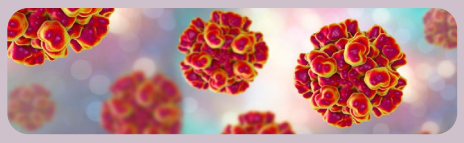
Poster Abstracts

Topics:



Hepatitis E virus Infection in Vietnamese Pregnant Women with Hepatitis B: Prevalence and Clinical Outcomes

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Abstract (244 words)

Background: Hepatitis E virus (HEV) infection during pregnancy is associated with obstetric complications and adverse maternal and neonatal outcomes. This study aimed to determine the seroprevalence of HEV and RNA positivity in both healthy pregnant women and women co-infected with hepatitis B virus (HBV).

Methods: A cross-sectional study was conducted involving 528 pregnant women (278 with and 250 without HBsAg) in their third trimester. Anti-HEV specific IgG and IgM antibodies were tested using ELISA, while HEV-RNA was detected by nested PCR. The status of anti-HEV antibodies was analysed regarding pregnancy outcomes and the risks of obstetric complications.

Results: The results indicated that 24% (127/528) of participants tested positive for anti-HEV IgG, while 2.5% (13/528) showed detectable anti-HEV IgM. Among HBV-positive women, 26% (55/250) had anti-HEV IgG, comparable to 22% (61/278) in HBV-negative controls. Notably, 28% (140/501) of cord blood samples were positive for anti-HEV IgG. No cases of HEV-RNA were detected. The prevalence of anti-HEV IgG increased with maternal age and was associated with higher birth weights. Anti-HEV IgM positivity was associated with shorter gestation ($p<0.05$), an increased risk of infant respiratory failure (OR=29; $p=0.03$) and neonatal infections (OR=27; $p=0.01$). Among HBsAg-positive women, those with anti-HEV IgG (26%) had higher gestational age at delivery, infant birth weights, but lower platelet counts and prothrombin times ($p<0.05$).

Conclusions: These findings highlight the endemic nature of HEV in Vietnam and underscore the potential risks of co-infection with HBV during pregnancy, which may lead to adverse obstetric outcomes.

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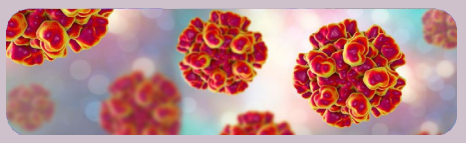

Hyper-E$_{INS}$: A tool for automated identification of insertions in the hepatitis E virus hypervariable region

Maximilian K Nocke[1,2], Michael Hermann Wißing[1], Leonard Knegendorf[1,3], Patrick Behrendt[3,4,5], Heiner Wedemeyer[4,5], Eike Steinmann[1,6], Daniel Todt[1,2]

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Abstract

Introduction:

Most hepatitis E virus (HEV) infections are asymptomatic and self-limiting, while immunocompromised or other risk group patients may develop chronic courses. The hypervariable region (HVR) within HEV's first open reading frame (ORF1) is known to integrate sequences of human origin. Several of previously identified insertions are linked to treatment failures.

With the development of Hyper-EINS, we aim to provide a time efficient bioinformatics pipeline tool that automates identification and validation of insertions in high-throughput sequencing (HTS) data, while offering an easy-to-use graphical user interface to simplify accessibility. Our tool will allow early identification of insertions possibly linked to treatment failure.

Methods:

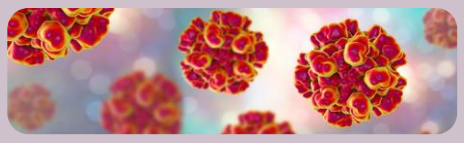
We collected longitudinal samples of a chronic infected patient experiencing ribavirin treatment failure. To gain insight on HVR insertions over the course of disease, we performed Illumina and Sanger sequencing on patient's samples. We applied our bioinformatics tool Hyper-Eins to identify insertions.

Results:

Hyper-EINS in silico analysis identified several novel insertions per sample. The most frequent ones were SERPINA1 and TRIM22 that were already detected pretreatment. During drug administration, the frequencies of viral genomes harboring insertions increased. SERPINA1 and TRIM22 insertions were shown to alter HEV replication capacity

Conclusions:

In conclusion Hyper-EINS has been designed as a user-friendly tool for detecting insertions of human or viral origin in the HVR of HEV from HTS data using computers with limited RAM and processing power. The graphical user interface visualizes the pipeline's output to make data interpretation and validation easier for the user. Early identification of HVR rearrangements in HEV infected patients can guide treatment decisions in a personalized medicine approach, based on both amplicon sequencing data specifically of the HVR or more spanned genomic regions covering the HVR. Hyper-E$_{INS}$ is written in Julia and will be publicly available in the future.



A 5-year serological follow-up study of hepatitis E virus: persistent IgM and declining IgG levels in asymptomatically infected blood donors

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Abstract (max. 300 words)

Introduction:

In recent years, the Hepatitis E virus (HEV) has received considerable attention in the field of transfusion medicine. The implementation of mandatory testing protocols in Europe has not only improved the safety of blood products, but has also provided valuable insights into the transmission and immunological aspects of HEV infection.

Methods:

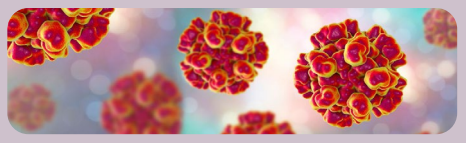
We followed a group of 497 blood donors who tested positive for HEV RNA at the time of donation. Follow-up samples were collected and serologically analysed for 370 of these donors over a period of up to five years after their index donation.

Results:

As expected, we found an initial increase in immunoglobulin M (IgM) and G (IgG) titres, followed by a gradual decline over the years. In particular, we found that 7.3% of participants remained positive for anti-HEV IgM (indicating long-term IgM positivity), while 9.1% were negative for anti-HEV IgG (a phenomenon known as seroreversion) during the five-year follow-up period, as determined by serological tests from three different manufacturers. These findings have implications for the assessment of the relationship between incidence and seroprevalence, which is influenced by the sensitivity and specificity of the serological tests used. In addition, there appears to be a gender bias, suggesting that women may have a stronger and more prolonged immune response.

Conclusions:

This research provides new perspectives on the long-term development of immunity against HEV, adding to the existing short-term epidemiological data on incidence and seroprevalence.



HBV Management: Challenges in Eastern Asia

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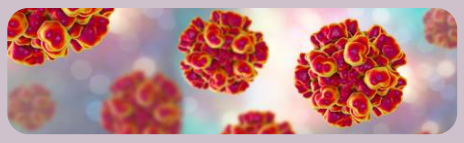
Hepatitis B virus (HBV) remains a critical global health issue, with over 290 million chronic infections worldwide, and poses a particularly significant public health concern in Eastern Asia, including countries like China and Vietnam. In these regions, HBV is a leading cause of liver cirrhosis (LC) and hepatocellular carcinoma (HCC). Advances in HBV management have improved prevention, diagnosis, and treatment outcomes. Universal vaccination programs, especially the inclusion of the hepatitis B vaccine in childhood immunization schedules, have substantially reduced new infections. Enhanced diagnostic tools, such as HBV-DNA quantification, have enabled better disease monitoring and treatment stratification. Antiviral therapies have shown high efficacy in achieving viral suppression with minimal resistance. However, a functional cure, defined as sustained loss of hepatitis B surface antigen (HBsAg), remains a significant challenge. Emerging therapies, including entry inhibitors, immune modulators, and gene-editing technologies, provide promising avenues for improved outcomes. Eastern Asia faces unique barriers to HBV management, including high rates of chronic infection, limited healthcare access in rural areas, socio-cultural problems to screening and treatment. Co-infections with other hepatotropic viruses, such as hepatitis C, D and E, and immune modulation during pregnancy further complicate clinical outcomes. Socioeconomic disparities and inadequate access to advanced diagnostic tools in resource-limited settings exacerbate these challenges. Addressing these issues requires region-specific strategies, such as integrating maternal healthcare services with HBV screening, expanding access to affordable treatments, and enhancing community awareness programs. Collaborative efforts among policymakers, healthcare providers, and researchers are essential to overcoming these obstacles, reducing the disease burden, and achieving global HBV elimination targets. This presentation will review the epidemiology of HBV in Eastern Asia with the focus on Vietnam, highlight advancements in management, and discuss the potential of innovative therapies to accelerate progress toward HBV elimination.



Acute hepatitis E virus infection is a relevant cause of decompensation and acute-on-chronic liver failure in patients with liver cirrhosis

Authors: Katja Dinkelborg [1,2,3]*, Christian Niehaus[1,2,4]*, Birgit Bremer[1], Christine Wundes[2], Anja Tiede[1], Natalie Petruch[1], Anke Kraft[1,2,3,4], Markus Cornberg[1,2,3,4,5], Heiner Wedemeyer[1,2,3,5], Henning Abels[6], Patrick Behrendt[1,2,3‡], Benjamin Maasoumy[1,3‡]

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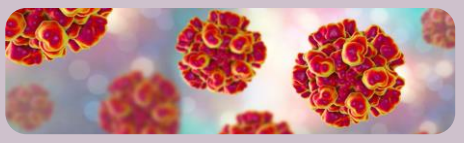
Abstract (max. 300 words)

Introduction:

Hepatitis E virus (HEV) infections are typically asymptomatic in immunocompetent individuals. However, in patients with pre-existing liver disease, HEV infection significantly increases the risk of developing acute-on-chronic liver failure (ACLF), a condition associated with high morbidity and mortality. This study investigates the impact of acute HEV infection on individuals with advanced liver cirrhosis in a hospital setting in northern Germany, where HEV genotype 3 predominates. The aim is to elucidate the role of HEV infection in exacerbating liver disease progression and severity in this high-risk population.

Methods:

A cohort of 698 patients with advanced liver cirrhosis was analyzed. Anti-HEV IgG seroprevalence was determined in 332 patients, while a subset of 184 patients was tested for anti-HEV IgM and HEV RNA. Additionally, a retrospective analysis was conducted on patients hospitalized for acute HEV infection at Hannover Medical School between 2014 and 2024.

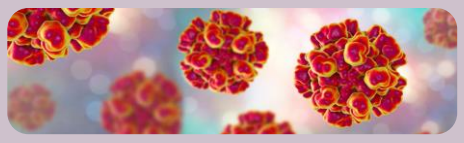


Results:

Anti-HEV IgG seroprevalence in patients with advanced liver cirrhosis was 32.8% (109/332). Among 249 tested sera, two were positive for HEV RNA, with one case remaining undetected during hospitalization. Additionally, ten patients tested positive for anti-HEV IgM, indicating recent HEV infection. Retrospective analysis identified 32 cirrhotic patients hospitalized with acute HEV infection over the past decade. Of these, 16 developed ACLF, resulting in a 31.3% mortality rate (5/16), while 18.8% (3/16) required liver transplantation to survive.

Conclusions:

Patients with advanced liver cirrhosis are vulnerable to acute HEV infection, which represents a significant precipitant of ACLF, characterized by high mortality and a substantial need for liver transplantation. These findings underscore the importance of early detection and management of HEV infections in this high-risk population.



A glycan-sensitive ORF2-ELISA and humoral immunity analysis in a large Hepatitis E virus cohort reveal determinants of chronicity

Lukas Fehlau*[1,2], Jonathan Garn*[1,2], Katja Dinkelborg[1,2,3], George Ssebyatika[4], Benjamin Maasoumy[2,3], Anke Kraft[2,5], Heiner Wedemeyer[2,3,6], Thomas Krey[3,4], Patrick Behrendt[1,2,3]

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*contributed equally

Abstract (max. 300 words)

Introduction:

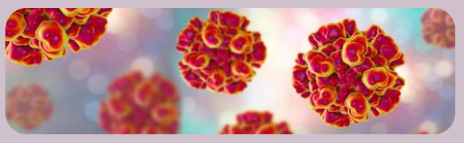
The hepatitis E virus (HEV) is the most common cause of acute viral hepatitis worldwide. Immunocompromised individuals are endangered to develop chronic infection potentially progressing to cirrhosis. Reasons for chronicity remain incompletely understood. Previous studies suggested the humoral immunity and HEV antigens as factors of chronicity. The main antigen of HEV, pORF2, exists in two forms: the capsid protein ORF2c and the secreted decoy protein ORF2s. To identify clinical and serological determinants of the course of infection, we analysed a cohort of HEV patients from a tertiary centre in Northern Germany.

Methods:

We collected serum samples from immunocompetent and immunocompromised patients with acute and chronic infection at various timepoints during and after infection. HEV antigen was assessed with commercial Wantai ELISA and our novel ELISA differentiating ORF2c and ORF2s. Humoral immunity was investigated using Wantai IgG ELISA, avidity ELISA and neutralisation assay.

Results:

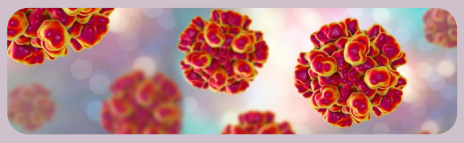
Clinically, solid organ transplantation and usage of Tacrolimus were found as determinants for HEV chronicity. Also, higher levels of liver enzymes were found in the acute patients and higher serum creatinine levels in chronic patients.



Serologically, both ORF2c and ORF2s were elevated in chronic patients. In contrast, higher levels of anti-HEV IgG and higher neutralisation capacity of sera were observed in self-resolving patients during viremia. Also, we could not show an increase of IgG avidity over time in the chronically infected patients. Our novel antigen ELISA enabled specific detection of ORF2c and showed stronger correlation with HEV-RNA than the commercial ELISA.

Conclusions:

Our study indicates the quality of humoral immunity as an important determinant of spontaneous HEV clearance. Also, it showed that high ORF2c and ORF2s levels are associated with HEV chronicity, presumably by hampering the humoral immune response. These parameters, observable early during infection, might be used to predict chronic infection courses in order to optimise individual therapy approaches.



Ribavirin treatment for severe hepatitis E: a case report from Portugal (2024)

Authors: Joana Costa[1], Rita de Sousa[2], Inês C. Conceição[1], Anton Gameiro[1]

Affiliations: [1]SAMS Hospital, Lisbon, Portugal; [2]National Institute of Health Doutor Ricardo Jorge, Lisbon, Portugal

Abstract

Introduction:

Hepatitis E is a liver infection caused by the hepatitis E virus (HEV). In Europe, HEV genotype 3 is the most prevalent, and the main route of human infection is through the consumption of pork meat products. Most infections are asymptomatic but severe acute hepatitis can occur in certain high-risk groups, including individuals with chronic liver disease, immunocompromised patients, and those with other underlying liver conditions. This report presents a unique case of a patient who developed severe HEV infection despite lacking any established risk factors.

Methods:

A 49-year-old man was admitted to the hospital presenting with flu-like symptoms lasting 5 days. He reported no recent travel abroad but mentioned consuming wild boar meat. ALT levels went from 4179 to 13737 U/L in 24 hours. HEV infection was confirmed by the presence of anti-HEV IgM and IgG antibodies, along with detection of HEV RNA in blood and stool. Further characterization identified the strain as HEV genotype 3c.

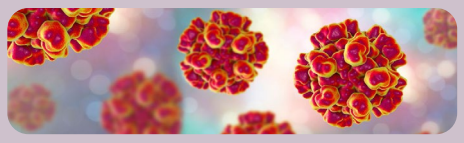
Results:

The patient was treated with ribavirin at 1000 mg/day for three weeks, resulting in a rapid decline in transaminase levels. Serum viral clearance was achieved within 6 weeks.

Conclusions:

HEV infection is an under-recognized cause of acute hepatitis and extrahepatic manifestations in high-income countries, including Portugal. Although increased clinician awareness and advancements in virological diagnostics have enhanced our understanding of the disease, uncertainties remain regarding the factors that trigger severe cases in patients without apparent risk factors.

The use of ribavirin for severe acute HEV infections remains controversial. While ribavirin has demonstrated efficacy in chronic HEV cases, evidence supporting its use in acute severe infections is limited, and its role in preventing progression to chronicity is still under debate. This case underscores the need for further research to identify predictors of severe disease and optimize therapeutic strategies.



Negative effect of early ribavirin discontinuation in chronically infected HEV patients and insights in whole-genome sequencing using metagenomics

Authors: Sergi Colomer-Castell (1,2,3), Marta Ibañez-Lligoña (1,2,3), Josep Gregori (1), Damir Garcia-Cehic (1,4), Mar Riveiro-Barciela (1,2,3), María Buti (1,2,3), Ariadna Rando-Segura (1,2), Carolina Campos (1,2,3), Álvaro Gónzalez-Camuesco (1,2), Caroline Melanie Adombi (5), Maria Isabel Costafreda (2,6), Susana Guix (2,6), Josep Quer (1,2,3)

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Abstract (max. 300 words)

Introduction:

HEV is a leading cause of acute viral hepatitis, particularly in low- and middle-income countries, and its incidence is rising in industrialized nations. While HEV usually causes acute infections, it can become chronic in immunocompromised individuals. Due to the lack of polymerase proofreading, HEV accumulates mutations during replication, forming a quasispecies. Ribavirin is the main treatment for chronic HEV, though resistance can develop.

Methods:

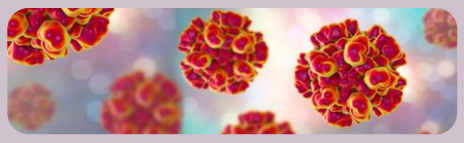
In this study, several samples from a chronic HEV patient treated at different stages with ribavirin –mutagen- have been collected during the course of the infection. To study HEV quasispecies, RNA was extracted and purified, a conserved fragment of ORF2 was amplified using consecutive RT-PCR, Nested-PCR and sequenced using NGS MiSeq platform, obtaining a high coverage for every sample. For the whole genome obtention, Viral MinElute Virus Spin kit nucleic acid extraction was applied to the same samples followed by shotgun metagenomics using NextSeq2000 platform.

Results:

HEV quasispecies showed high nucleotide complexity but limited protein-level variability. Ribavirin treatment increased rare haplotypes and significantly reduced the master haplotype, despite high doses, with resistance linked to rising viral loads. This raised questions about whether resistance arose from inherently resistant quasispecies or specific substitution combinations. Most ribavirin-induced variability involved synonymous mutations, preserving dominant protein-level haplotypes. To explore these mechanisms, a metagenomics sequencing method was developed, achieving 100% HEV genome coverage in samples with high viral loads (>10^4 part/mL). This method enables detailed investigation of resistance mutations, with current analysis ongoing and optimization underway to clarify the pathways driving ribavirin resistance.

Conclusions:

The HEV viral population is able to take advantage of the ribavirin-induced variability when ribavirin regime is stopped before HEV complete negativization. Also, shotgun metagenomics technique is capable to recover HEV whole genome when there is enough viral load, allowing variant calling analysis.



The road towards a decision support tool for HEV treatment failure and chronicity

Saskia Janshoff[1,2], Ricarda Plümers[2], André Gömer[1], Katja Dinkelborg[3,4,5], Patrick Behrendt[3,4,5], Daniel Todt[1], Tanja Vollmer[2] , Heiner Wedemeyer[3,5], Eike Steinmann[1]

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Abstract (max. 300 words)

Introduction:

The hepatitis E virus (HEV) is estimated to cause more than 420,000 acute hepatitis infections in Germany annually, leading to approximately 4,000 cases of severe liver disease. Immunocompromised patients, such as transplant recipients, are particularly at risk of developing severe chronic hepatitis, which cannot be effectively treated due to the lack of HEV-specific antivirals or the development of resistance variants. Although diagnostic methods have been successfully established, next-generation sequencing pipelines to analyze viral fitness and antiviral resistance in a personalized medicine approach are still lacking. In an attempt to characterize circulating HEV variants that may interfere with treatment success, we aim to improve next-generation sequencing-based diagnostic methods to detect HEV variants in a collaborative effort between clinicians, diagnostics and virologists.

Methods:

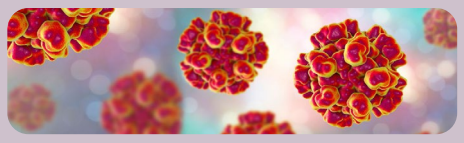
We established a longitudinal cohort of 90 patients with chronic HEV infection and a cohort of 497 blood donors who tested positive for HEV RNA. Clinical parameters including liver enzyme levels, HEV antibody status and demographics were collected for these patients. In addition, we generated amplicons spanning the polymerase and hypervariable region to analyze intra-host diversity and assess resistance profiles. These data will be used to correlate viral genomics, clinical parameters and disease progression to generate a decision support tool.

Results:

We were able to generate 106 amplicons from blood donors with acute HEV infection. Success in amplicon generation correlated with viral load. Initial results from amplicon sequencing suggest low viral heterogeneity during the course of acute infection. The majority of samples are currently being sequenced.

Conclusions:

Sequence information about viral population heterogeneity in chronic and acute patients will be correlated with clinical data in a machine learning approach to identify predictors of chronicity and treatment failure. This knowledge will be used in a decision support tool to aid clinical decision making.



Bioprinted Organ Models for Infection Studies

Beatrice Tolksdorf, Johanna Berg, Jens Kurreck

Affiliations: Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany

Abstract

Introduction:

The current research paradigm is based on 2D cell culture studies and animal models. Results from these experiments, however, are often not translatable to the human patients. Bioprinting technologies allow to produce sophisticated organ models composed of human cells. The aim of the present study is to develop organ models for application in infection studies.

Methods:

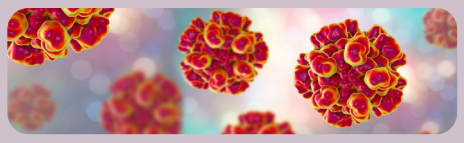
We used pneumatic extrusion bioprinting to generate 3D lung models. The models consisted of up to four cell types: an epithelial cell line (A549 or Calu-3), primary lung fibroblasts, THP-1 cells that were differentiated into macrophage-like cells, and HUVEC as endothelial cells. The lung models were cultured under Air-Liquid-Interface (ALI) conditions. Cell viability and infection with influenza A virus (IAV) were studied.

Results:

The lung model 2.0 consisted of an epithelial layer with A549 cells placed on a layer containing primary lung fibroblasts with THP-1 cells. The models retained high cell viability over a culture period of more than one month. The multi-cell type model had a higher cell viability than a single cell type model. IAV replicated in the epithelial cells of the bioprinted organ model. Antiviral treatment inhibited virus replication in a concentration-dependent manner. The latest version of the bioprinted model (lung model 3.0) contained an additional epithelial layer, and production of mucus was observed under ALI cultivation. Replacement of A549 cells by Calu-3 cells which express ACEII allowed infection with SARS-CoV-2, in addition to IAV.

Conclusions:

The current study provides a proof-of-principle that bioprinted organ models composed of human cells can be used to study virus replication and the antiviral activity of novel substances. The next step will be to use bioprinted liver models for the study of hepatitis E virus biology and its inhibition by newly developed antivirals.



Proliferative cell targeting and epithelial cell turnover fuels hepatitis E virus replication in human intestinal enteroids

Nanci Santos-Ferreira[1], Xin Zhang[2], Laura Corneillie[3], Jana Van Dycke[1], Claire Montpellier[3], Johan Neyts[2], Laurence Cocquerel[3], Suzanne J. F. Kaptein[2], Joana Rocha-Pereira[1]

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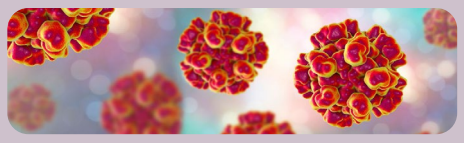
Abstract (max. 300 words)

Introduction: Hepatitis E virus (HEV) is a leading pathogen causing acute viral hepatitis globally. While HEV is primarily spread fecal-orally, the role of the gut in HEV pathogenesis remains largely unexplored, including how HEV disseminates from gut to liver, and whether the gut is an HEV reservoir. We here aimed to illuminate HEV biology in the gut using human intestinal enteroids (HIEs).

Methods: Three strategies were explored to establish an HEV-HIE infection model: three-dimensional (3D) HIEs, two-dimensional (2D) HIEs in transwell, and HEV RNA-electroporated HIEs. HEV particles produced by electroporated HIEs were characterized by western blot and gradient centrifugation. The intestinal tropism of HEV was investigated through confocal fluorescent microscopy and gene expression analysis.

Results: HEV infection in 3D-HIEs and 2D-HIEs showed limited replication, whereas HIEs electroporation led to a sustained increase in the release of non-enveloped infectious virions. These virions could re-infect new 3D-HIEs, yielding a ~2 log10 increase in HEV RNA. Remarkably, high expression of ORF2i capsid form was observed in the supernatant of HEV electroporated HIEs and abundant ORF2 staining was observed in proliferating cells, absorptive enterocytes, goblet and enteroendocrine cells. Additionally, HEV infected HIEs preserved the mature cell types and stem cell niche.

Conclusions: Overall, we established a robust HEV-HIE model that yields high titers of infectious non-enveloped virions. Proliferative cells and the fast intestinal epithelial cell turnover are important features that facilitate efficient HEV replication, and likely also its dissemination. This study suggests the gut is an HEV reservoir, capable of producing part of non-enveloped HEV shed in the feces.



Joint International Meeting on **Viral Infections of the Liver and the Heart, 17 – 18 January, 2024, Berlin, Germany**

Extrahepatic Manifestations of the Hepatitis E Virus: Replication in Kidney Cells and urine specific HEV-Variants Patterns *in vivo*

Avista Wahid[1,*], Nele Meyer[1,*], Lucas Hüffner[1], Christine Wundes[1], Saskia Janshoff[2,3], Martina Friesland[1], Katja Dinkelborg[1,4,5], Elmira Aliabadi[1], Fenja Laue[1], Markus Cornberg[4,5,6,7], Benjamin Maasoumy[4,5] , Birgit Bremer[4], Sven Pischke[8,9], Tobias Müller[10], Julian Schulze zur Wiesch[8,9], Julia Benckert[10], Rainer G. Ulrich[9,11], Svenja Hardtke[7,8,9], Petra Dörge[4,7], Florian Vondran[5,12], Ansgar Lohse[8,9], Michael Peter Manns[4,5], Daniel Todt[2,13], Heiner Wedemeyer[4,5], Thomas Pietschmann[1,5], Eike Steinmann[2], André Gömer[2,#], Patrick Behrendt[1,4,5,#]

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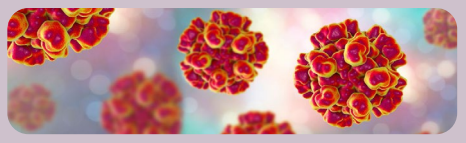


Abstract (max. 300 words)

Introduction:

Hepatitis E virus (HEV) is a leading cause of acute viral hepatitis worldwide. Additionally, HEV infections are associated with renal impairment, a notable extrahepatic manifestation, though the underlying mechanism remains elusive.

Methods:

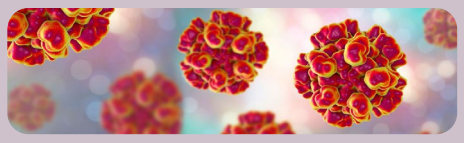
Two HEV-3 strains and one HEV-1 strain were used to study the viral replication cycle and the effect of antiviral agents in different human kidney cell lines in vitro. HEV RNA from urine, stool and plasma of chronically infected patients was sequenced to identify compartment specific variants. They were introduced into the parental HEV-3 p6 strain to assess their impact on virus replication, infectivity and response to antiviral therapeutics in liver and kidney cells in vitro.

Results:

Various kidney cell lines support the full replication cycle of the HEV-3 p6 strain in vitro. A reduced response to RBV was observed in kidney cells as compared to liver cells. In addition, replication capacity of HEV-3 83-2 and HEV-1 Sar-55 was demonstrated in kidney cells. Sequencing of HEV RNA from different body compartments of infected individuals revealed compartment-specific variants, that are currently characterized in vitro.

Conclusions:

Our results revealed that HEV can complete its full replication cycle in kidney cell lines in vitro, providing a potential mechanism for extrahepatic symptoms. Moreover, our results indicate that RBV efficacy is substantially reduced in kidney cell lines, which could possibly be correlated to antiviral resistance in patients. Sequencing of HEV RNA from different body specimens identified compartment-specific variants, the effects of which are currently being investigated, with the aim to enhance our understanding of how these variants may influence the replication dynamics of HEV and its overall pathogenicity in renal environments.



Joint International Meeting on **Viral Infections of the Liver and the Heart, 17 – 18 January, 2024, Berlin, Germany**

Phenotypic and Functional Shifts of CD8⁺γδ T-cells in acute and chronic hepatitis E virus infection

Authors: **Erich Freyer[1 2 3 4 5], Roni Souleiman[1 2 3 4 5], Katja S Steppich [1 2 3 4 5], Patrick Behrendt [1 3 4], Heiner Wedemeyer [1 3], Anke RM Kraft [# 1 2 3 4 5], Markus Cornberg [# 1 2 3 4 5]**

Affiliations: [1] Department of Gastroenterology, Hepatology, Infectious Diseases and Endocrinology, Hannover Medical School (MHH), Hannover, Germany. [2] Centre for Individualised Infection Medicine (CiiM), a joint venture between Helmholtz-Centre for Infection Research (HZI) and Hannover Medical School, Hannover, Germany. [3] German Centre for Infection Research (DZIF), partner site Hannover-Braunschweig, Germany. [4] TWINCORE, Centre of Experimental and Clinical Infection Research, a joint venture between Helmholtz-Centre for Infection Research (HZI) and Hannover Medical School, Hannover, Germany. [5] Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Hannover, Germany

Introduction:

Hepatitis E virus (HEV) is the leading cause of viral hepatitis worldwide, typically causing an acute, self-limiting infection in immunocompetent individuals. However, HEV genotype 3 can cause chronic hepatitis in immunocompromised patients. γδ T-cells, abundant in the liver, bridge innate and adaptive immunity and regulate chronic infections. This study investigates the phenotypic and functional adaptations of γδ T-cells during different stages of HEV infection.

Methods:

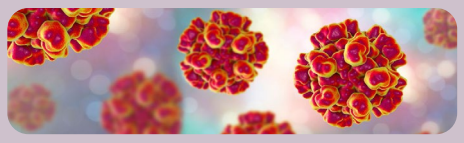
Peripheral blood mononuclear cells (PBMCs) from 53 patients (23 immunocompetent, 30 immunocompromised) with acute, chronic, or resolved HEV infections and 15 healthy controls were analyzed using high-parameter flow cytometry. Intracellular cytokine assays assessed functional responses following in vitro stimulation.

Results:

Dimensionality reduction revealed a distinct subset of CD8⁺γδ T-cells differing between acute and chronic HEV cases. In chronic HEV, CD8⁺γδ T-cells skewed towards Vδ1 and non-Vδ1Vδ2 subsets and displayed an effector phenotype with increased activation markers (CD38, CD69) and antiviral mediators (perforin, granzyme B, T-bet). Effector memory CD8⁺γδ T-cells (CCR7-/CD45RA-) correlated positively with liver inflammation (ALT) ($r=0.67$, $p<0.05$). These cells also expressed higher CD16 levels, indicating enhanced antibody-dependenT-cellular cytotoxicity (ADCC), but prolonged infection reduced CD16 expression. In chronic HEV, CD8⁺γδ T-cells showed reduced IFNγ production after TCR stimulation and weaker IL-17A and IL-10 responses following IL-12/IL-18 stimulation compared to acute HEV cases.

Conclusions:

CD8⁺γδ T-cells are a dynamic effector subset that adapts during HEV infection. In chronic HEV, their skewed effector phenotype and reduced regulatory functions may contribute to impaired antiviral responses and increased inflammation, promoting disease progression. These findings highlight their potential as biomarkers or therapeutic targets for chronic HEV infection.



Susceptibility of chimeric hepatitis E virus subtypes to antiviral treatment approaches

Rui Costa, Michelle Jagst, Alina Kohl, Daniel Todt, Eike Steinmann*, André Gömer*

Department of Molecular and Medical Virology, Ruhr University Bochum, Bochum, Germany

Abstract

Hepatitis E is an underestimated disease, causing a projected 20 million infections and up to 70,000 deaths annually. Infections are mostly asymptomatic but it can become chronic in immunocompromised patients and reach mortality rates up to 25% in pregnant women. HEV-3 is the most prevalent genotype in Europe and presently includes 14 subtypes. These can be grouped into three main clades: Clade 1 (3e-3g), Clade 2 (3a-3c, 3h-3m) and Clade 3 (3ra). Current treatment options are limited to off label use of antivirals ribavirin and sofosbuvir. Treatment responses have not been systematically evaluated against the different HEV subtypes due to the lack of robust viral systems. Therefore, we aimed to develop cell culture models for the incorporation of HEV subtypes into chimeric polymerase constructs to gain an in-depth mechanistic understanding of treatment responses.

Methods:

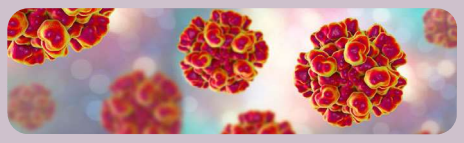
We performed a phylogenetic analysis of different HEV-3 subtypes and subsequently generated a library of 12 primary isolate derived polymerase chimera's representative of currently known HEV-3 subtypes in the backbone of the Kernow/C1 p6 subgenomic replicon (p6). We then assessed their replicative fitness in a time-kinetic experiment and their susceptibility to ribavirin and sofosbuvir.

Results:

Phylogenetic analysis revealed significant differences at the protein level between HEV-3 polymerases with up to 21 amino acid substitutions compared to p6. Of 12 polymerase chimeras, 11 showed replication at levels comparable to p6 or up to 3-fold higher. All subtypes were sensitive to clinically relevant antivirals. Importantly, ribavirin sensitivity was reduced in some chimeras.

Conclusions:

HEV showed high intra-genotypic genomic flexibility, allowing replication of different chimeric p6 constructs. This approach established as a robust method to assess intra-genotypic antiviral susceptibility of polymerase inhibitors. Understanding how genetic variants affect antiviral susceptibility remains an important aspect of HEV treatment regimens and disease burden.



Sentinel surveillance initiative for Hepatitis E virus (HEV) infection among cases attending four hospital sites located in four geopolitical zones of Nigeria (2019-2024).

Ikechukwu Nnaji[1],Johnson Ojo[1], Sikiru Badaru[1], Olajumoke Babatunde[1], Adetunji Adewusi[1], [1] Dominik Harms[2], Claus-Thomas Bock[2], Chidi Nnabuchi[3], Jacinta Akachukwu[3], Clementina Ngbadike[3], Sunday Ore[3], Garba Iliyasu[4], Amina Ibrahim-Mohammed[4], Rukayya Ibrahim-Hamza[4], Bala Musa[4], Charles Onyekwere[5], Peter Ogusanya[5], Adetilewa Onatola-Morakinyo[5], Segun Akinyoade[5], Shirley Chukwurah[6], Maria Onwunzo[6], Mercy Okere[6], Augustina Agbafuna[6], Adedeji Adebayo[1].

Nigeria Centre for Disease Control and Prevention (NCDC), Nigeria[1,] Robert

Koch Institute (RKI), Germany[2]

Asokoro District Hospital (ADH), Nigeria[3]

Aminu Kano Teaching Hospital (AKTH), Nigeria[4]

Lagos State University Teaching Hospital (LASUTH), Nigeria[5] Nnamdi

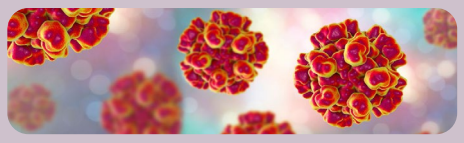
Azikiwe University Teaching Hospital (NAUTH), Nigeria[6]

Introduction: HEV is one of the leading causes of viral hepatitis in the world. Outbreak of this disease was recorded with significant morbidity and mortality among internally displaced population (IDP) of Borno State, Nigeria, in 2017. With poor understanding of the disease epidemiology among the general population, a surveillance programme was put in place, in 2019, consisting of four sentinel sites located in four geopolitical zones of Nigeria to, among others, determine the seroprevalence and distribution among acute cases of the disease.

Methods: The study was conducted between November, 2020 and June, 2024. A total of 301 patients who met the inclusion criteria of acute hepatitis were recruited into this study at four selected medical facilities located in the Northwest (AKTH), Northcentral (ADH), Southwest (LASUTH) and Southeast (NAUTH) geopolitical zones of Nigeria. Blood samples were collected from the cases and analysed at the National Reference Laboratory, Abuja for anti-HEV IgM and anti-HEV IgG using Enzyme-Linked Immunosorbent Assay (ELISA) technique. RNAs from positive samples were extracted and tested for presence of HEV genome by RT-PCR. Results were reported in seroprevalence rates.

Results: The overall seroprevalence of anti-HEV IgG and anti-HEV IgM among the cases was 17.6% (53/301) and 0.3% (1/301) respectively. Male has a higher prevalence of 20.1% (31/154) compared to female's 15.0% (22/147). The site distribution of anti-HEV IgG seropositivity are: AKTH 18.9% (20/106), LASUTH 6.3% (4/63), NAUTH 26.9%(25/93) and ADH 10.3% (4/39). Only 1 (0.9%) anti-HEV IgM positive case was recorded in AKTH. All serum samples tested negative for HEV RNA in PCR.

Conclusion: No significant differences occur in gender positivity to HEV anti-IgG, but in sentinel sites, highest specific positivity was recorded in NAUTH, a semi-urban population. Detection of anti-HEV- IgM is generally low signifying the need to intensify surveillance using this new sentinel surveillance approach in the country.



Title: The influence of the tissue-invasive intestinal nematode *Ascaris suum* on hepatitis E virus infection in pigs

A. Laubschat[1,4], L. Oser[1], I. Hrabal[2], M. Eiden[2], P. Geldhof [3], J. Schlosser-Brandenburg[4]

[1]Department of Veterinary Medicine, Institute of Immunology, Centre for Infection Medicine, Freie Universität Berlin, Berlin, Germany

[2] Institute for Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

[3] Laboratory of Parasitology, Department of Translational Physiology, Infectiology and Public Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

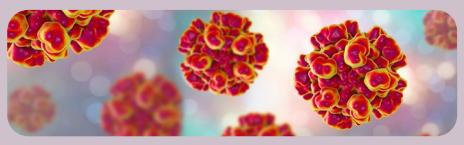
[4]MF 3, Animal Experimental Research and 3R, Robert Koch Institute, Berlin, Germany

Infections with the hepatotropic hepatitis E virus (HEV) and the tissue-invasive nematode *Ascaris suum* are widespread in pig production. Parasitic nematodes have been shown to weaken or override the host's immune response to viral infections. This can be attributed to dominant parasite-driven type 2 immune circuits counteracting type 1 immune mechanisms. Given the tropism of both pathogens for liver tissue, it seems likely that an interaction will occur. However, studies on the occurrence of *A. suum*/HEV coinfections in pigs are completely lacking.

We confirmed that repeated *A. suum* infections in pigs lead to the induction of hepatic Th2 immunity, as evidenced by a significant increase in the frequency of Gata3+ and IL-4-producing CD4+ T cells in the liver tissue and draining lymph nodes of *A. suum*-infected pigs compared to naïve controls. In light of these findings, we hypothesised that concurrent *Ascaris* infection might influence HEV pathogenesis in naturally coinfected pigs.

Therefore, blood and meat juice were collected from slaughtered fattening pigs to detect anti-*Ascaris* and anti-HEV antibodies. In addition, bile and liver tissue were analysed for the quantification of HEV RNA using qRT-PCR. Furthermore, pathological findings (e.g. 'milk spots', which are pathognomonic of *Ascaris*-induced parasitic hepatitis) were recorded for each animal examined.

Preliminary analyses revealed a high prevalence of HEV infection in pig production, with some pig farms simultaneously affected by *A. suum* infection. However, further research is needed to clarify the extent to which *Ascaris* can affect HEV pathogenesis and transmission, which may also have implications for public health.



Loss-of-function cDNA library screening for HEV restriction factors

Authors: Maximilian Beikirch, Richard J. P. Brown, Eike Steinmann, Daniel Todt

Affiliation: Department of Molecular and Medical Virology, Ruhr University Bochum

Abstract (max. 300 words)

Introduction:

HEV is an emerging disease worldwide, with an yearly incidence of 20 million infections. HEV's life cycle, its entry receptor and host restriction factors that suppress viral replication are poorly characterized. Infection with HEV can take severe courses in immunocompromised or pregnant women, whereas in healthy humans, the immune system clears the infection. Furthermore, mice are refractory to HEV infection. This study aims to ultimately identify host factors which help control HEV in healthy humans and mice.

Methods:

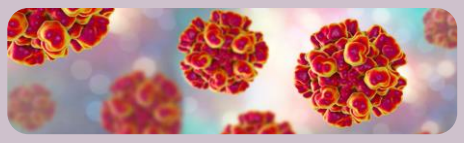
Human and mouse cDNAs were synthesized from liver RNAs using SMART technology and cloned into a lentiviral vector to produce cDNA libraries. To deliver cDNA libraries to cells, lentiviral pseudoparticles were produced in HEK-293T cells, followed by harvesting and titration. Hepatoma cells were transduced by library containing pseudoparticles and integrated cDNAs were assessed by RNA-seq.

Results:

After SfiI digestion and electrophoresis, vector backbone and insert smears are separated, suggesting successful ligation of cDNA pools. Insert smears indicate an unbiased distribution of genes, as expected. The mean insert size is ~1000 bp, with a low fraction of empty vectors. Sanger sequencing identified a high percentage of open reading frames in individual library clones, with diverse genes represented. Lentiviral pseudoparticles which deliver a GFP transgene in the vector backbone integrate well into Huh7 and Huh7.5 cells, seen clearly by immunofluorescence (IF) imaging. RNA-seq of library transduced cells was able to detect integrated cDNAs at around 1% of total cellular RNAs.

Conclusions:

The cDNA libraries were cloned, packaged and transduced into hepatoma cell-lines, with extensive quality checking at each stage. These characterized resources can now be used for unbiased loss-of-function screening for human and mouse HEV restriction factors.



Zoonotic Hepatitis E Virus in domestic pigs and farmed wild boars in Vietnam.

Le Chi Cao[1,2], Alexa Purgreth[1], Vo Minh Tiep[2], Tran Thi Giang[2], Le Nguyen Nhat Ha[2], Le Thi Kieu Linh[1,3], Truong Nhat My[3], Thirumalaisamy P Velavan[1,3]

[1] Institute of Tropical Medicine, University of Tübingen, Tübingen, Germany

[2] Hue University of Medicine and Pharmacy (HUMP), Hue University, Vietnam

[3] Vietnamese-German Center for Medical Research (VG-CARE), Hanoi, Vietnam

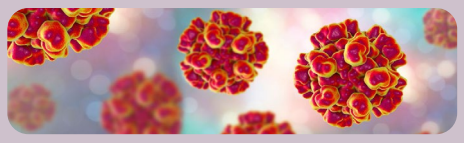
Abstract (244 words)

Introduction: Vietnam has unprecedented demand for meat from livestock such as pigs and farmed wildlife, which serve as zoonotic reservoirs for hepatitis E virus (HEV). This study aims to identify circulating zoonotic HEV in domestic pigs and farmed wild boar from the central and southern regions and subsequently characterise the genotypes to understand their distribution, transmission dynamics and associated human health burden

Methods: Rectal swabs, faeces and livers from pigs (n=427) and wild boars (n=101, excluding liver) were collected from different farms and slaughterhouses in central and southern Vietnam and analysed for HEV RNA by nested PCR. The HEV RNA-positive samples were then sequenced and characterised for HEV genotypes

Results: Of the pig samples tested, 7% (29/427) were positive for HEV RNA. Of these, 15% (14/92), 7% (6/88) and 4% (9/247) were positive in rectal swab, faecal and liver samples respectively. Of the wild boar samples, 25% (25/101) were positive for HEV RNA, with 29% (19/66) of rectal swabs and 17% (6/35) of faecal samples being positive. Sequencing showed that HEV subgenotype 3a was predominant, followed by subgenotype 4b and 3f2, which has high homology with human HEV 3 genotypes.

Conclusions: While there is still limited information on HEV genotypes infecting humans, our unpublished data offer insights into the dynamics of HEV transmission and show that domestic pigs and wild boar remain an important zoonotic reservoir for HEV. The non-enveloped naked HEV virions, which are transmitted enterically, may pose a risk of food-borne infection.



HOST FACTORS IN HEPATITIS E VIRUS INFECTION AND SPECIES BARRIERS

Authors: Leyla Sirkinti[1], Ian Perez Medina[1,2], Sarah Schlienkamp[1], Nicola Frericks[1], Volker Kinast[4], Maximilian K. Nocke[1,3], Eike Steinmann[4,5], Daniel Todt[1,3]

Affiliations: 1 Ruhr University Bochum, Department for Molecular and Medical Virology, Bochum, Germany

2 ESCI-UPF School of International Studies, Barcelona, Spain

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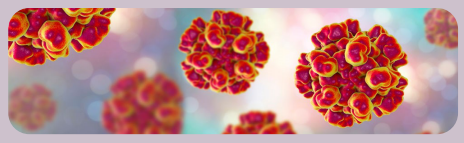
5 German Centre for Infection Research, External Partner Site, 44801 Bochum, Germany

Introduction: Hepatitis E virus (HEV) is a single-stranded RNA virus causing acute and chronic hepatitis, with around 20 million infections and 70,000 deaths annually. Zoonotic genotypes HEV-3 and HEV-4, primarily found in pigs and wild boars, are transmitted through animal products and can lead to chronic infections. Despite its global impact, HEV is often underdiagnosed, and a limited understanding of its replication cycle restricts the development of targeted therapies. HEV-3 is particularly notable for its ability to cross species, with pigs as primary reservoirs. While pigs remain asymptomatic, humans experience clinical outcomes. This study aims to explore the HEV-host interaction, focusing on the differences in antiviral responses between pigs and humans through transcriptomic analysis.

Methods: An optimized HEV cell culture system was used to produce infectious particles, which were then used to infect primary human (PHH) and porcine (PPH) hepatocytes for transcriptomic analysis at various time points, identifying pathways and genes involved in the antiviral response and host-virus interactions.

Results: Both PHH and PPH showed significant upregulation of interferon-stimulated genes (ISGs), indicating robust innate immune activation in response to HEV infection. Using the thresholds (p-value < 0.05, $|\log FC| > 1.5$), 381 significant genes were identified in PHH and 872 in PPH, with 47 genes overlapping between the two. GO enrichment analysis revealed strong viral response signatures in both PHH and PPH, highlighting the pathways involved in HEV-host interactions and viral control.

Conclusions: These findings are preliminary, and further analysis is required to validate the observed differences and explore the role of specific host factors during HEV infection. This study highlights the differences in antiviral responses between humans and pigs, advancing our understanding of the HEV replication cycle and host-pathogen interactions. It also identifies potential therapeutic targets for the development of effective antiviral strategies.



Higher seroprevalence hepatitis E IgG in the pig sector in Belgium: results of a cross-sectional case-control study.

Heidi Janssens[1,2], Lies Delameillieure[1], Stijn Jonckheere[3], Freya Van Houtte[4], Tom Geens[1], Philip Meuleman[4]

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[2] Department of Public Health and Primary Care, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium
[3] Department of Laboratory medicine, Jan Yperman Hospital,Ypres, Belgium
[4] Laboratory of Liver Infectious Diseases, Department of Diagnostic Sciences, Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium.

Abstract

Introduction:

Hepatitis E (HEV) is often a zoonotic disease in Western countries, with pigs being considered as the main reservoir. Consequently, workers in the pig industry may be a risk group for HEV infection. The transmission route is not always clear, but mainly transmission via consumption of food, in addition to contact with pigs and/or pork, has been described.
The clinical manifestations of HEV infection range from asymptomatic to (sub)acute hepatitis. Especially in patients with underlying liver disease or immune suppression, the disease course can be more severe and the infection may even evolve to chronicity.
There exist no Belgian HEV seroprevalence data from the pig sector. The current study aims to assess whether occupational exposure to pigs or pork is associated with a higher seroprevalence of HEV-specific IgG.

Methods:

A cross-sectional study was set up to which 92 employees occupationally exposed to pigs and/or pork (pig farmers, veterinarians, transport workers and slaughterhouse workers) and 217 control persons employed outside the pig sector participated.
Socio-demographic data, information on occupation and a number of additional variables (such as eating habits and history of blood transfusion) were collected using a questionnaire. Blood was sampled and the presence of HEV-specific IgG antibodies was determined via ELISA.

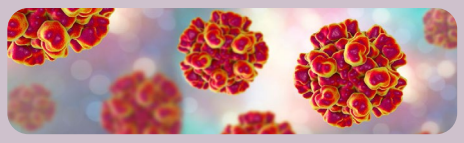
Results:

The results showed a significantly higher seroprevalence of HEV IgG in the occupational group (32.6%) compared to the control group (9.2%). The relationship between occupational exposure and HEV seropositivity remained significant in the multiple logistic regression analysis (OR: 3.30, 95%CI: 1.63-6.85), after adjusting for age, gender and eating habits. After adjustment for ‘living on a farm’, the relationship between occupational exposure and HEV IgG was nearly significant.

Conclusions:

The results of this first HEV IgG serology study in the Belgian pig industry showed that occupational exposure to pigs or pork is associated with a higher risk of HEV seropositivity.

Note: Unfortunately, no presentation on site possible due to travel issues



Detection of hepatitis E virus RNA in Black rats (*Rattus rattus*) trapped in pig farms in Bulgaria

Katerina Takova [1], Valeria Tonova [1], Gergana Zahmanova [1, 2]

Affiliations: [1] Department of Molecular Biology, Plovdiv University, Plovdiv, Bulgaria

[2] Center of Plant System Biology and Biotechnology, Plovdiv, Bulgaria

Introduction:

Hepatitis E virus (HEV) is an emerging zoonotic pathogen, that poses a significant public health concern. Rodents, especially rats, carry the rat HEV (Rocahepevirus genus, genotype C1). Rats are also frequently exposed to HEV-3 (Paslahepevirus genus, genotype 3), a zoonotic genotype prevalent in humans and widely prevalent in domestic and wild pigs. Recently, rat HEV has been reported to cause hepatitis infections in humans. Human infections with HEV-C1 are underestimated worldwide due to the paucity of data on HEV-C1 transmission mechanisms and genome epidemiology.

Methods:

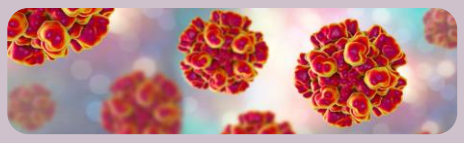
A total of 28 Black rats (*Rattus rattus*) were collected from pig farms, in the province of Plovdiv. Total RNA was extracted from the liver samples using the Trizol reagent method. Broad spectrum one-step reversetranscription polymerase chain reaction (RT PCR) with primers HEV-cs and HEV-cas (Johne et al., 2010) and Nested PCR with HEV-csn и HEV-casn (Johne et al. 2010) were used for the detection of HEV RNA.

Results:

Genome fragments with sizes 335 of nt were amplified in 15 liver samples. The prevalence of HEV-like RNA was 15 of 28 (53%) by One-step RT PCR followed by Nested PCR.

Conclusions:

Further investigations are necessary to clarify the genome sequences and identify the circulating HEV strains in rats, to assess the role of rats in the epidemiology of HEV transmission to pigs or humans.



Rat HEV in humans in Germany: presence but low detection rate, a screening study 2022 - 2023

Authors: Lisa J. Mueller[1], Marie L. Schmidt[2], Tatjana Schwarz[2], Till D. Best[1,2], Tobias Bleicker[2], Jessica Panajotov[3], Jenny Jansen[2], Julia Melchert[2], Tiina Mauno[2], Christian Drosten[1,2], Reimar Johne[3], Victor M. Corman[1,2]

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Abstract (max. 300 words)

Introduction:

Hepatitis E Virus (HEV) is a major cause of acute hepatitis and, in immunocompromised patients, chronic hepatitis. In 2018, descriptions of human infections caused by HEV strains belonging to the viral species *Rocahepevirus ratti* (RocaHEV) were published. Several cases have since been reported across Asia, Europe and North America raising discussions of RocaHEV as an emerging cause of hepatitis. *Rocahepevirus ratti* was found predominantly in the rodent genus *Rattus* and is only distantly related to the human pathogen *Paslahepevirus balayani*.

Our study aimed at testing human cohorts in and around Berlin, Germany, to assess the significance of RocaHEV.

Methods:

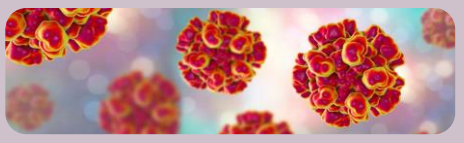
We conducted a retrospective screening of 2,520 anonymised left-over plasma and serum samples submitted for Paslahepevirus testing via PCR in 2022 and 2023. From all samples with negative Paslahepevirus PCR, we selected samples depending on the sending department for an immunocompromised cohort (Hematology/Oncology, Nephrology/Rheumatology, Transplant Surgery, n=858) and an acute hepatitis cohort (Gastroenterology, n=225). These samples were tested for RocaHEV by in-house real-time PCR and subsequent sequencing.

Results:

RocaHEV-RNA was detected in one patient (0.12%) within the immunocompromised cohort with a viral load of approximately 1x10^6 RNA copies/mL. Full genome sequencing of the patient's isolate revealed a close phylogenetic relationship to sequences originating from the species *Rattus norvegicus* in Germany and other European countries. The patient showed elevated transaminases at the time of positive PCR, consistent with acute hepatitis.

Conclusions:

With a growing number of case reports and screening studies worldwide describing infections with RocaHEV, this virus is increasingly recognized as a potential emerging pathogen. In line

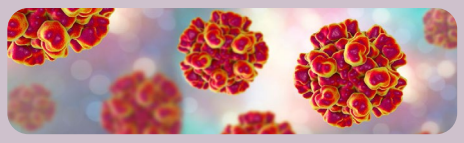


with these findings, RocaHEV was detected in humans in our retrospective analysis. However, one single positive case among 1,083 tested samples indicates a low detection rate of RocaHEV in humans in Germany. Implementing comparable inclusion criteria could facilitate the comparison of prevalence studies across different geographic regions.

Figures: attached Images

Figure 1: Study diagram. Left: inclusion criteria for screening study. Right: summary of additional samples

Figure 2: Phylogenetic Tree. Fast Tree, NJ 500 bootstraps, orange = human sequences, blue = rattus spec. sequences, black = eothenomys melanogaster sequence



Characterization of HEV-1 Sar55 Replication in Caco-2 Cells

Authors: Alexander Falkenhagen and Reimar Johne

Affiliations: Department of Biological Safety, German Federal Institute for Risk Assessment, Berlin, Germany

Abstract

Introduction:

The development of efficient cell culture and reverse genetics systems for hepatitis E virus genotype 3 (HEV-3) has greatly advanced our understanding of the virus. However, systems with comparable efficiency were lacking for HEV-1. We have recently shown that the colon-derived cell line Caco-2 supports the generation and propagation of HEV-1_{Sar55}, whereby HEV-1_{Sar55} replicated to higher titers in comparison to HEV-$3_{47832mc}$. Here, we further characterized the replication of HEV-1_{Sar55} in Caco-2 cells.

Methods:

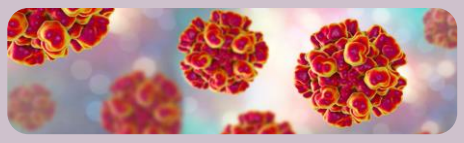
HEV-1_{Sar55} and HEV-$3_{47832mc}$ were generated by transfection of in vitro-transcribed and -capped RNA genomes into Caco-2 cells. Culture supernatant-derived virus from transfected or infected cells was used to infect Caco-2 cells. HEV RNA titers in culture supernatants over time as well as the number of HEV particles that attached to or entered Caco-2 cells were detected by RT-qPCR. The genome sequence of HEV-1_{Sar55} was determined by next-generation-sequencing.

Results:

HEV-1_{Sar55} could readily be passaged on Caco-2 cells, reaching 10^8 RNA copies/ml in culture supernatants of infected cells. Sequencing of passage 2 virus showed that neither insertions nor mutations were present in the pre-dominant virus population. Using this virus, productive infection of Caco-2 cells was possible with as little as 10^5 RNA copies. However, neither DMSO nor JAK inhibitor, which have previously been shown to increase HEV replication, seemed to have a positive effect on HEV-1_{Sar55} RNA titers in culture supernatants of infected Caco-2 cells. Additionally, no differences in the attachment or entry of HEV-1_{Sar55} and HEV-$3_{47832mc}$ were evident.

Conclusions:

HEV-1_{Sar55} efficiently infects and replicates in a human colon-derived cell line. Experiments with JAK inhibitor suggest that replication is not inhibited by innate immune responses in these cells. As HEV-1_{Sar55} and HEV-$3_{47832mc}$ attachment and entry were similar, the observed differences in replication kinetics are likely due to post-entry factors.



Polarized secretion and palmitoylation of the hepatitis E virus ORF3 protein

Charlotte C. Syren[1], Patrick Bröscky[1], Carola Krug[2], Leonid Kostrykin[2], Di Ge[3], Antonio Piras[3] ,Huanting Chi[1], Andrew Freistaedter[1], Karl Rohr[2], Andreas Pichlmair[3], Viet Loan Dao Thi[1]

[1]Schaller Research group at Department of Infectious Diseases and Virology, Heidelberg University Hospital, 69120 Heidelberg, Germany

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[3]Technical University of Munich, School of Medicine, Institute of Virology, 81675 Munich, Germany.

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Abstract

Introduction:

Hepatitis E virus (HEV) is a leading cause of acute hepatitis worldwide. The HEV genome encodes three open reading frames (ORFs): The replicase ORF1, the capsid ORF2 and ORF3, which is critical for progeny secretion. The latter contains palmitoylated cysteine residues that are critical for membrane association and subcellular localization of ORF3.

HEV is primarily transmitted by the fecal-oral route. The polarity of the cells in the tissues involved, i.e. the gut (intestinal epithelial cells) and the liver (hepatocytes), plays a critical role in HEV transmission: These cells, which normally act as a barrier, have developed finely tuned trafficking machinery. Simply put, HEV must enter these cells from one side and exit from the other. In this study, we aim to identify the viral and cellular determinants of directional HEV secretion.

Methods:

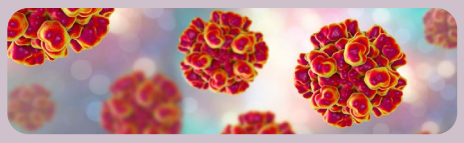
GFP-tagged ORF3 was studied in different polarity cell systems and analyzed by high resolution microscopy. The effect of mutating charged residues along the ORF3 protein was quantitatively analyzed by automated segmentation and quantification of the staining intensity of polarized cells. Co-immunoprecipitation followed by mass spectrometry (CoIP-MS) was used to identify ORF3-interacting cellular proteins. Identified candidates were confirmed by siRNAs and titration of infectious particles by qRT-PCR and foci counting.

Results:

In polarized epithelial cells the ORF3 protein localizes mainly to the apical membrane, compared to ORF2. To identify the viral determinants of apical ORF3 localization, we screened alanine mutants of ORF3-GFP and identified two mutations of N-terminal basic residues (RHR23-25AAA and R29A) with reduced apical localization and accumulation in subapical vesicles. We also found that these mutations affected the palmitoylation of ORF3. Using CoIP-MS, we identified the palmitoyltransferase ZDHHC17 as a potential interaction partner of ORF3. Knockdown of this palmitoyltransferase inhibited the secretion of infectious HEV particles.

Conclusions:

We have identified positively charged residues at the N-terminus of ORF3 that affect its apical localization in polarized cells. We are currently investigating whether these residues affect the interaction with palmitoyltransferase 17, which may be a target for future antiviral intervention strategies.



Investigating ORF1 protein processing and its potential role in HEV replication

Huanting Chi[1,2], Piero Giansant[3], Andreas Pichlmair[4,5], Viet Loan Dao Thi[1,2]

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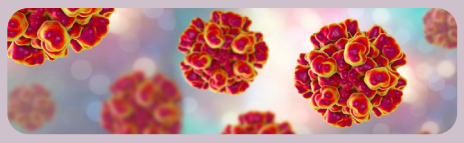
Introduction: Hepatitis E virus (HEV) infection is the most common cause of acute hepatitis worldwide. HEV has a positive-strand RNA genome encoding three open reading frames (ORF1-3). The ORF1 protein, which mediates genome replication, contains several functional domains that are homologous to the non-structural proteins of other positive-strand RNA viruses. It is not clear whether these domains are processed into biochemically distinct entities or act as a single polyprotein with multiple functions. Current treatment options are suboptimal and a direct-acting antiviral treatment against HEV is urgently needed. The identification of ORF1 cleavage products could lead to the identification of the responsible cellular proteases, which could be a direct target for antiviral intervention.

Methods: Terminal amine isotopic labelling of substrates (TAILS) coupled with tandem mass tag (TMT) analysis was performed on HepG2/C3A electroporated with the HEV GT3 Kernow-C1 P6 genome. Potential cleavage sites were confirmed using an image-based fluorescence cleavage reporter. V5 tagged-ORF1 protein processing, either ectopically expressed or by viral replication, was analysed by Western blot (WB).

Results: WB analysis of infected or ectopically expressing cells revealed a predominance of unprocessed ORF1 protein, with only a small fraction potentially processed into smaller fragments. TMT-TAIL analysis of HEV replicating cells revealed three putative cleavage sites in ORF1 (CS 1-3), within the methyltransferase, the S17 insertion in the HVR domain and the helicase domain, respectively. We introduced the identified sites into an ER-anchored fluorescent protein fused to a nuclear localisation sequence, allowing GFP to translocate to the nucleus upon successful cleavage. We observed nuclear translocation of GFP with CS2 and 3, but not with CS1, independent of HEV infection. Mutation of CS3, but not CS2, abolished viral replication.

Conclusions: Our preliminary data indicated that the majority of the HEV ORF1 protein is expressed in its full-length, unprocessed form. However, our results suggest that a small fraction of the ORF1 protein is truncated at the carboxyl terminus, probably mediated by a



Interferon-independent host factors define the hepatitis E virus species barrier in murine hepatocytes

Nicola Frericks[1], Leyla Sirkinti[1], Hoang Duy Nguyen[2], Yannick Brüggemann[1], Thomas Burkard[1], Richard Brown[1], Tran Tuoc[2], Huu Phuc Nguyen[2], Daniel Todt[1,3], Eike Steinmann[1]

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Abstract (max. 300 words)

Introduction:

The zoonotic potential of hepatitis E virus (HEV) poses a significant public health threat. Specifically, infections with the distantly related rat HEV are increasingly reported in humans. Interestingly, HEV infections in mice have only rarely been documented. The determinants of HEV species tropism are poorly understood due to limited understanding of virus-host interactions during the replication cycle. This study aims to uncover the molecular mechanisms underlying the murine species barrier to HEV infection.

Methods:

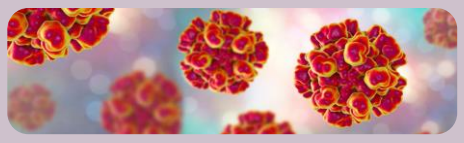
Using a sub genomic reporter replicon and full-length molecular clone of the HEV-3 strain Kernow-C1 p6, we investigated susceptibility and permissiveness of murine hepatoma cells and authentic primary mouse hepatocytes to HEV infection. To assess the role of innate immunity in restricting HEV infection, type I interferon signaling was monitored using fluorescent-based ISRE reporter cell lines and RNA sequencing was conducted to investigate innate immune-dependent gene expression patterns. Further, viral replication and particle production were examined in presence of Janus kinase inhibitors.

Results:

Our results indicate that murine hepatoma cells support zoonotic HEV-3 replication and infectious virion production upon transfection of viral RNA, albeit with significantly lower efficiency compared to the human hepatoma cell line HepG2. Inoculation of murine hepatocytes with infectious HEV particles did not result in productive infection. Moreover, inhibition of type I interferon signaling did not affect host susceptibility, viral replication, or virus production in murine hepatocytes. Consistently, ISRE-dependent reporter activity and interferon-stimulated gene expression were detected in HepG2 cells but not in murine hepatoma cells.

Conclusions:

These findings underscore the role of intrinsic cellular barriers, rather than innate immune responses, as key restriction factors in murine hepatocytes. These barriers likely prevent HEV transmission to mice and limit efficient viral replication and virus production.



Adaptation of HuH-7 Cells to Chemically Defined Medium

Ahmed Ali[1], Johanna Berg[1], Viola Roehrs[1], Dongwei Wu[1] Johannes Hackethal[2], Lisa Woelken[3], Cornelia Rauh[3], Albert Braeuning[4], Jens Kurreck[1]

[1] Department of Applied Biochemistry, Institute of Biotechnology, Technische Universität Berlin, Berlin, Germany

[2] THT Biomaterials, Vienna, Austria

[3] Department of Food Biotechnology and Food Process Engineering, Technische Universität Berlin, Berlin, Germany

[4] Department Food Safety, German Federal, Institute for Risk Assessment (BfR), Berlin, Germany

Abstract (max. 300 words)

Introduction:

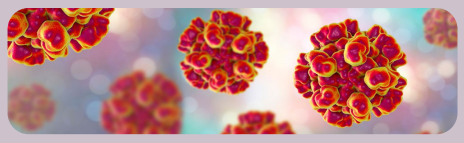
HuH-7 cells, a well-characterized human hepatocellular carcinoma cell line, serve as a valuable model for studying hepatitis E virus (HEV) biology. Their permissiveness to HEV replication allows researchers to investigate viral entry, replication mechanisms, and host-pathogen interactions. However, most reported models to study liver function, toxicity and viral infection rely on animal-derived components, limiting their suitability for fully defined and reproducible experimental conditions.

Methods:
A xeno-free bioprinted liver model was developed based on HuH-7 cells [1]. Cells were adapted to a chemically defined medium (CDM) using both direct and sequential approaches. Furthermore, three different chemically defined freezing media were prepared and optimized for the cryopreservation of adapted cells. Additionally, a xeno-free bioink was formulated based on sodium alginate, human collagen (I), and nutrient supplements to avoid the use of animal components in bioprinting.

Results:
The adapted cells demonstrated high survival rates (85–92%) after cryopreservation in chemically defined freezing media, comparable to the survival rates observed with standard freezing media (86–92%). The resulting xeno-free bioprinted liver models demonstrated high cell viability and metabolic activity, comparable to that of the Matrigel-based liver model. To assess the applicability of this xeno-free model, it was used to test the hepatotoxicity of okadaic acid, and results were compared to conventional 2D cell culture.

Conclusions:
HuH-7 cells were successfully adapted to CDM and used to construct a 3D liver model. This model demonstrated its effectiveness in assessing the hepatotoxicity of Okadaic acid and holds potential for broader applications, including studies of liver viral infections.

[1] Ali, A.S.M.; Berg, J.; Roehrs, V.; Wu, D.; Hackethal, J.; Braeuning, A.; Woelken, L.; Rauh, C.; Kurreck, J. Xeno-Free 3D Bioprinted Liver Model for Hepatotoxicity Assessment. Int. J. Mol. Sci. 2024, 25, 1811. https://doi.org/10.3390/ijms25031811



siRNA as an Alternative Antiviral Therapy: Insights from Influenza Virus Studies

Melissa Pires-Alves, Luisa Feldmann, Jens Kurreck

Affiliations: Institute of Biotechnology, Technische Universität Berlin, Berlin, German

Abstract

Introduction:

Current vaccines for influenza virus, hepatitis E virus (HEV), SARS-CoV-2, and other viral infections provide only partial protection due to limitations in specificity and efficiency. Studies have shown siRNA as a promising strategy for controlling viral infections. This study aimed to establish conditions for inhibiting influenza A virus (IAV) replication using siRNA in a monoclonal human alveolar epithelial cell line (Arlo), with the goal of applying these conditions to a human cell-based 3D lung model.

Methods:

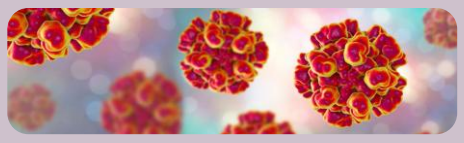
A siRNA targeting the nucleoprotein of IAV was transfected into Arlo cells using Lipofectamine RNAiMAX. Total virus yield, viral genome copy number, and nucleoprotein expression levels were used to analyze the antiviral activity. To investigate the effect of IAV infection on barrier integrity, Arlo cells were cultured under air-liquid interface (ALI) conditions. Barrier function of airway epithelial cells was analyzed by transepithelial electrical resistance (TEER) measurements and immunostaining with antibodies against the tight junction protein ZO-1.

Results:

A multi-step growth curve demonstrated that Arlo cells are susceptible to IAV, reaching a viral titer of $10^{6.5}$ PFU/ml at 48 hours post-infection with MOI of 0.25. Treatment with siRNA suppressed viral replication in a dose-dependent manner, reducing viral titer by 2.5 to 3 log units. This inhibition was corroborated by decreases in both viral mRNA and protein levels. The barrier integrity of Arlo cells generated a TEER of 1119 $\Omega.cm^2$. This barrier was disturbed during the course of viral infection, as revealed by immunostaining against ZO-1.

Conclusions:

We estabilish the conditions to study influenza virus replication inhibition by siRNA in 2D monolayer culture. These conditions are essential for investigating the antiviral activity in a multicellular human cell-based 3D lung model. Combining this model with siRNA-based therapy provides a valuable platform for studying virus supression, highlighting its potential as a therapeutic strategy to combat viral infections.



Hepatitis E virus entry requires the cholesterol transporter Niemann–Pick C1

Authors:
Emely Richter[1], Mara Klöhn[1], Viktoria Kowalzick[1], André Gömer[1], Rebecca Menhua Fu[2,3], Alexander Falkenhagen[4], Viet Loan Dao Thi[2,3], Reimar Johne[4], Yannick Brüggemann[1], Eike Steinmann[1,5#]

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#corresponding author

Abstract

Introduction:

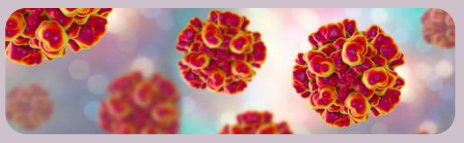
The hepatitis E virus (HEV) presents a significant global health concern with an estimated 20 million infections occurring annually, while no specific antiviral treatments are available. While our current understanding of the viral life cycle is limited, advancing this knowledge is essential for the development of novel antiviral strategies.
The lysosomal cholesterol transporter Niemann-Pick-C1 (NPC1) has been identified as an entry factor of different viruses, including Ebola virus and SARS-CoV-2. Here, we investigated the role of NPC1 during the HEV infectious cycle and evaluated the antiviral potential of the clinically approved NPC1 inhibitor Itraconazole.

Methods:

Using a robust HEV cell culture model, we investigated the dose-dependent effect of the two NPC1 inhibitors, Itraconazole and U18666A, on the infection with (non-)enveloped HEV. In addition, we performed time-of-addition as well as subgenomic replicon assays to gain insights into the mode of action of NPC1 inhibition. We further performed quantitative RNA fluorescence *in situ* hybridization (RNA-FISH) to localize incoming HEV virions upon NPC1 inhibition. The requirement of NPC1 during the HEV life cycle was further confirmed via siRNA-mediated knockdown and CRISPR/Cas9-mediated knockout.

Results:

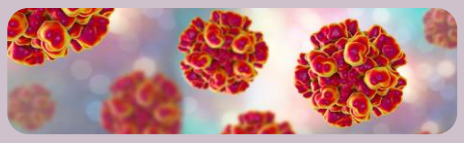
Both NPC1 inhibitors, Itraconazole and U18666A, lowered HEV infectivity at nanomolar efficacy in hepatoma cells. Consistent with this, siRNA-mediated knockdown of NPC1 and CRISPR/Cas9-mediated knockout lowered the susceptibility to infection with (non-)enveloped HEV. Time-of-



addition experiments showed that NPC1 inhibition compromises HEV entry and does not affect viral replication. In agreement with this, we observed cholesterol accumulation and enlargement of lysosomal compartments upon NPC1 perturbation and a blockage of viral RNA trafficking and productive infection.

Conclusions:

Our data suggests that NPC1 plays a crucial role during the viral entry of HEV. Considering that Itraconazole is clinically used in immunocompromised patients (unrelated to viral conditions), the pharmacological targeting of NPC1 may guide novel antiviral strategies to treat HEV infections in the future.



Inhibition of PIKfyve kinase inhibition prevents infection by Hepatitis E virus

Maria Laura Goussain Darido[1], Julian Ring[1], Sarah Schlienkamp[1], Xin Zhang[2], Jil Alexandra Haase[1], Rebecca Menhua Fu[3,4], Viet Loan Dao Thi[3,4,5], Mara Klöhn[1], Daniel Todt[1,6], Johan Neyts[3], Suzanne J. F. Kaptein[3], Eike Steinmann[1,7#], Yannick Brüggemann[1#]

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[2]KU Leuven Department of Microbiology, Immunology and Transplantation, Rega Institute for Medical Research, Laboratory of Virology and Chemotherapy, Leuven, Belgium
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[5]German Centre for Infection Research (DZIF), Partner Site Heidelberg, Heidelberg, Germany
[6]European Virus Bioinformatics Centre (EVBC), Jena, Germany
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Introduction:

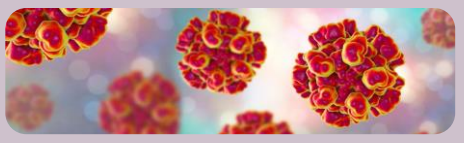
Hepatitis E virus (HEV) infections are a leading cause of acute viral hepatitis in humans. Current standard of care treatment is limited to the off-label use of nucleoside-analog ribavirin (RBV) and PEGylated interferon-α, which have significant side effects and provide only limited efficacy. Consequently, novel strategies are needed to effectively and safely target HEV. The phosphoinositide kinase PIKfyve is a critical regulator of the endolysosomal morphology, function, and biogenesis. PIKfyve further plays a critical role during cellular entry of different viruses, however its role during HEV infections remains unclear. Here evaluated the antiviral potential of PIKfyve inhibitors against HEV *in vitro* and *in vivo*.

Methods:

Using a robust HEV cell culture model, we assessed the impact of PIKfyve kinase inhibitors on HEV infections in human hepatoma cells. We further employed a HEV subgenomic replicon and performed time-of-addition experiments to identify the HEV live cycle step affected by PIKfyve. Moreover, we evaluated the antiviral potential of the PIKfyve kinase inhibitor apilimod in a rat HEV infection model.

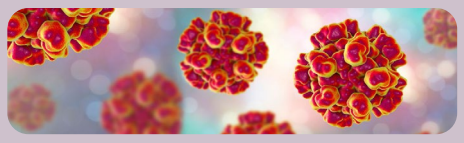
Results:

We observed that the PIKfyve kinase inhibitors apilimod and YM-2016-36 hindered HEV infection at nanomolar efficacy in hepatoma cells, without affecting HEV replication. Through time-of-addition experiments, we confirmed that HEV entry is potently blocked upon PIKfyve inhibition. Moreover, the antiviral potential of apilimod was evaluated in a rat HEV infection model. Animals treated with apilimod showed lowered viral replication kinetics as determined by the viral load within the feces as compared to animals treated with a vehicle control. In addition, a strong reduction of the viral loads within the liver, feces, spleen and intestine was observed.



Conclusions:

Overall, our data suggest an essential role for PIKfyve during HEV infection and highlight the potential of PIKfyve kinase inhibition as a novel antiviral strategy against HEV. Considering the successful safety testing of apilimod in previous human clinical trials unrelated to viral conditions, our findings could further uncover novel avenues for innovative pharmacological approaches in targeting HEV.



Therapeutic treatment of Hepatitis E Virus infection in pigs with a neutralizing monoclonal antibody

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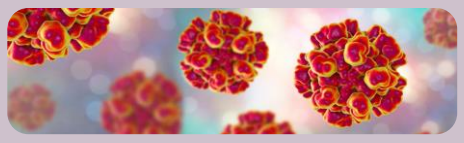
Hepatitis E virus (HEV) represents an important public health concern. In Europe, infection with the zoonotic genotype 3 (HEV-3) can lead to chronic hepatitis E in immunocompromised patients with a potentially fatal outcome. This study evaluates the therapeutic potential of a monoclonal antibody (mAb)-based treatment strategy using an HEV-3-infected pig model. A panel of monoclonal antibodies targeting the viral capsid protein was generated, with mAb 5F6A1 exhibiting the highest neutralizing activity in vitro. Based on these findings, mAb 5F6A1 was selected for in vivo evaluation. Pigs were infected with HEV-3 and treated intravenously with mAb 5F6A1 on days 1 and 7 post-infection. The treatment resulted in a significant reduction in both viremia and viral shedding compared to untreated controls. These results underscore the promise of mAb-based therapies for combating HEV infections in animal models and highlight their potential application in human medicine.



A novel class of human monoclonal antibodies neutralizing HEV

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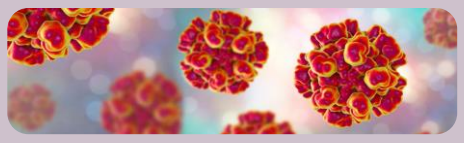
Abstract (max. 300 words)



Introduction:

Effective therapies for HEV infections remain elusive. Neutralizing antibodies (nAbs) have emerged as a potential therapeutic strategy for various viral infections, including HEV. The protruding (P) domain of the HEV capsid protein, pORF2, serves as a key epitope for nAbs. However, in HEV-infected individuals, the predominant antigenic form in circulation consists of secreted, glycosylated pORF2 dimers, which are non-infectious and possibly act as decoy molecules by sequestering nAbs.

Methods:

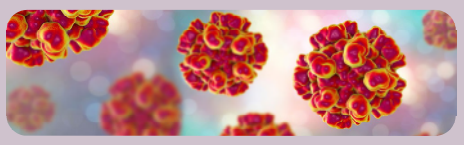
Memory B cells from two acutely HEV-infected individuals were isolated based on their reactivity to the P domain of pORF2. The heavy and light chain sequences of the B cell receptors were determined, and recombinant monoclonal antibodies (mAbs) were expressed. Binding affinities were assessed using ELISA and SPR. Neutralization efficacy was evaluated against various HEV strains and patient-derived isolates. Selected scFvs were crystallized in complex with the HEV-3 P domain, and their structures were resolved. In vivo neutralization assays were conducted in humanized liver chimeric mice.

Results:

Seven human mAbs demonstrated neutralization against both naked and quasi-enveloped HEV-3 particles. Of the four most potent nAbs, two showed glycan sensitivity. Pre-incubation of glycosylated pORF2 dimers with glycan-insensitive nAbs abrogated their neutralization in a dose-dependent manner, whereas glycan-sensitive nAbs remained effective. Structural analysis revealed that the conserved epitope targeted by glycan-sensitive nAbs encompasses the glycosylation site on the P domain. These nAbs recognized circulating pORF2 in HEV-infected patients and neutralized patient-derived HEV isolates. Prophylactic administration of the nAbs in liver chimeric mice prevented HEV-3 and -1 infection, as evidenced by the absence of viral RNA in stool samples and serum.

Conclusions:

This study identifies a novel class of human mAbs with broad and potent neutralizing activity against HEV. Glycan-sensitive nAbs effectively target a conserved epitope on pORF2, bypassing the neutralization blockade posed by glycosylated pORF2 dimers. These findings support the potential clinical application of these nAbs.



Characterization of TMEM62 as a novel restriction factor of Hepatitis E virus

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Abstract (max. 300 words)

Introduction:

Hepatitis E virus (HEV) infections are a major cause of acute viral hepatitis in humans and are mainly transmitted via the fecal-oral route. Most infections remain asymptomatic and self-limiting but can lead to fulminant hepatitis, liver cirrhosis, or liver failure, especially in immunocompromised individuals. The interferon (IFN) response is reported to contribute to the restriction of HEV. However, the specific interferon-stimulated genes (ISGs) involved and the precise mechanisms through which they mediate this restriction remain unknown.

Methods:

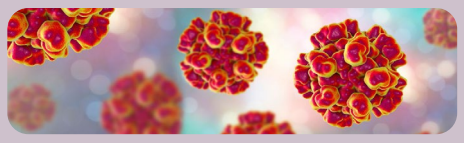
To assess the restriction capacity of individual ISGs, we took advantage of arrayed ISG libraries. For further characterization of one potent inhibitor of HEV, TMEM62, we performed CRISPR/Cas9-mediated gene editing or stably overexpressed the protein in human hepatoma cells. We further generated TMEM62 mutants with individual amino acid substitutions to evaluate their functional importance for the restriction. HEV infection and subgenomic replicon systems, along with RT-qPCR and microscopic analysis were used for the detailed characterization of the restriction capacity and mechanism.

Results:

The uncharted hydrolase TMEM62 was identified as an ISG with a strong anti-HEV phenotype. The absence of endogenous TMEM62 led to a higher number of HEV-infected cells. Transfection of subgenomic HEV reporter replicons and full length HEV RNA, showed that HEV RNA replication and progeny virus production were not affected by the presence or absence of TMEM62, suggesting that the protein restricts the entry process of HEV.

Conclusions:

We identified the so far uncharacterized ISG TMEM62 as a potent inhibitor of HEV infection. Our data suggest that TMEM62 restricts an early life cycle step of HEV, prior to the onset of HEV RNA replication. These findings offer valuable insights into the IFN-mediated antiviral response against HEV and facilitate our understanding of virus-host interactions, serving as essential groundwork for the development of targeted antiviral drugs.



Identification of Hepatitis E Virus Inhibitors

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Introduction

Hepatitis E Virus (HEV) is a prevalent cause of viral hepatitis. Only non-specific therapy with ribavirin (RBV) is available but ineffective in some patients, creating a need for specific antivirals. Viral macrodomains hydrolyze ADP-ribose (ADPr) from viral proteins, thus inhibiting the cellular immune response. Some mutations of viral macrodomains impair Hepeviridae, Togaviridae and Coronaviridae infection. Drugs targeting viral macrodomains could be promising broad-spectrum antivirals. Here, we screened a drug library *in vitro* and in cell culture models to identify inhibitors against HEV.

Methods

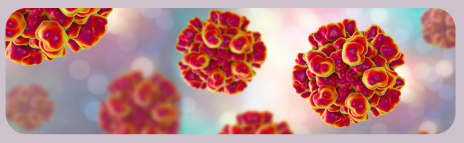
HepG2/C3A cells were infected with HEV and simultaneously treated with a library of 774 drugs at 20µM per well. Infection was quantified by immunofluorescence microscopy. Drugs were excluded when treatment resulted in <90% cell count or >10% increase in intracellular accumulation of phospholipids (phospholipidosis, PLD). PLD was quantified by adding a phospholipid reporter to treated cells for 24h. To identify macrodomain inhibitors, drugs and HEV macrodomain were added to protein-bound ADPr *in vitro* before time-resolved quantification of ADPr-specific FRET signal.

Results

20 drugs inhibited HEV infection by >90% while cell count remained >90% compared to untreated controls. Treatment with >100 other compounds resulted in >50% inhibition without significant cytotoxicity *in vitro*. 9/20 drugs increased PLD by 37-100% (others 0-7%) compared to control drug. All 20 drugs have been described as antagonists/agonists of histamine, dopamine or serotonin receptors. 1/20 drug increased ADPr-specific FRET signal by 94% in the presence of HEV macrodomain, suggesting specific inhibition. Treatment with this drug resulted in dose-dependent reduction of HEV infection (IC_{50} ~7 µM) compared to RBV (IC_{50} ~25 µM). Treatment of HEV-producing cells reduced infectivity of HEV by ~50% compared to RBV (>80%).

Conclusion

By screening a drug-repurposing library for their antiviral activity against HEV, we identified potential novel HEV inhibitors. One drug inhibited macrodomain activity of HEV *in vitro*.



NRF2 activator inhibits hepatitis E virus propagation in vitro

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Introduction:

Hepatitis E virus (HEV) is a major cause of acute viral hepatitis worldwide, with over 20 million cases and 70,000 deaths annually. Chronic HEV infection can arise in approximately 50% of infected immunosuppressed individuals. Currently, there is no approved antiviral treatment for HEV, highlighting the urgent need for effective antiviral therapies. Activators of the nuclear factor erythroid 2-related factor 2 (NRF2) have demonstrated antiviral activity against various viruses, including HBV and HCV. We aimed to identify safe and effective compounds for HEV treatment and to explore the underlying host mechanism that could reveal potential host factors involved in HEV infection.

Methods:

We investigated the role of several NRF2 activators including itaconate, citraconate, mesaconate, dimethyl itaconate, 4-octyl itaconate, bardoxolone methyl (Bard) and sulforaphane (SFN) during HEV infection in a cell culture-based system. The inhibitory effects on HEV replication were assessed using different subgenomic reporter constructs. HepG2/C3A cells and primary human hepatocytes (PHHs) were inoculated with authentic HEVcc GT3 and treated with Bard to analyze the effect of NRF2-activation on viral growth.

Results:

Bard and SFN inhibited HEV replication in a dose-dependent manner. Time-of-addition experiments confirmed that Bard functions as replication inhibitor. Additional assays demonstrated broad antiviral activity of Bard against GT1 and rat HEV. In infected PHHs, a strong decrease in viral titres was observed after Bard treatment. Interestingly, induction of canonically regulated mRNAs seem not to correlate with antiviral effect, suggesting that other mechanisms may be involved. These results show that Bard is a promising antiviral candidate for the treatment of HEV infections.

Conclusions:

Bard showed the strongest antiviral effect against HEV, possibly by non-canonical NRF2 activation. RNA sequencing will provide further insight into upregulated host mechanisms after Bard treatment and could contribute to the identification of host factors essential in HEV infection.