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SHORT COMMUNICATION

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Proof of infectivity of hepatitis E virus particles from the ejaculate of chronically infected patients

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Abstract

Recently, hepatitis E virus (HEV, *Paslahepevirus balayani*) particles were detected for the first time in the ejaculate of two chronically infected patients. Since then, we have been able to detect HEV in ejaculate in five further patients, and thus in a total of seven out of nine (78%) chronically infected men (age 36–67 years, median 56 years). In five patients, the HEV RNA concentration was more than 100-fold higher compared to the serum, while in two patients, the viral load was more than 10-fold lower. However, it has remained unclear whether viral particles shed in the ejaculate were infectious, as a previous cell culture model had failed to demonstrate the infectivity. In the current study, we employed an optimized HEV cell culture system based on overconfluent PLC/PRF/5 cells to investigate the infectivity of HEV particles from ejaculate and other body fluids. With this approach, we were able to show for the first time that HEV particles in the ejaculate from several patients were infectious. HEV replicated to high viral loads of 1e9 HEV RNA copies per ml. This indicates that HEV-positive ejaculate could bear a risk of infection for sexual partners.

KEYWORDS

chronic infection, ejaculate, hepatitis E virus (HEV), immunosuppression, *Paslahepevirus balayani*, STD

Preliminary results from this study have been presented (as posters) at the following congresses:International Liver Congress™ 2022, June 22-26, 2022; 32nd Annual Meeting of the Society for Virology, March 28–31, 2023; 18th Global Hepatitis Summit, April 25–28, 2023.

1 | INTRODUCTION

The hepatitis E virus (HEV, *Paslahepevirus balayani*) is a virus with many different aspects, mainly affecting the liver. Acute HEV genotype 1 and 2 (HEV-1 and -2) infections are caused by contaminated water and lead to outbreaks in resource-limited countries. In contrast, HEV genotype 3 and 4 (HEV-3 and -4) are mainly transmitted zoonotically via pork products and rarely via blood transfusion and solid organ transplants in industrialized countries.[1–4] Chronic HEV-3 infections in immunocompromised people play a major role, mainly in Europe.[5] About 80%–90% of these infections can be cured with ribavirin.[6] Furthermore, assumed extrahepatic manifestations of HEV infections and, in particular, extrahepatic replication is a topic of emerging interest. In 2021, Horvatits et al. showed that HEV-3 infections in chronically infected individuals can replicate in the male genital tract beyond the blood–testis barrier. Furthermore, the clinical course of one of the patients described indicated that the virus can spread from the male genital tract to the rest of the body.[7] However, until now, it was not possible to prove that HEV particles in ejaculate can be infectious. This study aimed to determine the infectivity of HEV in the ejaculate using an optimized cell culture system.

2 | MATERIALS AND METHODS

2.1 | Patients

All patients gave written informed consent, and samples were analyzed pseudonymously at the University Hospital Hamburg Eppendorf. Patients from other centers agreed to donate ejaculate and be tested for HEV. These patients have been included anonymously into the study. The study was reviewed and approved by the Ethics Committee of the Medical Council of Hamburg (WF-138/20).

2.2 | HEV RT-qPCR

Patient samples were tested in a laboratory-developed real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR) using the cobas 6800 system (Roche) according to Allweiss et al.[8] HEV RNA was quantified as IU/mL (limit of quantification 24 IU/mL) using the WHO standard.[9] Nucleic acid was isolated from cell culture supernatants and inoculates using the EZ1® Advanced XL workstation with the EZ1 Virus Mini Kit v2.0 (Qiagen). Eluted nucleic acid was tested by RT-qPCR according to Wenzel et al.[10] HEV RNA was quantified as genome copies per mL (c/mL).

2.3 | HEV subtyping

HEV-positive patient specimens analyzed via cell culture were sequenced in the National Consultant Laboratory. For this purpose, purified nucleic acid was reverse-transcribed and amplified in two genomic regions by a first round, and consecutive nested PCR: a 242 bp fragment within open reading frame (ORF1), and a 493 bp fragment within ORF2 (primers excluded). The ORF1 fragment was amplified according to Wenzel et al.,[10] the ORF2 fragment according to Boxman et al.[11] Amplification products were purified and sequenced on an ABI 3130xl sequencer. The resulting electropherograms were analyzed and assembled with CodonCode Aligner v10.0.2 (www.codoncode.com, CodonCode Corporation). Sequences were deposited in the GenBank (accession numbers PP748650–PP748675). Subtypes were assigned by identifying the best matching reference sequence from the latest *P. balayani* reference set[12] using the fasta36 algorithm.[13] HEV from some patients had been subtyped in other institutions before this study was conducted. Besides the subtype information, neither sequence data nor stored surplus material was available from these cases.

2.4 | Inoculum preparation

Patient materials (in the case of different body fluids per patient, these were taken on the same day) were diluted with 0.2% bovine serum albumin (BSA); (Sigma-Aldrich) (w/v) in PBS without Ca^{2+} and Mg^{2+} (PBS^{-}; Lonza) to a total volume of 1.5 mL, resulting in dilution factors of 1.5–3.3. Samples were vortexed vigorously and centrifuged at 8000×g for 10 min. The supernatant was sterile filtered through a 0.20 µm polyether sulfone (PES) membrane (Sarstedt). The remaining cell pellets of ejaculate samples were further processed to generate cell lysate to obtain intracellular virus particles. Therefore, the pellets were vortexed in 100 µL of deionized water followed by incubation on ice for 15 min with intercalated vortex steps every 3 min. The cell suspension was then sonicated for 5 min using the Diagenode Bioruptor® Standard waterbath sonicator (Hologic) at medium intensity and on/off cycles of 30 s each. Finally, lysis was completed by three freeze-thaw cycles in liquid nitrogen. Subsequent, 0.2% BSA (w/v) in PBS^{-} was added to the cell lysate to a total volume of 1.5 mL, vortexed vigorously, centrifuged at 8000×g for 10 min. The supernatant was sterile filtered through a 0.20 µm PES membrane.

2.5 | HEV cell culture

HEV isolation experiments were performed in a cell culture system optimized for HEV propagation.[14] Briefly, PLC/PRF/5 cells (ATCC CRL-8024, LGC Standards) were seeded in T12.5 flasks at a cell density of 1e5 viable cells per cm^2. Cells were maintained at 37°C and 5% CO_2 in Eagle minimum essential medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 1% nonessential amino acids, 100 U/mL penicillin G, 100 µg/mL streptomycin, 2.5 µg/mL amphotericin B (PAN Biotech) and 30 mM $MgCl_2$ (Sigma-Aldrich). The medium was completely exchanged every 3–4 days. After 2 weeks, the overconfluent 3D cell layers were inoculated with 250 µL of HEV RNA positive patient material per T12.5 flask. Characterized HEV strains 14-16753 (subtype 3c, GenBank MK089849), 14-22707 (subtype 3e, GenBank MK089848), and 15-22016 (subtype 3f, GenBank

MK089847) served as positive controls and 0.2% BSA (w/v) in PBS^- as negative control. After an incubation of 75 min at room temperature, medium was added, and cells were incubated at 34.5°C and 5% CO_2. After 24 h, the supernatant was completely replaced with fresh medium, which was then refreshed every 3–4 days. Supernatants were collected on days 1, 4, and 7 postinoculation (dpi) and from then on weekly until 70 dpi.

2.6 | HEV ORF2 antigen ELISA

Supernatants from suspected robustly infected cell cultures (i.e., steadily increasing amounts of HEV RNA over time) were retested for ORF2 antigen using the HEV-Ag ELISA^{Plus} kit (Wantai). The ELISA was performed according to the manufacturer's protocol.

2.7 | HEV ORF2 immunofluorescence assay (IFA)

HEV RNA positive and control cell cultures were detached after different time points, seeded in a 96-well µ-plate (Ibidi) and grown to ~80% confluency within 2 days. Afterward, cells were washed with 0.05% Tween-20 (Sigma-Aldrich) (v/v) in PBS with Ca^{2+} and Mg^{2+} (PBS^+; Lonza) and fixed with 2% formaldehyde (Merck) (v/v) in PBS^+. After another washing step, cells were permeabilized with 0.1% Triton X-100 (Sigma-Aldrich) (v/v) in PBS^+ followed by blocking with 5% goat serum (Jackson ImmunoResearch) (v/v) in PBS^+. The blocking solution was then replaced by 10 µg/mL primary antibody anti-HEV ORF2 clone 1E6[15] (Merck) diluted in 5% goat serum (v/v) in PBS^+ and incubated for 1 h at room temperature. After washing, cells were incubated with 3.75 µg/mL secondary antibody Alexa Fluor® 488 AffiniPure™ goat anti-mouse IgG H + L (Jackson ImmunoResearch) and Texas Red™-X phalloidin (Thermo Fisher Scientific) diluted in 1% BSA (w/v) in PBS^+ in the dark for 1 h at room temperature. After washing, nuclei were stained with 0.3 µM DAPI (Thermo Fisher Scientific) in the dark for 5 min at room temperature. After washing, the stained cells were preserved in mounting medium (Ibidi). Fluorescent images were taken with a Keyence BZ-9000 microscope.

3 | RESULTS

3.1 | Detection and genotyping of HEV in the ejaculate of chronically infected patients

In addition to the ejaculate of three previously described patients (patients 1–3),[7] the ejaculate of six further immunosuppressed, chronically HEV-infected men was tested (Table 1). All patients received ribavirin treatment. Overall, HEV was detected in the ejaculate of seven of nine (78%) patients. In the majority of these patients (4/7, 57%), the viral load in the ejaculate was higher compared to other body fluids (median fold change of 106 vs. blood, 11 vs. feces and 558 vs. urine). Only patient 7 (1/7, 14%) had the lowest viral load in ejaculate, while in the remaining two patients (2/7, 28%) it was between the viral loads measured in blood and feces (patients 5 and 6). The dominant HEV subtype in this patient cohort was HEV-3c ($n = 7$, 78%). HEV fragments sequenced from patient 3 were most closely related to HEV-3i, while those from patient 7 could not be assigned to a distinct subtype and were therefore classified as monophyletic clade 3abjk.

3.2 | Proof of infectivity of HEV particles in the ejaculate

The first isolation experiment was performed with ejaculate from patients 1 and 2 in overconfluent PLC/PRF/5 cell layers. An

TABLE 1 Clinical characteristics of patients chronically infected.

Patient	Age (years)	Underlying Disease	HEV Subtype	HEV RNA (IU/mL)			
				Blood	Feces	Urine	Ejaculate
1	48	Heart transplant	3c	<2.4e1	n.t.	3.9e4	6.3e7
2	67	Lymphoma	3c	5.4e5	1.3e6	2.3e4	1.6e7
3	63	Heart transplant	3i	5.5e4	n.t.	n.t.	n.d.
4	56	Kidney transplant	3c	3.1e5	3.1e6	1.8e5	3.3e7
5	39	Kidney transplant	3c	5.1e4	1.1e2	n.t.	5.3e2
6	59	Lymphoma	3c	6.4e3	6.1e5	n.t.	1.5e5
7	61	Lymphoma	3abjk	4.9e7	9.0e7	n.t.	1.4e2
8	61	Heart transplant	3c	3.3e3	1.0e4	n.d.	n.d.
9	36	Kidney transplant	3c	6.0e3	n.d.	2.0e4	8.4e6

Note: HEV fragments sequenced from patient 7 could not be assigned to a distinct subtype and were therefore classified as monophyletic clade 3abjk.
Abbreviations: HEV, hepatitis E virus; n.d., not detected; n.t., not tested.

increase of HEV RNA in the supernatant usually within days to weeks post-inoculation is confirmative of infectious HEV in the inoculum, as shown for established and passaged positive control strains (Figure 1A). Cells inoculated with patient 1's ejaculate resulted in a steady increase of HEV RNA in the supernatant after 2 weeks postinoculation, indicating infectivity while no replication was observed for patient 2's ejaculate (Figure 1B). Although the HEV RNA concentration in patient 1's ejaculate lysate was almost 100-fold lower as compared to ejaculate supernatant, the measured viral RNA in cell culture supernatant was equally high as of 49 dpi. This suggests either faster replication kinetics of HEV derived from lysate or more infectious HEV particles being present in lysate. The infectivity and replication dynamics were confirmed by testing the supernatants for ORF2 antigen by ELISA (Figure 1C). In addition, intracellular ORF2 antigen was detected by IFA staining (Figure 1D).

3.3 | Infectivity of HEV particles varies depending on the patient's material

To compare the infectivity of studied particles originating from the male urogenital tract with particles from other body regions, HEV particles derived from different patient materials taken on the same day were studied. Patient materials were prepared for cell culture inoculation. Sterile-filtered materials were tested for HEV RNA, and viral loads were adjusted to the material with the lowest viral load by 10-fold serial dilutions. All diluted and undiluted materials, as well as positive control HEV strains, were inoculated onto overconfluent PLC/PRF/5 cell layers (Figure 2A–D). HEV from patient 4's feces and ejaculate but not from the urine, replicated in cell culture. Cells inoculated at a concentration of 1e6 HEV RNA c/ml suggested that more infectious viral particles were present in ejaculate lysate compared to supernatant since HEV from lysate replicated at an

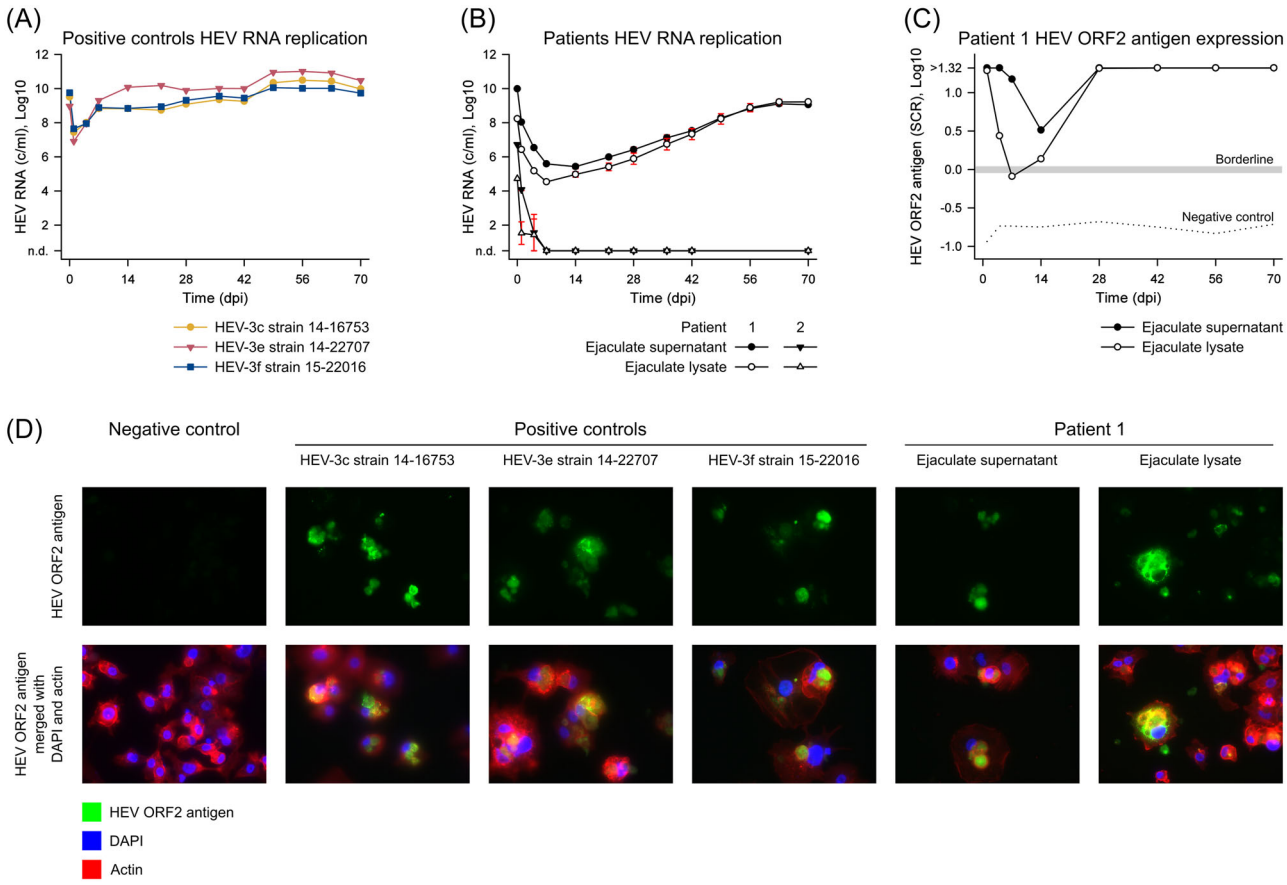

FIGURE 1 Infectivity of hepatitis E virus (HEV)-positive ejaculate of patients 1 and 2. Overconfluent PLC/PRF/5 cell layers were inoculated with (A) characterized HEV-3 strains and (B) HEV RNA positive ejaculate supernatant and lysate. Cell culture supernatants were tested for HEV RNA on days 1, 4, and 7 postinoculation and then once a week until day 70 postinoculation. PBS without Ca^{2+} and Mg^{2+} containing 0.2% BSA (w/v) served as negative control (all time points were PCR negative; data not shown). Patient results are shown as the mean of technical triplicates, error bars represent standard error of the mean. (C) Supernatants of the first of three technical replicates from cell cultures inoculated with patient 1's ejaculate were tested by ELISA for ORF2 antigen together with positive (all time points positive with OD > 4, which is equivalent to $\log_{10}$(SCR) > 1.32; data not shown) and negative controls. (D) In addition, cell cultures were tested for ORF2 antigen via immunofluorescence staining at 1050 dpi. BSA, bovine serum albumin; dpi, days postinoculation; n.d., not detected; OD, optical density; ORF2, open reading frame; PCR, polymerase chain reaction; SCR, signal to cutoff ratio.

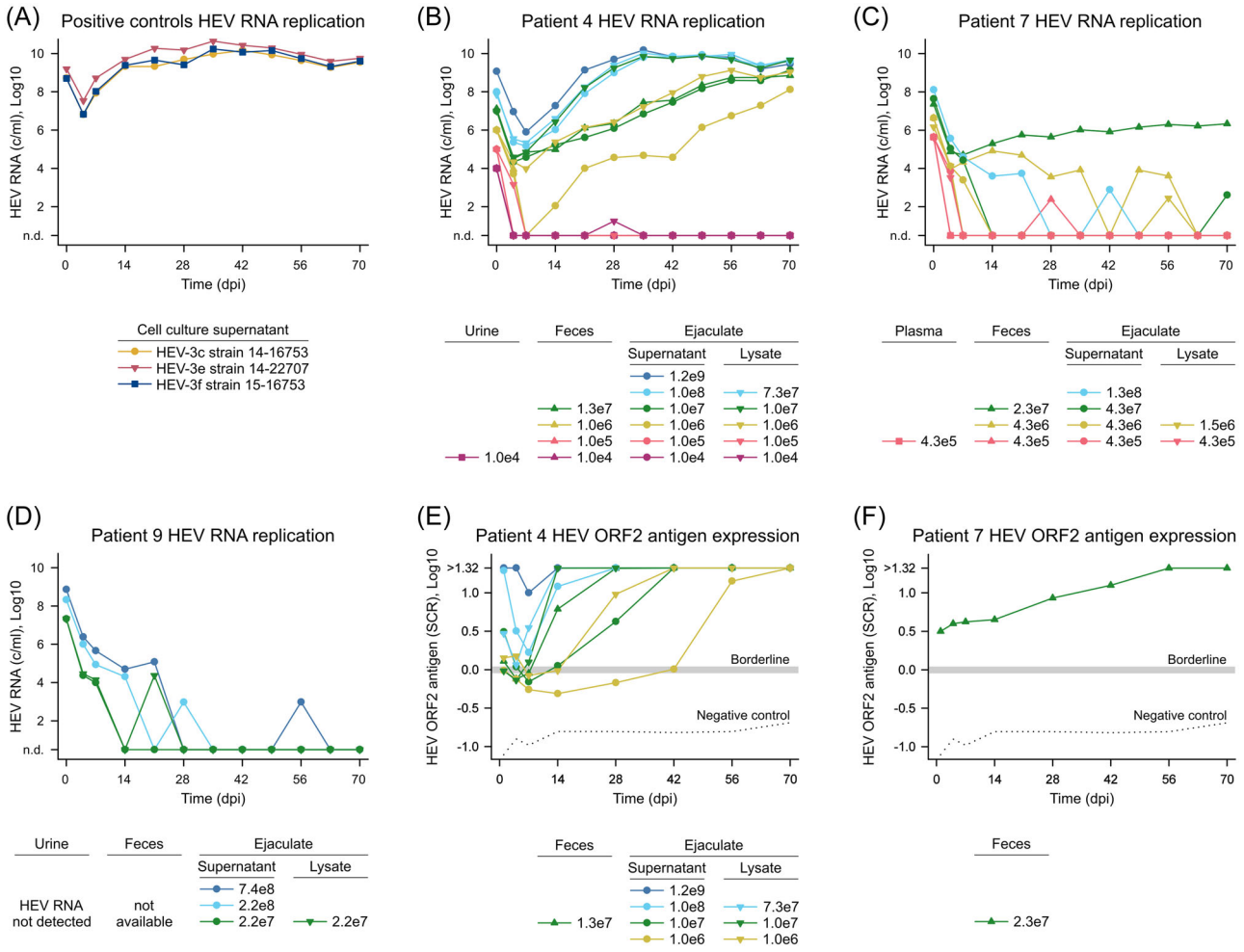

FIGURE 2 Comparison of the infectivity of hepatitis E virus (HEV) derived from different patient materials. Overconfluent PLC/PRF/5 cell layers were inoculated with (A) characterized HEV-3 strains and (B–D) various materials of three different patients (4, 7, and 9, respectively). Materials were patient-specific 10-fold serial diluted to match the viral load of the material with the lowest HEV RNA concentration. Cell culture supernatants were tested for HEV RNA on day 1, 4, and 7 postinoculation and then once a week until day 70 postinoculation. PBS without Ca^{2+} and Mg^{2+} containing 0.2% BSA (w/v) served as negative control (all time points were PCR negative; data not shown). (E, F) Supernatants with persistently detectable HEV RNA were tested by ELISA for ORF2 antigen together with positive (all time points positive with OD > 4, which is equivalent to $\log_{10}$(SCR) > 1.32; data not shown) and negative controls. BSA, bovine serum albumin; dpi, days postinoculation; n.d., not detected; OD, optical density; ORF2, open reading frame; PCR, polymerase chain reaction; SCR, signal to cutoff ratio.

average of 2.3 orders of magnitude higher level (as of 14 dpi), while too few infectious particles were present in feces to establish a persistent infection. However, at an inoculum concentration of 1e7 c/mL, HEV from feces and ejaculate supernatant replicated at a comparable level, which was still surpassed by HEV from ejaculate lysate at an average of 2.4 orders of magnitude between 14 and 35 dpi. No infection was established after inoculation at viral loads ≤1e5 HEV RNA c/mL. HEV derived from patient 7 stands in contrast to patient 4: only robust infection could be established by HEV from feces at a viral load of 2.3e7 HEV RNA c/mL. HEV from plasma, ejaculate supernatant, and lysate at viral loads of 4.3e5, 4.3e7–1.3e8, and 1.5e6 HEV RNA c/mL, respectively, infected the cell layer but did not result in persistent infection. Similarly, HEV from patient 9 derived from ejaculate supernatant and lysate at viral loads of 2.2e8–2.2e9 and 2.2e7, respectively, resulted in transient but not persistent infection. All persistent infections were confirmed by detecting ORF2 antigen in the cell culture supernatants by ELISA (Figure 2E,F), as well as intracellularly via IFA (Figure S1).

4 | DISCUSSION

Despite the detection of high concentrations of HEV RNA, and virions in the ejaculate of two chronically infected men, infectivity could not be proven in a previous cell culture model.[7] In the present work, five further cases with detectable HEV particles in the ejaculate could be identified. By using an optimized HEV cell culture system,[14] infectivity of HEV particles originating from male reproductive system could be proven. Furthermore, the infectivity of HEV from different body fluids was compared.

Depending on the patient, HEV replicated more efficiently from ejaculate or feces.

While HEV from patient 4's ejaculate was most infectious compared to feces, replication of HEV from feces of patient 7 was much more effective to replicate in vitro in comparison to particles from the ejaculate. At present, it remains unclear whether the observed differences in the infectivity of HEV particles from different patient's materials are due to methodological conditions (i.e., storage time, transport, cooling, etc.), different virus subtypes (HEV-3c from patient 4 vs. HEV-3abjk from patient 7), individual ribavirin treatment course and physical or biological properties. Furthermore, there is evidence that HEV from ejaculate lysate is more infectious than from ejaculate supernatant. HEV particles are naked in feces, bile duct, and cell culture lysate, while in serum, urine, ejaculate, and cell culture supernatant they appear quasi-enveloped.[7,16–18] Since, the HEV RNA distribution of ejaculate and cell culture supernatant is nearly equal across the density gradient, one could assume, that the particles in ejaculate lysate are non-enveloped compared to the enveloped particles from the ejaculate supernatant. This would also be in line with previous observations which had demonstrated that naked HEV is more infectious than quasi-enveloped HEV.[19]

HEV-3 is known to be transmitted mostly through consumption of insufficiently cooked pork but also to a much lesser extent via contaminated blood products and solid organ transplants.[2–4] We now show that HEV particles in the ejaculate of chronically infected men are infectious. Thus, chronic HEV infection in men could eventually be considered a sexually transmitted disease (STD). This would further blur the traditionally sharp categorical distinction between HBV, HCV, and HDV on the one side versus HAV and HEV on the other. To date, however, there is no epidemiological evidence of sexual transmission of HEV—at least not in immunocompetent individuals.[20,21] Future studies should focus on the standardized testing of anti-HEV IgG and IgM positivity in sexual partners of chronically HEV-infected men.

We identified seven out of nine chronically infected HEV patients in whom the virus was detectable in their ejaculate. This high rate implies a common new route of shedding in this patient cohort. Furthermore, we show for the first time that HEV in the ejaculate is infectious, suggesting a novel possible transmission risk and considering hepatitis E as a potentially sexually transmittable disease.

AUTHOR CONTRIBUTIONS

Mathias Schemmerer: Conceptualization; methodology; software; validation; formal analysis; investigation; data curation; visualization; writing—original draft preparation. **Hans H. Bock**: Investigation; writing—review and editing. **Jörn M. Schattenberg**: Investigation; writing—review and editing. **Samuel Huber**: Investigation; writing—review and editing, **Susanne Polywka**: Investigation; writing—review and editing. **Maria Mader**: Methodology; writing—review and editing. **Ansgar W. Lohse**: Conceptualization; writing—review and editing; supervision. **Daniel Todt**: Methodology; investigation; writing—review and editing. **Eike Steinmann**: Methodology; writing—review and editing. **Jürgen J. Wenzel**: data curation; writing—review and editing; supervision. **Thomas Horvatits**: Conceptualization; investigation; writing—review and editing. **Sven Pischke**: Conceptualization; validation; investigation; writing—original draft preparation. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

Hans H. Bock received consulting fees from Gilead, Abbvie, and Ipsen; payment or honoraria for lectures or presentations from Gilead and Abbvie. Jörn M. Schattenberg received consulting fees from Akero, Alentis Therapeutics, Astra Zeneca, 89Bio, Boehringer Ingelheim, GSK, Ipsen, Inventiva Pharma, Madrigal, MSD, Northsea Therapeutics, Novartis, Novo Nordisk, Pfizer, Roche, Sanofi and Siemens Healthineers; owns stock options from AGED diagnostics, Hepta Bio; received speaker fees from Gilead Sciences, Advanz, Echosens and MedPublico GmbH. Mathias Schemmerer received a lecture fee from Mikrogen GmbH. The remaining authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Pallerla SR, Harms D, Johne R, et al. Hepatitis E virus infection: circulation, molecular epidemiology, and impact on global health. *Pathogens*. 2020;9(10):856. doi:10.3390/pathogens9100856
2. Ji Y, Li P, Jia Y, et al. Estimating the burden and modeling mitigation strategies of pork-related hepatitis E virus foodborne transmission in representative European countries. *One Health*. 2021;13:100350. doi:10.1016/j.onehlt.2021.100350
3. Cordes AK, Goudeva L, Lütgehetmann M, et al. Risk of transfusion-transmitted hepatitis E virus infection from pool-tested platelets and plasma. *J Hepatol*. 2022;76(1):46-52. doi:10.1016/j.jhep.2021.08.018
4. Schlosser B, Stein A, Neuhaus R, et al. Liver transplant from a donor with occult HEV infection induced chronic hepatitis and cirrhosis in the recipient. *J Hepatol*. 2012;56(2):500-502. doi:10.1016/j.jhep.2011.06.021
5. Ma Z, de Man RA, Kamar N, Pan Q. Chronic hepatitis E: advancing research and patient care. *J Hepatol*. 2022;77(4):1109-1123. doi:10.1016/j.jhep.2022.05.006
6. Kamar N, Abravanel F, Behrendt P, et al. Ribavirin for hepatitis E virus infection after organ transplantation: a large European retrospective multicenter study. *Clin Infect Dis*. 2020;71(5):1204-1211. doi:10.1093/cid/ciz953

7. Horvatits T, Wißmann J-E, Johne R, et al. Hepatitis E virus persists in the ejaculate of chronically infected men. *J Hepatol*. 2021;75(1):55-63. doi:10.1016/j.jhep.2020.12.030
8. Allweiss L, Gass S, Giersch K, et al. Human liver chimeric mice as a new model of chronic hepatitis E virus infection and preclinical drug evaluation. *J Hepatol*. 2016;64(5):1033-1040. doi:10.1016/j.jhep.2016.01.011
9. Baylis SA, Blümel J, Mizusawa S, et al. World Health Organization International Standard to harmonize assays for detection of hepatitis E virus RNA. *Emerging Infect Dis*. 2013;19(5):729-735. doi:10.3201/eid1905.121845
10. Wenzel JJ, Preiß J, Schemmerer M, Huber B, Plentz A, Jilg W. Detection of hepatitis E virus (HEV) from porcine livers in Southeastern Germany and high sequence homology to human HEV isolates. *J Clin Virol*. 2011;52(1):50-54. doi:10.1016/j.jcv.2011.06.006
11. Boxman ILA, Jansen CCC, Hägele G, et al. Porcine blood used as ingredient in meat productions may serve as a vehicle for hepatitis E virus transmission. *Int J Food Microbiol*. 2017;257:225-231. doi:10.1016/j.ijfoodmicro.2017.06.029
12. 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. doi:10.1099/jgv.0.001435
13. Pearson WR. Finding protein and nucleotide similarities with FASTA. *Curr Protoc Bioinformat*. 2016;53:3.9.1-3.9.25. doi:10.1002/0471250953.bi0309s53
14. Schemmerer M, Johne R, Erl M, Jilg W, Wenzel JJ. Isolation of subtype 3c, 3e and 3f-like hepatitis E virus strains stably replicating to high viral loads in an optimized cell culture system. *Viruses*. 2019;11(6):483. doi:10.3390/v11060483
15. Riddell MA, Li F, Anderson DA. Identification of immunodominant and conformational epitopes in the capsid protein of hepatitis E virus by using monoclonal antibodies. *J Virol*. 2000;74(17):8011-8017. doi:10.1128/jvi.74.17.8011-8017.2000
16. Takahashi M, Yamada K, Hoshino Y, et al. Monoclonal antibodies raised against the ORF3 protein of hepatitis E virus (HEV) can capture HEV particles in culture supernatant and serum but not those in feces. *Arch Virol*. 2008;153(9):1703-1713. doi:10.1007/s00705-008-0179-6
17. Marion O, Capelli N, Lhomme S, et al. Hepatitis E virus genotype 3 and capsid protein in the blood and urine of immunocompromised patients. *J Infect*. 2019;78(3):232-240. doi:10.1016/j.jinf.2019.01.004
18. Todt D, Friesland M, Moeller N, et al. Robust hepatitis E virus infection and transcriptional response in human hepatocytes. *Proc Natl Acad Sci USA*. 2020;117(3):1731-1741. doi:10.1073/pnas.1912307117
19. Yin X, Ambardekar C, Lu Y, Feng Z. Distinct entry mechanisms for nonenveloped and quasi-enveloped hepatitis E viruses. *J Virol*. 2016;90(8):4232-4242. doi:10.1128/JVI.02804-15
20. Migueres M, Ducours M, Dimeglio C, et al. No evidence of sexual transmission of HEV among individuals using HIV pre-exposure prophylaxis. *J Viral Hepatitis*. 2020;27:1495-1501. doi:10.1111/jvh.13367
21. Spada E, Costantino A, Pezzotti P, et al. Hepatitis E virus infection prevalence among men who have sex with men involved in a hepatitis A virus outbreak in Italy. *Blood Transf*. 2019;17(6):428-432. doi:10.2450/2019.0209-19

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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