

## **Final report on the DZIF project**

Project number: TI 07.012\_Pischke  
Title of the project: Advanced Clinician Scientist

Individual project

### **Project partner and project duration**

Projekt Coordination	Start of term	End of term
Universitätsklinikum Hamburg-Eppendorf	01.05.2022	- 31.07.2024

  

Project partner	Start of term	End of term
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## Chapter 1: Short report (will be published)

Please summarize the project in a generally understandable form in this Chapter.

### a) Original task and scientific/technical status built on

Please indicate the original task/objective. Add any deviations that occurred in the course of the project. Information can be taken from Chapter 4 "Project objectives" of the current project description (VHB) (no reference please).

This project aimed to investigate new factors that pose a risk for exposure to HEV contact, for the transition of the virus from the gut to the bloodstream and for a severe course of infection.

In 5 work packages the importance of various aspects have been studied:

- (1) Domestic and farm animals as a source of HEV transmission
- (2) human-to-human transmission as a source of infection (including surface contacts)
- (3) intestinal microbiome, IgA and beta-defensin-2 levels as a protective barrier and total serum IgA levels
- (4 and 5) genetic and hormonal factors as determinants of the clinical course are investigated.

Due to the Covid pandemic and its consequences, the project experienced some delays and obstacles, but all questions were answered adequately. Only minimal modifications were necessary. The duration of the project was only slightly extended due to the pandemic. This was done on a cost-neutral basis and after approval by the DZIF.

#### Ad (1)

The investigation of pets and livestock as a possible source of HEV infection was a complete success: it has already been published that dogs and cats carry anti-HEV antibodies significantly more often than horses and thus play a greater role as a "spillover host" or even reservoir. Further data are even more interesting but have not yet been published, for example it has been shown that 2/3 of all pig stool samples and thus pig bio-fertilizer contain HEV particles, in some cases with virus loads of up to millions of copies per ml. the genetic and morphological characterization (density centrifugation followed by PCR) of these HEV particles was also successful (will be published soon).

#### Ad (2)

Testing family members for anti-HEV IgG and questioning them about hobbies and diet did not clearly identify the risk of HEV human-to-human transmission, as serologically positive family members of HEV-infected individuals often shared the same potential sources of exposure.

However, the examination of toilet furniture (toilet seat, flush button, faucet, and door handle) in patients with HEV infection was very conclusive: HEV was only detectable in such low concentrations that there is only a very low risk of transmission via this route (to be published soon).

#### Ad (3)

Although this sub-project has generated a lot of data, relevant differences between clearly defined groups of HEV infected persons (acute hepatitis E vs. asymptomatic infection) could not be identified.

#### Ad (4)

Unfortunately, the investigation into the frequency of various genetic factors in patients with genuine acute hepatitis E vs. asymptotically infected patients did not reveal any significant difference. Unfortunately, due to the pandemic, the cohort had to be reduced slightly. This sub-project could therefore still end successfully, as more patients will be analyzed in the coming months and years.

#### Ad (5)

Testing for hormonal factors in patients with acute and chronic hepatitis E, as well as asymptomatic infection, was successful in a much larger group than initially planned. Well over 100 patients were included in this subproject, resulting in an immensely extensive data set. The detailed evaluation of this data set, including longitudinal investigation of various hormones at different phases of an HEV infection, is extremely complex and will unfortunately take several months.

### b) Course of the project

Please provide information on the practical and temporal implementation of the project. If applicable, information can be taken from Chapter 6 "Work program" of the VHB (no references or results, please). Please add any adjustments/changes to the original work program, additional work packages, adjusted study starts or similar.

- ➔ In Work Package 1, it was initially planned to test 400 animals for HEV and anti-HEV. Due to stricter regulations and formalities, this was not exactly achieved; "only" 365 animals were tested (dogs, cats and horses). However, the testing was carried out within the planned time period. In order to generate further data on the relevance of animals as a source of HEV infection, the project was supplemented by testing a cohort of pigs for HEV in their stool. However, the planned testing of pets of patients with HEV infections (n=50) could not be carried out because it would have been irresponsible to make the planned home visits during the covid pandemic.
- ➔ In work package 2, it was planned to test contact persons of HEV infected people (family members, nursing staff, etc.) for anti-HEV antibodies in order to evaluate the possibility of human-to-human transmission. Within the first 6 months, it became clear that testing family members, unfortunately, did not produce any significant results, as family members often ate the same food as the patients and thus shared the risk of exposure. Thus this project was therefore slightly modified and focused on a comparison of anti-HEV IgG rate in nursing staff (n=248) vs employees without direct patient contact (n=154). This project demonstrated the possibility of human-to-human transmission.

Furthermore, toilet furniture (toilet seat, flush button, faucet, and door handle) in patients with HEV infection was tested by PCR:

The overall cohort experienced a slight reduction due to Covid but it clearly demonstrated that the risk of HEV transmission resulting from toilet furniture is low.

- ➔ Work package 3 was planned to test factors of the intestinal barrier as well as genetic factors in blood donors with and without frequent consumption of raw pork ("Mett"): Testing a cohort of blood donors was not responsible during the COVID pandemic. There was often a shortage of blood products and the risk of scaring off blood donors with questionnaires and tests was too great, so a group of 40 patients with HEV infection (asymptomatic and symptomatic) were tested for IgA, beta-defensin-2 and the genetic factors Apo E3/E4, IL28b, PROGENS and the HLA type. The also planned testing for the stool microbiome was not carried out because another working group had already published this.
- ➔ In work package 4, it was planned to compare HEV-infected people with symptomatic and asymptomatic HEV infection concerning genetic factors (20 vs 20). Unfortunately, this analysis did not lead to valid results as the difference between symptomatic and asymptomatic patients was not significant.
- ➔ In Work Package 4, the initial plan was to test 40 HEV-infected people for various hormone levels and to correlate them with the severity of the infection. Since the cohort size had to be slightly reduced in some of the other work programs, this cohort was able to be greatly enlarged: well over 100 patients were recruited, but. This is an immensely complex data set, the evaluation of which will take about three months.

#### c) Main results and cooperation with other research institutions, if applicable

Please provide a short, generally understandable summary of the main results. If applicable, also mention cooperation with other research institutions.

It has been shown that dogs and cats, but not horses, can be relevant hosts for the hepatitis E virus (WP1). Furthermore it could be demonstrated that there is only a low risk of HEV exposure by toilet furniture (WP2).

In order to characterize the risk of human-to-human transmission of HEV, the frequency of anti-HEV antibodies in UKE employees, both in patient care and in administration, was determined and risk factors (diet, hobbies, travel, etc.) were determined by means of a questionnaire. This showed that work in patient care is associated with an increased likelihood of contact with HEV (WP2).

A test of factors that influence the intestinal barrier, hormones, and genetic factors that could be associated with HEV infection (like IgA, beta-defensin-2, TSH, Cortisol, anti-Thyreoglobulin, Vitamin D and the genetic factors Apo E3/E4, IL28b, PROGENS and the HLA type) and a comparison with healthy controls (pre-data) unfortunately did not reveal any relevance. So, we assume that none of these factors is associated as relevant with the uptake or course of HEV infection (WP3 and WP4).

## Chapter 2: Detailed description (will be published)

Please provide detailed information in Chapter 2. In subchapters 2a) - 2c), detailed information should always be presented in comparison to the current project description (VHB).

### a) Detailed presentation of the results achieved

The work carried out as part of the project must be described in detail here, particularly in comparison with the current VHB. The individual results achieved and the use of funding (with regard to content, no numbers necessary) should be comprehensible. Please structure the presentation of the results according to the work packages defined in chapter 6 of the VHB. Please also explain if there have been any changes in the objectives during the course of the project or if the objectives were not achieved during the project duration.

This project was able to show that dogs (n=124) and cats (n=119), but not horses (n=122), are often exposed to HEV (anti HEV 10% and 6% vs 2%). It was also able to show that 68% of pig manure samples (n=34) contain HEV but 0% HAV. It has thus identified new relevant aspects for the origin of a HEV infection.

It was also shown that nursing staff (n=248) come into contact with HEV more frequently than other hospital employees (anti-HEV IgG positive 12% vs 4%,  $p < 0.01$ ), but that the environmental contamination of HEV on toilet furniture of infected people is negligible: While 6/13 toilet seats (45%) tested PCR positive for HEV, only 3/13 of the flush buttons (23%), 4 of 13 of the faucet fittings (31%) and 1/13 of the door handles (8%) were positive for HEV. However, the virus loads were so low that they usually did not pose a relevant risk of exposure (CT values between 35 and 39 cycles). This makes a realistic and detailed assessment of the risk of human-to-human transmission possible. This data is of great value to hygiene facilities worldwide.

The influence of genetic factors (HLA-type, PROGINs, apolipoprotein, IL28b) on the course of an HEV infection E was investigated in 32 patients. Heterozygote apolipoprotein E3/E4 status was present in 58%, homozygote E4/E4 in 17%, E3/E3 in 10% and the remaining patients had rare constellations. The Progin mutation was present in 10% and the IL28b homozygote variant in 59%. The evaluation of the results from the HLA typing and the comparison with healthy controls are still ongoing but will be finished within the next 3 months.

The distribution of hormone levels (TSH, anti Thyroglobulin, cortisol) did not show relevant impact on HEV infection.



6	Examination of 20 anti-HEV IgG-positive and 20 IgG-negative individuals who frequently consume raw pork for genetic markers, as well as stool and serum IgA, microbiome and beta-defensin-2	3	UKE	01.06.2023		not achievable	A survey and recruitment of blood donors was not responsible during and immediately after the Covid pandemic, therefore the extension of this project to patients
7	Inclusion of 50% of subjects to MS 6	3	UKE	31.12.2022			See comment milestone 6
8	Start of recruitment of 40 subjects for the examination of genetic and hormonal markers in acute HEV infection	4 and 5	UKE	01.05.2022		completed	
9	Investigation of genetic and hormonal markers in 40 patients with acute HEV infection	4 and 5	UKE	30.02.2024	30.02.2024	completed	
10	Inclusion of 50% of subjects to MS 8	4 and 5	UKE	01.05.2023	01.08.2023	completed	Delayed but successfully finished

### Deliverables

No.	Title	Work package	Institution	Date as per project description	Corrected date	Status	Comment/reason
1	Extension of an existing ethics vote to include new aspects	1-5	UKE	01.05.2022	01.05.2022	completed	The decision of the ethics committee on an addendum to the approved ethics application (PV 7049) took too long, so we reverted to the clarification documents and the approved ethics application of the

							<b>“Livernet” in Hamburg (this covers all liver diseases and was already approved in 2013 and 2018, see appendix)</b>
<b>2</b>	<b>Clarification of the relevance of pets as possible sources of HEV infection</b>	<b>1</b>	<b>UKE</b>	<b>01.04.2024</b>	<b>01.08.2023</b>	completed	<b>Published (Pischke et a. Sci Rep 2023)</b>
<b>3</b>	<b>Clarification of the relevance of human-to-human transmission of HEV</b>	<b>2</b>	<b>UKE</b>	<b>01.04.2024</b>	<b>01.07.2024</b>	completed	<b>Will soon be published</b>
<b>4</b>	<b>Clarification of the role of the intestinal barrier as a barrier to the transfer of HEV into the bloodstream</b>	<b>3</b>	<b>UKE</b>	<b>01.06.2023</b>	<b>01.06.2024</b>	completed	
<b>5</b>	<b>Clarification of the importance of genetic and hormonal factors for the course of a HEV infection</b>	<b>4 and 5</b>	<b>UKE</b>	<b>30.02.2024</b>	<b>01.07.2024</b>	completed	<b>Final analysis and publication pending</b>
<b>6</b>	<b>Submissions for publications of deliverables 2 and 3</b>	<b>2 and 3</b>	<b>UKE</b>	<b>30.04.2024</b>		not achievable	<b>Not achievable in time. Delayed. Will be reached October 2024</b>
<b>7</b>	<b>Submissions for publications of deliverable 4</b>	<b>3</b>	<b>UKE</b>	<b>15.07.2023</b>		not achievable	<b>Not achievable in time. Delayed. Will be reached January 2025</b>
<b>8</b>	<b>Submissions for publications of deliverable 5</b>	<b>4 and 5</b>	<b>UKE</b>	<b>30.05.2024</b>		not achievable	<b>Not achievable in time. Delayed. Will be reached January 2025</b>

## c) Most important items of the financial report

Please refer to the entire duration of the project. Here, key words or enumerations are sufficient, coherent text and concrete numbers (in EUR) are not necessary.

Please note that the information in this chapter should correspond to the information in the numerical proof ("Beleglisten").

### Personnel costs

In the interests of data minimization, it is not necessary to provide people's real names. Please use function descriptions including classification/pay level, job scope and duration and, if available, personnel numbers (e.g. employment of technical staff 1, E9/level 3, 50%, three years (01/2020-12/2022)).

**Use:** Salary of Advanced Clinician Scientist fellow, 05/2022-01/2024 Ä3/3 50%; 02/2024-07/2024 Ä3/3 25%.

**Circumstances:** Due to Covid-related delays and emergency replacements, the scholarship had to be extended slightly with the approval of the DZIF (cost-neutral).

### Consumables (also contracts)

Please use general terms, e.g. mouse husbandry, antibodies, NGS, chemicals or similar. Please also indicate the awarding of contracts here.

**Use:** Consumables

- DNA sequencing
- PCR-testing
- Anti-HEV IgG and IgM testing

**Circumstances:** All consumables (antibodies, ELISA Kits, RT-PCR and genotyping kits) were consumed as planned.

### Investment funds

Please check the approved equipment list, if applicable.

**Use:** none

**Circumstances:** none

### Travel expenses

E.g. meetings with project partners, DZIF annual meetings, TTU/site meetings, scientific symposia.

**Use:** none

**Circumstances:** none

d) Necessity and adequacy of the work done

Please explain whether the use of resources was appropriate and necessary for the work performed or the results achieved (or whether the objective would have been achieved without the funding).

All resources were used up. The scholarship enabled me to pursue countless scientific questions regarding HEV and to accelerate my scientific career to the point where I have now received a professorship. I am extremely grateful to the DZIF for this excellent program.

e) Prospective benefit of the project, in particular the usability of the results according to the updated exploitation plan

Please also consider specific plans for the near future (e.g., use of results in follow-up projects/further development, who needs the result).

The data obtained in this project formed the basis for a multi-center One Health application with a budget of almost 1.2 million euros, of which over 200,000 are earmarked for the UKE to continue the projects. A decision on the application will be made in November. In particular, the aspects concerning pets and farm animals, as well as the relevance of the exposure of medical personnel and the consumption of raw pork are planned to be investigated in much more detail.

f) Progress/Advances in the field of the project by third parties during implementation of the project

Please comment if research results relevant to this DZIF project were made known by third parties that had a (possibly direct) influence on the course of the project.

Initially it has been planned in Work package 3 to test factors of the intestinal barrier as well as the stool microbiome. However, as this has already been published by another group, we modified the initial plans. The also planned testing for the stool microbiome was not carried out because another working group had already published (Wu et al. J. Med. Virol. 2022) this part was waived.

g) Successful or planned publication of the results according to No. 5 of the "Nebenbestimmungen für Zuwendungen" (NABF/NKBF 2017)

The following publications, which were produced during the scholarship period, include data from the scholarship:

1. Serological indication of chronic inflammatory demyelinating polyneuropathy as an extrahepatic manifestation of hepatitis E virus infection. Pischke S, Kjasimov A, Skripuletz T, Casar C, Bannasch J, Mader M, Huber S, Konen F, Wolski A, Horvatits T, Gingele S, Peine S, Hiller J, Seeliger T, Thayssen G, Lütgehetmann M, Schulze Zur Wiesch J, Golsari A, Gelderblom M. *Sci Rep.* 2024 Aug 20;14(1):19244. doi: 10.1038/s41598-024-70104-3.
2. Proof of infectivity of hepatitis E virus particles from the ejaculate of chronically infected patients. Schemmerer M, Bock HH, Schattenberg JM, Huber S, Polywka S, Mader M, Lohse AW, Todt D, Steinmann E, Wenzel JJ, Horvatits T, Pischke S. *J Med Virol.* 2024 Jun;96(6):e29735. doi: 10.1002/jmv.29735.
3. Lack of evidence of acute HEV infections as a sexually transmitted disease: Data from a German cohort of PrEP users. Schäfer G, Lübke R, Degen O, Mader M, Scheiter R, Wolski A, Addo MM, Schulze Zur Wiesch J, Pischke S. *Braz J Infect Dis.* 2024 Jan-Feb;28(1):103720. doi: 10.1016/j.bjid.2024.103720.
4. Anti-HEV seroprevalence and rate of viremia in a German cohort of dogs, cats, and horses. Pischke S, Knoop EV, Mader M, Kling L, Wolski A, Wagner A, Mueller K, Horvatits T, Stiller J, Wisnewski K, Kohn B, Schulze Zur Wiesch J, Groschup MH, Eiden M. *Sci Rep.* 2023 Nov 7;13(1):19240. doi: 10.1038/s41598-023-46009-y.
5. Emergence of resistance-associated variants during sofosbuvir treatment in chronically infected hepatitis E patients. Gömer A, Klöhn M, Jagst M, Nocke MK, Pischke S, Horvatits T, Schulze Zur Wiesch J, Müller T, Hardtke S, Cornberg M, Wedemeyer H, Behrendt P, Steinmann E, Todt D. *Hepatology.* 2023 Dec 1;78(6):1882-1895. doi: 10.1097/HEP.0000000000000514.
6. Higher Risk of HEV Transmission and Exposure among Blood Donors in Europe and Asia in Comparison to North America: A Meta-Analysis. Wolski A, Pischke S, Ozga AK, Addo MM, Horvatits T. *Pathogens.* 2023 Mar 8;12(3):425. doi: 10.3390/pathogens12030425.
7. No link between the prevalence of hepatitis E virus infection and the diagnosis of schizophrenia. Beisel C, Pischke S, Mader M, Moritz S, Schöttle D, Lüdecke D. *Infection.* 2022 Dec;50(6):1623-1624. doi: 10.1007/s15010-022-01871-2.