acute jaundice syndrome			64.62
Salemane *et al*. 2024 [73]	South Africa	Wastewater	Urban and rural areas	HEV-3c, 3f, 4b		
Sayed *et al*. 2020 [68]	Egypt	Food	Camel meat and derived meat products	HEV-3a		
Simani *et al*. 2022 [35]	South Africa	Human	Pregnant women			3.13
Suluku *et al*. 2024 [65]	Sierra-Leone	Pig	Livestock farming	HEV-3		4
Temmam *et al*. 2013 [39]	Madagascar	Human/pig	Butchers	HEV-3		Human : 14.1
			Livestock farming			Pig : 71.2
Tene *et al*. 2024 [62]	Senegal	Food	Pork meat and derived products	HEV-3		
Tialla *et al*. 2022 [54]	Burkina Faso	Cow/pig	Livestock farming			Cow : 5.1
						Pig : 80.7
Traoré *et al*. 2015 [37]	Burkina Faso	Human	Community and butchers	HEV-3		Human : 76
		Pig	Livestock farming			Pig : 80
Tricou *et al*. 2020 [50]	Central African Republic	Human	Patients with acute jaundice syndrome	HEV-1e, HEV-2b	26	
Twagirumugabe *et al*. 2019 [45]	Rwanda	Human	Community members, blood donors, and patients with liver disorders			11.9
Vernier *et al*. 2018 [19]	Chad	Human	Community		6.7	45.2
Y. Ben-Ayed *et al*. 2015 [29]	Tunisia	Human	Hemophilic and hemodialysis patients			Blood donors: 4.5 Hemophilic: 7.5 Hemodialysis: 10.2

Beyond prevalence estimates, it is noteworthy that there are few, if any, published data on the pathogenicity of HEV infection in pregnant women in Africa. In regions where HEV-3 is endemic, virtually no cases of exacerbated disease have been reported in this group. It remains unclear whether this reflects an actual epidemiological reality or a lack of case investigation. These observations emphasize the need for studies specifically focused on pregnant women to guide the implementation of tailored preventive measures.

Regular medical follow-up is therefore recommended to prevent and detect HEV infection and associated risks. Preventive measures, such as proper hand hygiene, access to safe drinking water, and thorough cooking of food, are essential to reduce transmission. In cases of infection, appropriate treatment and monitoring are required to protect both maternal and fetal health. Collaboration among healthcare professionals is critical to ensure optimal management.

At-risk occupations

Intensive pig farming under suboptimal hygienic conditions also facilitates the spread of HEV. In Burkina Faso, studies revealed very high seroprevalence among butchers (76%) compared to the already high seroprevalence in the general population (47.8%) [37]. This higher rate is likely linked to the handling of pork products. In Uganda and Madagascar, lower seroprevalence was observed among slaughterhouse workers, at 13.3% and 14.1%, respectively [38,39]. In Ghana, where seroprevalence rates were relatively close between pig farmers (15.2%) and the surrounding community (12.4%), HEV circulation was detected in 2.9% and 0.7% of these groups, respectively [40]. In Nigeria, one study reported IgG seroprevalence of 1.3% among villagers living near pig farms, 14.9% IgG and 1.3% IgM among pig farmers, and 31.1% IgG and 2.2% IgM among butchers. These results clearly demonstrate that occupations related to pig farming, particularly butchery, are high-risk, with IgM positivity indicating ongoing infection [4].

In Guinea, where epidemic crises such as Ebola (2014–16) and the Covid-19 pandemic (2020–21) overshadowed HEV surveillance, a study on the circulation of HIV and hepatitis viruses reported HEV serological traces among healthcare workers, with low IgG (2.7%) and IgM (1.35%) prevalence. Although viral RNA was not detected by PCR, this study underscores the importance of maintaining broad-spectrum infectious disease surveillance, even during health emergencies, particularly for healthcare personnel at higher risk of infection [41].

Diagnostic confusion or co-infections with other viruses: hepatitis viruses, human immunodeficiency virus (HIV), or yellow fever virus (YFV)

Several viruses can cause hepatitis, and HEV can act alone or in co-infection with other pathogens, which complicates both diagnosis and treatment. In Egypt, 72% of patients positive for hepatitis C virus (HCV) also had anti-HEV IgG [42]. Conversely, in South Africa's Free State Province, 60% of patients with acute hepatitis tested negative for hepatitis A (HAV), B (HBV), and C (HCV) viruses, but were positive for anti-HEV IgG, and 0.3% for anti-HEV IgM [43]. In northern Uganda, surveillance showed that 42% of acute jaundice syndrome cases were attributable to HEV [44]. Results can sometimes be contradictory when comparing the general population with patients suffering from hepatitis. For instance, in Tunisia, one study detected no HEV infection markers either in blood donors or in patients with acute hepatitis [28] while another reported a 19.5% anti-HEV IgG prevalence among acute hepatitis patients [2]. A similar comparison in Rwanda found that 11.9% of adult blood donors tested positive for anti-HEV antibodies and 0.5% carried low viral RNA loads, with no significant difference between healthy individuals and those with liver disease [45]. By contrast, in Kenya, higher seroprevalence was observed among patients with acute febrile illnesses compared to the general population, reaching 25.7% for IgM and 37.8% for IgG, although no virus was detected by PCR [46]. These differing, and even conflicting results, clearly underscore the need to include HEV testing in patients presenting with acute hepatitis.

With respect to HIV/HEV co-infection, in Uganda (2008–09), people living with HIV exhibited a similar anti-HEV serological profile (46% IgG) to that of HIV-negative individuals (48%). Given that HEV may cause more severe or prolonged hepatitis in immunocompromised patients, including those living with HIV, genotype HEV-3 was identified in one individual who also tested positive for anti-HEV IgM [47]. In Tanzania, a study revealed that 8% of HIV-positive pregnant women carried anti-HEV IgG, indicating past exposure. Although these data do not provide estimates of symptomatic infection, they highlight the heightened vulnerability of pregnant women to potential active HEV infection [48].

In sub-Saharan Africa, jaundice, a clinical marker of liver injury, can also result from malaria or yellow fever virus (YFV) infection. However, many suspected cases may actually be attributable to HEV. In the Democratic Republic of Congo (DRC), 10.4% of suspected cases were positive for anti-HEV IgM [49]. In the Central

African Republic (CAR), among 3,181 suspected cases sampled between 2008 and 2009 and testing negative for YFV, 48.9% were positive for anti-HEV IgM, with HEV RNA detected in 2.5%, revealing viruses closely related to HEV-1 as well as one HEV-2b strain [50]. A follow-up survey conducted about a decade later in the same patient group revealed anti-HEV seroprevalence of 40.3% IgM and 49.7% IgG, again with circulation of genotype HEV-1e [51]. In Burkina Faso (2013–15), among 900 patients with febrile jaundice who tested negative for YFV, 18% and 2.6% were positive for anti-HEV IgG and IgM, respectively, and genotyping confirmed circulation of HEV-2b [52]. In Cameroon in 2024, among individuals clinically suspected of yellow fever but testing negative in the laboratory, 8.1% were positive only for anti-HEV IgM, 5.9% only for anti-HEV IgG, and 8.3% for both. Furthermore, viral RNA was identified in 15.2% of sera, confirming circulation of HEV-1e, HEV-3f, and HEV-4b. These findings demonstrate that HEV is likely responsible for a substantial proportion of acute febrile jaundice cases among patients enrolled in Cameroon's yellow fever surveillance program [53].

Circulation of HEV in animals

High prevalence in pigs and/or pork products

Several studies report high HEV seroprevalence in pigs across various African countries: 80% in Burkina Faso [54], 71.2% in Madagascar [39], 66% in Nigeria where genotype HEV-3 is circulating, and 47.7% in Zambia, also affected by genotype HEV-3e [55]. In Guinea, overall seroprevalence was lower (22%), though peaks of up to 50% were observed in farms located in the Forest Region, where part of the population is Christian and raises and consumes pork contaminated with genotype HEV-3c [56]. In Cameroon, pig seroprevalence is also significant (43.2%) [57] and a study conducted in 2017–18 in slaughterhouses across three cities reported 21% anti-HEV IgM and 17.7% anti-HEV IgG positivity, with HEV closely related to genotype HEV-3 detected in 5.9% of fecal samples from seropositive animals [58]. Variable levels of HEV detection in pig feces have been described elsewhere in Africa: lower in Lagos, Nigeria (2.9%) [59], higher in Ethiopia (12%) [60] and particularly elevated in the Central African Republic (CAR, 36.1%), where genotype HEV-3h circulates in five districts of Bangui [61].

Pork products represent another potential source of contamination. Genotype HEV-3 was detected in 0.8% of pig livers sampled in three regions of Cameroon [9], in 1–1.2% of pig livers in Burkina Faso [37] and Madagascar [39], and in 3% of meat and 22% of pig livers sold at the Saint-Louis market in Senegal [62]. In South Africa, several studies identified HEV in pork products, with genetic sequences closely related to those found in humans [63], suggesting a potential zoonotic transmission risk [64], although direct evidence remains limited.

Taken together, these findings demonstrate that, in addition to human contamination of water sources, zoonotic transmission of HEV plays an important role in West Africa [37]. They highlight the endemicity of genotype HEV-3 in many African countries, including those with low seroprevalence such as Sierra Leone (4%) [65]. They further emphasize the importance of the "One Health" approach, with pig surveillance being essential given the clear zoonotic risk posed by this reservoir, as illustrated by the heterogeneity of HEV genotypes circulating among humans and pigs in Africa (*figure 4*). Moreover, the variability of results between countries and regions indicates the need to adapt surveillance and control strategies to local contexts. Additional studies are required to better understand the dynamics of HEV transmission from pigs to humans.

HEV prevalence in other domestic animals

Beyond pigs, HEV has also been detected in several domestic animal species such as rabbits, dromedaries, cattle, sheep, and goats. In Egypt, HEV-3 was found in 15.2% of rabbit fecal samples, while 12.8% tested positive for anti-HEV IgG [66]. In Burkina Faso, seroprevalence reached 52% in hares and 60% in rabbits, and was also observed in other livestock species such as cattle (26.4%), sheep (12%), and goats (28.4%) [27]. Phylogenetic analysis showed no relationship between HEV-3 strains in rabbits and those found in pigs or humans. Proximity to pig farms has been identified as a risk factor for HEV exposure in livestock and for wider disease spread. For instance, while the average seroprevalence in cattle is 5.1%, it can reach 32.4% in farms located near pig holdings, where 80.7% of pigs carry anti-HEV antibodies. This has raised concerns among health authorities in Burkina Faso regarding the risk of HEV transmission through dairy products originating from mixed cattle–pig farming systems [54]. Across the border from Burkina Faso, Ghana reported HEV circulation in 2011 among five animal species (dromedaries, cattle, goats, sheep, and pigs) in the Kumasi region, with HEV-3 identified in pigs and HEV-7 in dromedaries [67]. The virus has also been detected in 2.2% of dromedaries in Ethiopia. In Egypt, HEV-3 RNA was identified in cow's milk sold in rural communities, raising potential public health concerns even though no transmission to humans has yet been demonstrated [68]. However, a

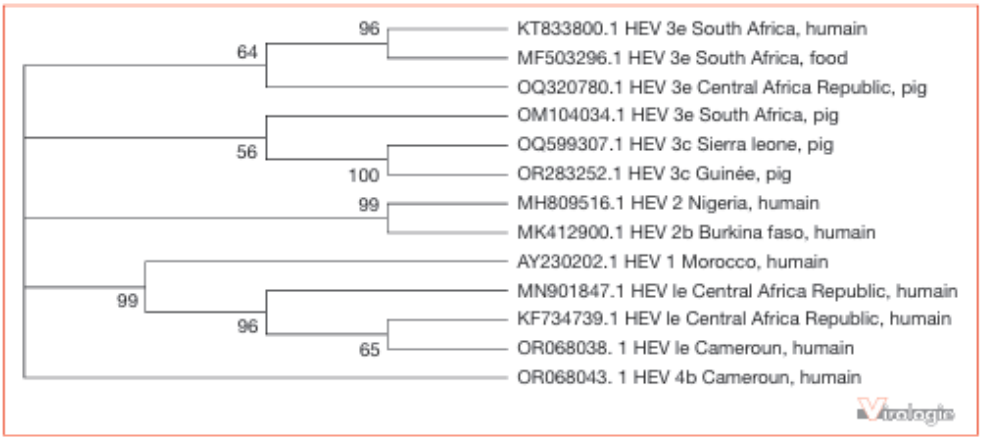


Figure 4. Phylogenetic tree of ORF2 sequences of Hepatitis E virus (HEV) obtained from human and animal samples in Africa. Reference sequences representing different genotypes were retrieved from GenBank. Sequences were aligned using the MUSCLE algorithm in MEGA 12. The tree was constructed using the Neighbor-Joining method with the p-distance model and 500 bootstrap replicates. Bootstrap values above 70% are indicated at the corresponding nodes.

confirmed case of HEV-7 transmission *via* camel milk was documented in the United Arab Emirates in 2016 [69]. The avian genotype (genus *Avihepevirus*), which has not yet been associated with human disease, was detected in Nigeria in 12.5% of serum and 9.1% of fecal samples from healthy laying hens [70]. Its presence was further confirmed in 2.9% of poultry sampled across three districts of Lagos between 2017 and 2019 [59].

Overall, it appears that several domestic animals in close contact with humans may serve as potential HEV reservoirs. This underscores the need for expanded surveillance across different animal species and region-specific approaches to better understand infection dynamics and reduce human transmission risk. Farming practices and sanitary conditions play a major role in shaping HEV prevalence among animals and may facilitate viral spread. Co-infection or superinfection with other viruses further increases carriage rates and prolongs the duration of viral shedding [71, 72].

Detection in wastewater

Due to its fecal–oral transmission route, it is not surprising that HEV is detected in both urban and rural wastewater. Wastewater surveillance is indeed a key tool for tracking active virus circulation in human populations and the environment, with waste management practices also influencing viral dissemination. A study carried out in wastewater treatment plants, rivers, and lavatory facilities across seven regions of South Africa reported circulation of HEV genotypes 3c, 3f, and 4b [73]. In Tunisia, both HEV-1 and HEV-3 were detected in wastewater. While the presence of HEV-1, a strictly human genotype, is expected, the detection of HEV-3 mainly transmitted through pigs is surprising in a predominantly Muslim country where pork consumption is low. Further studies are needed to clarify the diversity of HEV strains circulating in humans and animals, as well as the dynamics of their transmission [74].

Conclusion

Hepatitis E represents a major public health challenge in Africa. The genetic diversity of the virus and the multiplicity of its reservoirs highlight the urgent need for in-depth research to better understand and control the disease and its transmission dynamics. Beyond the strictly human HEV-1 and HEV-2 genotypes, pigs act as major reservoirs for zoonotic HEV-3 and HEV-4, facilitating their spread. Recurrent detection of HEV-3 across the continent and HEV-4 in Central Africa is coupled with high seroprevalence in certain populations. The recent identification of HEV-7 in dromedaries in Madagascar and HEV-4b in Southern Africa further broadens the range of zoonotic genotypes with transmission potential.

According to the WHO, hepatitis E is endemic in many developing countries, with epidemic outbreaks

exacerbated by poor sanitation. In Africa, both human-to-human and zoonotic transmission are intensified by inadequate sanitation infrastructure and the socioeconomic importance of pig farming. The high prevalence of HEV in pigs and in human populations either in close contact with pigs or consuming undercooked pork products underlines the need for greater public awareness and improved hygiene practices. Moreover, other domestic animals including rabbits, goats, and cattle—may act as intermediate hosts between pigs and humans, either directly or *via* derived products such as meat or milk. Although rabbit-to-human transmission of HEV-3 has not yet been demonstrated, the potential exists, just as with HEV-7, which has been reported in Asia. The detection of HEV-4, originally identified in Japan, in pigs from Cameroon further illustrates the risks posed by international travel and trade, although a direct link with commercial pork transport has not been established to date.

Given these multiple risks, a multidisciplinary approach rooted in the "One Health" framework is essential to address the persistence of hepatitis E, improve hygiene and farming practices, raise community awareness of transmission risks, and strengthen human infection prevention and management. In particular, close monitoring of at-risk populations is critical—most notably pregnant women, who face severe complications and significantly higher mortality rates than the general population. Regular medical surveillance, preventive hygiene measures, and coordinated action between health professionals are crucial to protect this vulnerable group.

From a research perspective, the application of next-generation sequencing technologies could help map HEV genetic diversity across animal and human populations, while also identifying mutations linked to virulence or resistance to treatment. Integration of geospatial and environmental data with animal and human surveillance would allow a better understanding of interactions between animal reservoirs and human populations. Finally, training modules should be developed to strengthen the clinical management of hepatitis E, from recognition of atypical symptoms—which may mimic other diseases—to the development of specific diagnostic tools capable of distinguishing HEV cases from other causes of jaundice or acute hepatitis such as malaria or yellow fever.

Such research will contribute to a better understanding of HEV transmission dynamics in Africa, help identify vulnerable populations, guide the development of tailored prevention and control strategies, and ultimately support the design of effective public health policies for the continent.

Links of interests: none.

References

1. Arankalle VA. Hepatitis E in India. *Proc Natl Acad Sci India Sect B - Biol Sci* 2012; 82 (1): 43-53.

2. Hannachi N, Hidar S, Harrabi I, Mhalla S, Marzouk M, Ghzel H, *et al*. Séroprévalence et facteurs de risque de l'hépatite virale E chez la femme enceinte dans le centre tunisien. *Pathol Biol* 2011; 59 (5): 2009-2012.

3. Bagulo H, Majekodunmi AO, Welburn SC. Hepatitis E in Sub-Saharan Africa – a significant emerging disease. *One Health* 2020; 11: 100186.

4. 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: 1-8.

5. Amar N, Bendall R, Legrand-Abravanel F, Xia N, Ijaz S, Izopet J, *et al*. Hepatitis E. *Lancet* 2012; 379 (9835): 2477-2488.

6. Bergløv A, Hallager S, Weis N. Hepatitis E during pregnancy: Maternal and foetal case-fatality rates and adverse outcomes—A systematic review. *J Viral Hepat* 2019; 26 (11): 1240-1248.

7. Ciglenecki I, Rumunu J, Wamala JF, Nkemenang P, Duncker J, Nesbitt R, *et al*. The first reactive vaccination campaign against hepatitis E. *Lancet Infect Dis* 2022; 22 (8): 1110-1111.

8. Primadharsini PP, Nagashima S, Okamoto H. Genetic variability and evolution of hepatitis E virus. *Viruses* 2019; 11 (5): 456.

9. de Paula VS, Wiele M, Mbunkah AH, Daniel AM, Kingsley MT, Schmidt-Chanasit J. Hepatitis E virus genotype 3 strains in domestic pigs, Cameroon. Vol. 19, *Emerging Infectious Diseases* 2013; 19 (4): 666-668.

10. Uchida T. Hepatitis E. *Ryōikibetsu shōkōgun shirīzu* 1995; 17-19.

11. Hu X, Wang J, Wang Y, Yuan W, Wang J, Xu X. Detection and Characterization of Paslahepevirus balayani (Hepatitis E Virus) in Dairy Products from Hebei Province, China. *Pathogens* 2025; 14 (6): 1-9.

12. Smith DB, Simmonds P, Izopet J, Oliveira-Filho EF, Ulrich RG, Johne R, *et al*. Proposed reference sequences for hepatitis E virus subtypes. *J Gen Virol* 2016; 97: 537-542.

13. Purdy MA, Harrison TJ, Jameel S, Meng XJJ, Okamoto H, Van Der Poel WHMM, *et al*. ICTV virus taxonomy profile: Hepeviridae. *J Gen Virol* 2017; 98 (11): 2645-2646.

14. Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, *et al*. A novel virus in swine is closely related to the human hepatitis E virus. *Proc Natl Acad Sci U S A*. 1997; 94 (18): 9860-9865.

15. Smith DB, Izopet J, Nicot F, Simmonds P, Jameel S, Meng XJ, *et al*. Update: Proposed reference sequences for subtypes of hepatitis E virus (species Orthohepevirus A). *J Gen Virol* 2020; 101 (7): 692-698.

16. Vina-rodriguez A, Schlosser J, Becher D, Kaden V, Groschup MH, Eiden M, *et al*. Hepatitis E Virus Genotype 3 Diversity: Phylogenetic Analysis and Presence of Subtype 3b in Wild Boar in Europe. *Viruses* 2015; 2704-2726.

17. Wang B, Meng XJ. Hepatitis E virus: host tropism and zoonotic infection. *Curr Opin Microbiol* 2021; 59: 8-15.

18. Aubry P. Hépatites virales en zones tropicales. Actualités 2012. *Médecine Trop* 2013; 1-8.

19. Vernier L, Lenglet A, Hogema BM, Moussa AM, Ariti C, Vollmer S, *et al*. Seroprevalence and risk factors of recent infection with hepatitis E virus during an acute outbreak in an urban setting in Chad, 2017. *BMC Infect Dis* 2018; 18 (1): 1-10.

20. Kim JH, Nelson KE, Panzner U, Kasture Y, Labrique AB, Wierzba TF. A systematic review of the epidemiology of hepatitis E virus in Africa. *BMC Infect Dis* 2014; 14: 308.

21. Azman AS, Bouhenia M, Iyer AS, Rumunu J, Laku RL, Wamala JF, *et al*. High hepatitis E seroprevalence among displaced persons in South Sudan. *Am J Trop Med Hyg* 2017; 96 (6): 1296-1301.

22. Lagare A, Ibrahim A, Ousmane S, Issaka B, Zaneidou M, Kadadé G, *et al*. Outbreak of Hepatitis E virus infection in displaced persons camps in Diffa region, Niger, 2017. *Am J Trop Med Hyg* 2018; 99 (4): 1055-1057.

23. Owolodun OA, Gerber PF, Giménez-Lirola LG, Kwaga JKP, Opriessnig T. First report of hepatitis E virus circulation in domestic pigs in Nigeria. *Am J Trop Med Hyg* 2014; 91 (4): 699-704.

24. Bouscaillou J, Komas N, Tricou V, Nakouné E, Sélékon B, Fontanet A, *et al*. Imported hepatitis E virus, Central African Republic, 2011. *Emerg Infect Dis* 2013; 19 (2): 335-337.

25. Sadio BD, Faye M, Kaiser M, Diarra M, Balique F, Diagne CT, *et al*. First hepatitis E outbreak in Southeastern Senegal. *Sci Rep* 2022; 12 (1): 1-10.

26. Behloul N, Zhang M, Meng J. Binding preference of anti-HEV antibodies in sera collected in Algeria for antigens derived from HEV genotype 1. *Hepat Mon* 2016; 16 (8): 0-6.

27. Ouoba JB, Traore KA, Rouamba H, Setondji KVM, Minoungou GL, Ouoba BL, *et al*. Prevalence of anti-hepatitis E virus antibodies in domestic animals from three representative provinces of Burkina Faso. *Vet Anim Sci* 2019; 7: 100059.

28. Houcine N, Jacques R, Salma F, Lyon D, Nord V. Seroprevalence of hepatitis E virus infection in rural and urban populations , *J Infect Dev Ctries* 2012; 6 (3): 234-239.

29. Bartholomeusz A, Locarnini S. Hepatitis E virus: molecular virology and associated antiviral therapy. *Antivir Ther* 2006; 11 (1): 52-55.

30. Andersson MI, Stead PA, Maponga T, van der Plas H, Preiser W. Hepatitis E virus infection: An underdiagnosed infection in transplant patients in Southern Africa? *J Clin Virol* 2015; 70: 23-25.

31. Mbachu CNP, Ebenebe JC, Okpara HC, Chukwuka JO, Mbachu II, Elo-Ilo JC, *et al*. Hepatitis E prevalence, knowledge, and practice of preventive measures among secondary school adolescents in rural Nigeria: a cross-sectional study. *BMC Public Health* 2021; 21: 1-8.

32. Ababa A, Abebe M, Ali I, Ayele S, Overbo J, Aseffa A, *et al*. Seroprevalence and risk factors of Hepatitis E Virus infection among pregnant women in. *PLoS One* 2017; 12 (6): 1-9.

33. Obiri-Yeboah D, Awuku YA, Adu J, Pappoe F, Obboh E, Nsiah P, *et al*. Sero-prevalence and risk factors for hepatitis E virus infection among pregnant women in the Cape Coast Metropolis, Ghana. *PLoS One* 2018; 13 (1): 1-13.

34. Diouara AAM, Lo S, Nguer CM, Senghor A, Diop Ndiaye H, Manga NM, *et al*. Hepatitis E Virus Seroprevalence and Associated Risk Factors in Pregnant Women Attending Antenatal Consultations in Senegal. *Viruses* 2022; 14 (8): 1-12.

35. Simani OE, Seipone TP, Selabe G, Seheri LM, Mphahlele MJ, Mayaphi SH, *et al*. Low seroprevalence of hepatitis E virus in pregnant women in an urban area near Pretoria, South Africa. *IJID Reg* 2022; 2: 70-73.

36. De Paschale M, Ceriani C, Romanò L, Cerulli T, Cagnin D, Cavallari S, *et al*. Epidemiology of hepatitis E virus infection during pregnancy in Benin. *Trop Med Int Heal* 2016; 21 (1): 108-113.

37. Traoré KA, Ouoba JB, Huot N, Rogée S, Dumarest M, Traoré AS, *et al*. Hepatitis E virus exposure is increased in pork butchers from Burkina Faso. *Am J Trop Med Hyg* 2015; 93 (6): 1356-1359

38. Ukuli AQ, Mugimba KK. Seroprevalence of hepatitis E in swine abattoir workers1. Ukuli AQ, Mugimba KK. Seroprevalence of hepatitis E in swine abattoir workers. *Afr Health Sci* 2017; 17 (4): 1022-1028.

39. Temmam S, Besnard L, Andriamandimby SF, Foray C, Rasamoelina-Andriamanivo H, Héraud JM, *et al*. High prevalence of hepatitis E in humans and pigs and evidence of genotype-3 virus in Swine, Madagascar. *Am J Trop Med Hyg* 2013; 88 (2): 329-338.

40. Bagulo H, Majekodunmi AO, Welburn SC, Bimi L. Hepatitis E seroprevalence and risk factors in humans and pigs in Ghana. *BMC Infect Dis* 2022; 22: 1-12.

41. Ostankova YV, Shchemelev AN, Boumbaly S, Balde TAL, Zueva EB, Valutite DE, *et al*. Prevalence of HIV and viral hepatitis markers among healthcare workers in the Republic of Guinea. *Diagnostics* 2023; 276 (7): 1-11.

42. Elhendawy M, Abo-Ali L, Abd-Elsalam S, Hagras MM, Kabbash I, Mansour L, *et al*. HCV and HEV: two players in an Egyptian village, a study of prevalence, incidence, and co-infection. *Environ Sci Pollut Res* 2020; 27 (27): 33659-33667.

43. Maphumulo SS, Goedhals D, Van Rooyen C, Vawda S. High seroprevalence of hepatitis E virus in the Free State province of South Africa. *South African J Infect Dis* 2024; 39 (1): 1-4.

44. Gerbi GB, Williams R, Bakamutumaho B, Liu S, Downing R, Drobeniuc J, *et al*. Hepatitis E as a cause of acute jaundice syndrome in northern Uganda, 2010-2012. *Am J Trop Med Hyg* 2015; 92 (2): 411-4.

45. Twagirumugabe T, Saguti F, Habarurema S, Gahutu JB, Bergström T, Norder H. Hepatitis A and E virus infections have different epidemiological patterns in Rwanda. *Int J Infect Dis* 2019; 86: 12-14.

46. Furukawa NW, Teshale EH, Cosmas L, Ochieng M, Gikunju S, Fields BS, Montgomery JM. Serologic evidence for hepatitis E virus infection among patients with undifferentiated acute febrile illness in Kibera, Kenya. *J Clin Virol* 2016; 77: 106-108.

47. Boon D, Redd AD, Laeyendecker O, Engle RE, Nguyen H, Ocama P, *et al*. Hepatitis E virus seroprevalence and correlates of anti-HEV IgG antibodies in the Rakai District, Uganda. *J Infect Dis* 2018; 217 (5): 785-789.

48. Harritshøj LH, Theilgaard ZP, Mannheimer E, Midgley SE, Chiduo M, Ullum H, *et al*. Hepatitis E virus epidemiology among HIV-infected women in an urban area in Tanzania. *Int J Infect Dis* 2018 ; 73 : 7-9.

49. Makiala-Mandanda S, Le Gal F, Ngwaka-Matsung N, Ahuka-Mundeke S, Onanga R, Bivigou-Mboumba B, *et al*. High prevalence and diversity of hepatitis viruses in suspected cases of yellow fever in the democratic Republic of Congo. *J Clin Microbiol* 2017; 55 (5): 1299-312.

50. Tricou V, Bouscaillou J, Laghoe-Nguembe GL, Béré A, Konamna X, Sélékon B, *et al*. Hepatitis E virus outbreak associated with rainfall in the Central African Republic in 2008-2009. *BMC Infect Dis 2020*; 20 (1): 1-7.

51. de Marguerite Nombot-Yazenguet MP, Doté JW, Koyaweda GW, Zemingui-Bembete PA, Selekon B, Vickos U, *et al*. Hepatitis E outbreak in the health district of Bocaranga-Koui, Central African Republic, 2018-2019. *BMC Infect Dis* 2024; 24 (1): 1-10.

52. Dimeglio C, Kania D, Mantono JM, Kagoné T, Zida S, Tassembedo S, *et al*. Hepatitis E virus infections among patients with acute febrile jaundice in Burkina Faso. *Viruses* 2019; 11 (6): 3-11.

53. Modiyinji AF, Tankeu LTA, Monamele CG, Moumbeket MHY, Ngoupo PAT, Simo HT, *et al*. Hepatitis E virus infections among patients with acute febrile jaundice in two regions of Cameroon: first molecular characterization of hepatitis E virus genotype 4. *PLoS One* 2024; 19 (2): 1-12.

54. Tialla D, Cissé A, Ouédraogo GA, Hübschen JM, Tarnagda Z, Snoeck CJ. Prevalence of hepatitis E virus antibodies in cattle in Burkina Faso associated with swine mixed farming. *J Vet Sci* 2022; 23 (3): 1-10.

55. Chambaro HM, Sasaki M, Muleya W, Kajihara M, Shawa M, Mwape KE, *et al*. Hepatitis E virus infection in pigs: a first report from Zambia. *Emerg Microbes Infect* 2021; 10 (1): 2169-72.

56. 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 (8): 540-545.

57. Modiyinji AF, Atsama MA, Monamele GC, Nola M, Njouom R. High seroprevalence of hepatitis E among pigs suggests an animal reservoir in Cameroon. *J Infect Dev Ctries* 2018; 12 (8): 676-679.

58. Modiyinji AF, Sanding GMAM, Atsama MA, Monamele CG, Nola M, Njouom R. Serological and molecular investigation of hepatitis E virus in pig reservoirs from Cameroon reveals elevated seroprevalence and presence of genotype 3. *PLoS One* 2020; 15 (2): e0229073

59. Hospital M, Ikeja A, Ikeja A, Local A. Molecular detection of hepatitis E virus among swine and poultry birds in Lagos, Nigeria. *Int J Vet Sci* 2024; 25 (2): 201-209.

60. Bari FD, Wodaje HB, Said U, Waktole H, Sombo M, Leta S, *et al*. First molecular detection of hepatitis E virus genome in camel and pig faecal samples in Ethiopia. *Virol J* 2021; 18 (1): 160.

61. de Marguerite Nombot-Yazenguet MP, Modiyinji AF, Tricou V, Manirakiza A, Njouom R, Komas NPJ. Investigating animal reservoirs for hepatitis E virus in Bangui, Central African Republic. *PLoS One* 2024; 19 (3): 1-10.

62. Tene SD, Diouara AAM, Kane A, Sané S, Coundoul S, Thiam F, *et al*. Detection of Hepatitis E Virus (HEV) in Pork Sold in Saint-Louis, the North of Senegal. *Life* 2024; 14 (4): 1-12.

63. Korsman S, Bloemberg J, Brombacher M, Giuricich A, Halley-Stott RP, Kaba M. Hepatitis E in pig-derived food products in Cape Town, South Africa, 2014. *South African Med J* 2019; 109 (8): 584-586.

64. Chauhan RP, Gordon ML. Characterization of a Near Full-Length Hepatitis E Virus Genome of Subtype 3c Generated from Naturally Infected South African Backyard Pigs. *Pathogens* 2022; 11 (9).

65. Suluku R, Jabaty J, Fischer K, Diederich S, Groschup MH, Eiden M. Hepatitis E seroprevalence and detection of genotype 3 strains in domestic pigs from Sierra Leone collected in 2016 and 2017. *Viruses* 2024; 16 (4): 558.

66. El-Adly AM. Serological and genetic diversity of hepatitis E virus among rabbits population in Egypt. *Open Vet J* 2023; 13 (5): 515-522.

67. El-Duah P, Dei D, Binger T, Sylverken A, Wollny R, Tasiame W, *et al*. Detection and genomic characterization of hepatitis E virus genotype 3 from pigs in Ghana, Africa. *One Heal Outlook* 2020; 2 (1): 10.

68. Sayed IM, Hammam ARA, Elfaruk MS, Alsaleem KA, Gaber MA, Ezzat AA, *et al*. Enhancement of the molecular and serological assessment of hepatitis E virus in milk samples. *Microorganisms* 2020; 8 (8): 1-14.

69. Africa E, Emirates UA, Rasche A, Saqib M, Liljander AM, Bornstein S, *et al*. Hepatitis E Virus Infection in Dromedaries, North and East Africa, United Arab Emirates, and Pakistan, 1983-2015. *Emerg Infect Dis* 2016; 22 (7): 1249-1252.

70. Osamudiamen FT, Akanbi OA, Zander S, Oluwayelu DO, Bock CT, Klink P. Identification of a putative novel genotype of avian hepatitis E virus from apparently healthy chickens in southwestern nigeria. *Viruses* 2021; 13 (6): 954.

71. Salines M, Demange A, Stéphant G, Renson P, Bourry O, Andraud M, *et al*. Persistent viremia and presence of hepatitis E virus RNA in pig muscle meat after experimental co-infection with porcine reproductive and respiratory syndrome virus. *Int J Food Microbiol* 2019; 292: 144-149.

72. Salines M, Dumarest M, Andraud M, Mahé S, Barnaud E, Cineux M, *et al*. Natural viral co-infections in pig herds affect hepatitis E virus (HEV) infection dynamics and increase the risk of contaminated livers at slaughter. *Transbound Emerg Dis* 2019; 66 (5): 1930-1945.

73. Salemane K, Coetzee LZ, Pocock G, Genthe B, Taylor MB, Mans J. Water-based epidemiological investigation of hepatitis E virus in South Africa. *Food Environ Virol* 2024: 16 (3): 338-350.

74. Béji-Hamza A, Hassine-Zaafrane M, Khélifi-Gharbi H, Della Libera S, Iaconelli M, Muscillo M, *et al*. Hepatitis E virus genotypes 1 and 3 in wastewater samples in Tunisia. *Arch Virol* 2015; 160 (1): 183-189.