VILNIUS UNIVERSITY

Martynas
SIMANAVIČIUS

The prevalence studies and diagnostics of hepatitis E virus

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Natural Sciences,
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Hepatito E viruso diagnostika ir paplitimo tyrimai

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CONTENTS

LIST OF ABBREVIATIONS

aa – amino acid
ELISA – enzyme-linked immunosorbent assay
HEV – hepatitis E virus
HRP – horseradish peroxidase
IgA, IgM, IgG – immunoglobulin A, M, G
MAb – monoclonal antibody
NBS-RT-PCR – nested broad-spectrum reverse transcription PCR
ORF – open reading frame
ROC – receiver operating curve
RT-qPCR – reverse transcription-quantitative PCR
VLP – virus-like particle

INTRODUCTION

Hepatitis E is a human disease caused by the hepatitis E virus (HEV). HEV genome is a positive-sense single-stranded RNA. The virus belongs to the family *Hepeviridae*. Currently, seven HEV genotypes that infect various mammals are classified into the genus *Orthohepevirus*. HEV genotypes 1, 2, 3, 4, and 7 infect humans. HEV genotype 1 (HEV-1) and HEV-2 circulate exclusively within the human population and spread through contaminated water. These HEV variants cause outbreaks of infections with mortal consequences. HEV-3 is a zoonotic virus that infects not only humans but pigs, wild boars, deers, and rabbits as well. HEV-3 primary source of transmission to humans is poorly processed contaminated food products. This virus variant could also spread through blood products and organ transplants. When compared to HEV-1 and HEV-2, HEV-3 is considered as a less pathogenic virus that is dangerous to immunocompromised individuals, for instance, patients receiving treatment with blood products, immunosuppressants, or carrying organ transplants. HEV-3 is an emerging threat to public health.

The family *Hepeviridae* also consists of HEV-like viruses isolated from chicken, ferret, fox, bat, moose. Rat HEV was identified in Germany in 2010 and has been detected in other countries since then. Rats are commensals living near human dwellings. Rat HEV zoonosis was first described in 2018. Rat HEV is now being recognized as a human pathogen. There is also a discussed possibility of HEV-3 to infect rats, which are carriers of various other pathogens.

Epidemiologic studies of HEV infections are performed using molecular and serologic methods. The most popular means for HEV infection investigation is a standardized molecular assay. Serologic assays for epidemiologic research could be more cost-effective and straightforward means. Unfortunately, there is no serologic assay

approved for diagnostic use. Researchers use serologic systems or developed in-house or by some manufacturers. Results gathered using these different methods are variable and depend on the manufacturer. Thus, there is a need for novel serologic assays.

There are many unanswered questions in HEV biology about virus replication and transmission. The major limiting factor is the lack of a tissue culture system for effective HEV replication. Such research could be upgraded by the employment of monoclonal antibodies specific to HEV.

The dissertation aims to develop and apply tools for the investigation of HEV infection and its prevalence in Lithuania.

Tasks of the dissertation:

1. To generate and characterize monoclonal antibodies against yeast-expressed HEV-3 and rat HEV capsid proteins and evaluate their immunogenicity and antigenicity;
2. To develop serologic assays for detection of rat HEV-specific IgM and IgG antibodies and apply them along with molecular assays to investigate HEV prevalence in wild rats.
3. To evaluate HEV specific-serologic tests based on yeast-expressed HEV capsid proteins and perform a serologic investigation of HEV infection in pigs and humans
4. To evaluate the efficiency of the newly developed monoclonal antibodies for detection of HEV capsid protein in rat, pig, and human samples.

Scientific novelty:

Hepatitis E caused by HEV infection is a widespread infectious disease. HEV-1 and HEV-2 infect only humans, while HEV-3 and HEV-4 could infect humans and other mammals, such as pigs, wild boar, deer, rabbits. HEV-1 and HEV-2 infections are prevalent in developing countries and are spreading through contaminated water. The infections caused by these HEV genotypes are detected in

developed countries as well but are mainly travel-associated. In contrast, HEV-3 infections are widespread in developed countries. The main source of HEV-3 is undercooked meat products. HEV-3 is a zoonotic virus. Recently rat HEV zoonoses causing hepatitis E were described as well. Thus, rat HEV is considered a zoonotic virus. Serologic assays for the detection of rat HEV infection markers are lacking, and there is a growing demand for such assays.

Studies on HEV infections and their prevalence are performed using molecular and serologic methods. Molecular studies are based on reverse transcription PCR, which is standardized for all HEV genotypes. Serologic studies are not standardized, thus, there is a demand for novel tools and methods designed to investigate HEV infections. In this dissertation HEV capsid proteins forming virus-like particles (VLPs) synthesized in yeast for the first time are described. In the yeast expression system, protein post-translation modifications differ compared to other eukaryotic or prokaryotic expression systems. Thus, proteins expressed in yeast may possess unique properties. For instance, yeast-expressed HEV capsid proteins-formed VLPs could contain unique conformational epitopes, which may impact the sensitivity of a virus-specific antibody detection system.

Monoclonal antibodies (MAbs) developed and characterized in this dissertation are tools suitable for HEV investigation. The newly generated MAbs enrich the toolbox available for virus research. The novel MAbs are described that possess distinguished characteristics. All previously described antibodies to HEV capsid protein bind to one of the three domains. In that domain, the epitopes of neutralizing antibodies are located. In our study, MAb binding sites are located in all three domains of the HEV capsid protein. This is the first time when such MAbs are described thus providing a potential opportunity to develop more efficient and widely adaptable means for HEV research.

HEV prevalence studies in Lithuania are scarce. Recently, researchers from the Lithuanian University of Health Sciences have evaluated HEV prevalence in pigs, wild boars, and other ungulates (Spancerniene et al., 2016, Spancerniene et al., 2018). In this dissertation, the investigation of HEV prevalence in pigs enriches knowledge about HEV infections in Lithuanian pigs. Importantly, in our study, the prevalence of HEV in humans and wild rats is described for the first time. Investigation of wild rats significantly contributed to knowledge about rat HEV infections in Northeastern Europe.

1. LITERATURE OVERVIEW

The Hepatitis E virus (HEV) belongs to the *Hepeviridae* family. HEV infectious particles exist in two forms. The unenveloped virions are found in the feces while the enveloped virions circulate in the blood (Kamar et al., 2017). HEV genome is a positive-sense single-stranded RNA. It contains three open reading frames (ORFs). ORF1 encodes functional domains that participate in the replication of the viral genome. ORF2 encodes the capsid protein that packs the viral genome into virions. ORF3 encodes a phosphoprotein involved in virion formation and release (Ding et al., 2017). Virus strains are classified into the *Orthohepevirus* genus, which is comprised of four species (A-D) (Fig. 1). *Orthohepevirus A* is the main virus species which hosts are humans. HEV genotypes 1 (HEV-1) and HEV-2 belonging to *Orthohepevirus A* infect only humans while HEV-3 and HEV-4 are also endemic in pigs, wild boar, deer, and rabbits. HEV-3 and HEV-4 are zoonotic viruses. HEV-5 and HEV-6 infect only wild boar and HEV-7 was reported to infect camels and humans who consumed camel milk or meat. HEV strains belonging to HEV-3 have been identified in Europe, North America, South America, and Japan (Larrue et al., 2020). HEV strains belonging to the species *Orthohepvirus C* were first described to infect rat (Johne et al., 2010b, Johne et al., 2010a), then later was detected in the ferret (Raj et al., 2012), mink (Krog et al., 2013, Xie et al., 2018) and other rodents. Recently, *Orthohepevirus C* strains have also been identified to infect and cause symptoms in humans in contact with rats (Sridhar et al., 2018, Andonov et al., 2019, Sridhar et al., 2020). It allows evaluating rat HEV as a likely human zoonotic pathogen.

HEV epidemiology in developing and developed countries is diverse. In developing countries, HEV causes large epidemics and spreads through water contaminated by human feces (Kamar et al., 2017). In these regions, pregnant women are at the highest risk. HEV infection in the second and the third trimester of pregnancy may

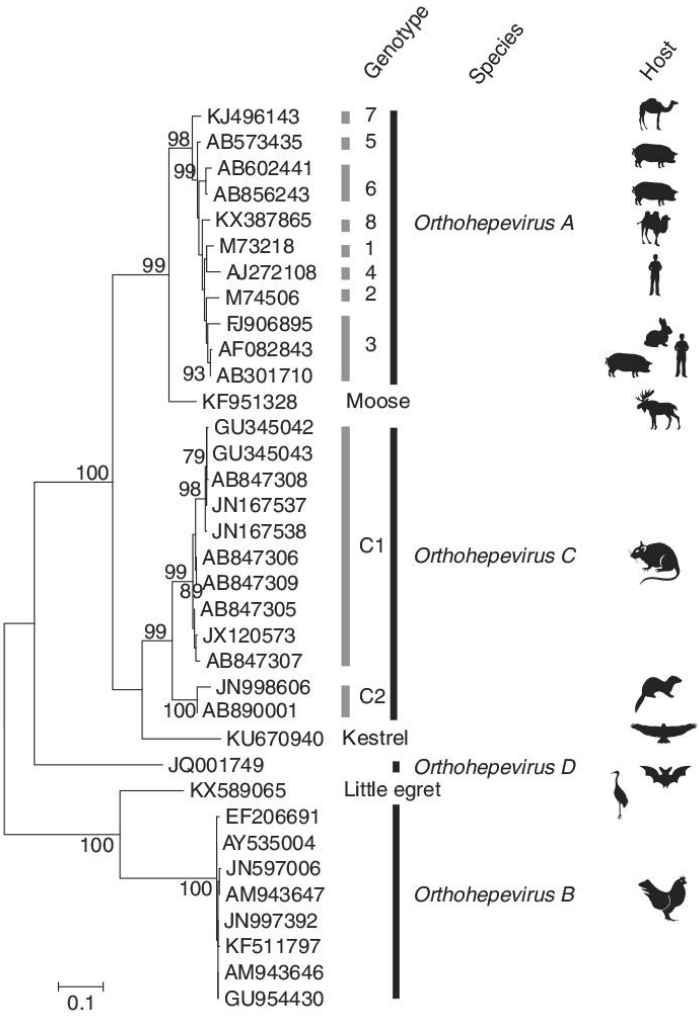


Figure 1. Phylogenetic relationship between *Orthohepevirus* species (Smith and Simmonds, 2018).

cause liver dysfunction. The mortality could reach 25% during the third trimester of pregnancy (Navaneethan et al., 2008). HEV could also be transmitted from mother to child. It increases neonatal

morbidity and mortality (Khuroo et al., 2009). In developed countries, HEV is a zoonosis and the main source of infection is the consumption of HEV-3 contaminated meat, mainly pork (Kamar et al., 2017). HEV transmission by blood transfusion of blood products has been described as a potential source as well (Hewitt et al., 2014).

Hepatitis E is a disease caused by HEV. This disease classically is an acute viral infection accompanied by jaundice in 5-30% of cases. The main symptoms are fever, nausea, vomit, loss of appetite, and general illness. The disease is self-limiting and disappears after a few days or weeks. However, the mortality could reach 0.5-4% during the outbreaks (Wedemeyer et al., 2012). Immunocompromised patients are at high risk of developing chronic HEV-3 infection. Such patients are organ transplant recipients (Gérolami et al., 2008) or HIV-positive individuals (Kenfak-Foguena et al., 2011). HEV infection is considered chronic when HEV replication lasts longer than 3 months (Kamar et al., 2013). During such infection, chronic hepatitis develops that could progress to liver cirrhosis in 10% of cases (Kamar et al., 2011). HEV-3 infection could cause the worst condition for immunocompromised patients.

Extrahepatic manifestations of HEV infections are also described. The symptoms caused by such infections could be divided into neurologic and renal. There is limited knowledge about these manifestations. It is believed that the direct effect of HEV replication in different organs or immune response to HEV replication may cause the symptoms. Neurologic symptoms include neuralgic amyotrophy, *Guillain-Barré* syndrome, paralysis, and multiple radiculopathies (Dalton et al., 2017). Renal injuries could develop during acute and chronic HEV infections (Kamar et al., 2005, Kamar et al., 2012). HEV infections could cause or worsen conditions such as glomerulonephritis, IgA nephropathy, cryoglobulinemia (Kamar et al., 2012, Del Bello et al., 2015, Guinault et al., 2016, Marion et al., 2018). Mechanisms underlying these conditions are unknown. It

is believed that renal dysfunction could be caused by the formation of immunocomplexes in the glomerular. These immunocomplexes may consist of HEV antigen, IgG antibodies to HEV, and rheumatoidal factor. Studies in favor of this theory showed that HEV antigen and RNA are detected in the urine of patients with chronic HEV infection (Geng et al., 2016, Marion et al., 2019). Furthermore, high HEV antigen concentrations were detected in the urine of immunocompromised patients independently of HEV RNA detection even though high molecular weight HEV virions should not pass through glomerular filtration barriers (Robinson et al., 1998, Montpellier et al., 2018, Yin et al., 2018). Despite that, low molecular weight secreted forms of HEV capsid protein (Montpellier et al., 2018, Yin et al., 2018) could migrate to urine and be detected. It might be possible that HEV antigen is secreted to urine by kidney epithelial cells. It has been shown that HEV could replicate in non-human primate and swine kidney cells (Grigas et al., 2020). However, there is no direct proof of HEV nephrotoxicity and the capability to replicate in human kidney cells (Lhomme et al., 2020).

HEV could be detected directly and indirectly by measuring various markers present during infection (Fig. 2). Diagnosis of HEV infection is based on the direct detection of viral RNA in the serum and/or feces. RNA detection methods target the conserved overlapping region of ORF3 and ORF2 (Baylis et al., 2019). Reverse transcription PCR is commonly used for HEV RNA detection. An indirect diagnosis is based on the detection of antibodies to HEV. IgM class antibodies to HEV indicate the ongoing HEV infection and are the first to appear in blood serum. IgG class antibodies to HEV indicate clearing or past infection (Al-Sadeq et al., 2018). Even though several HEV genotypes exist, antibodies elicited to them during an infection are very similar (Engle et al., 2002), thus indirect detection of HEV infection markers using serologic methods might be universal diagnostic tools.

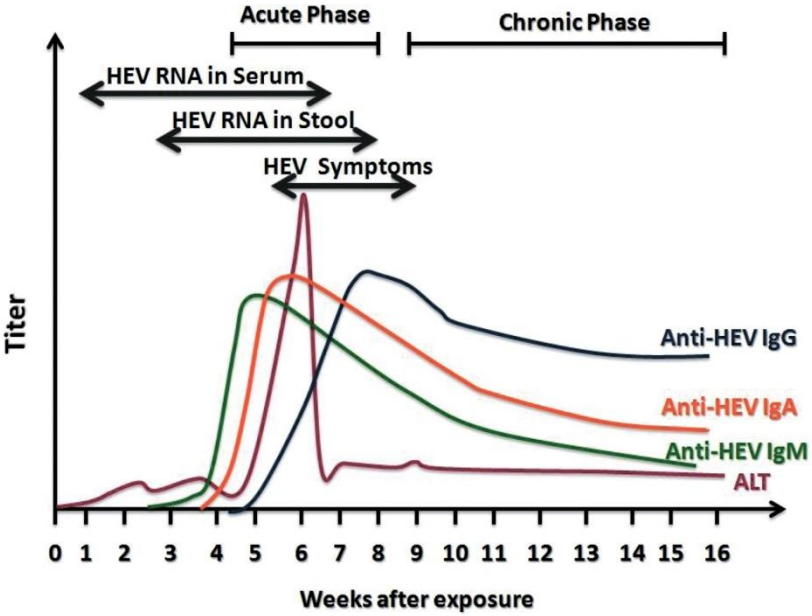


Figure 2. HEV infection markers levels during the course of human infection (Al-Sadeq et al., 2018).

Monoclonal antibodies (MAbs) could be an excellent tool for the research of HEV. MAbs could be employed to specifically detect viral capsid or proteins involved in viral replication to investigate HEV biology (Kumar et al., 2020). Furthermore, MAbs could be a basis of immunochemical techniques that are used for viral infection diagnostics. For instance, MAbs were used in capture ELISA to detect virus-specific IgM antibodies (Samuel et al., 2003). Besides, MAbs are used for capturing viral antigens in sandwich ELISA assays (Zhang et al., 2006).

2. MATERIALS AND METHODS

Materials. The reagents were purchased from Thermo Fisher Scientific, Fisher Scientific, Carl Roth, Merck (Millipore, Sigma-Aldrich) group, and Invitrogen unless otherwise stated. Primers and probes were purchased from Metabion International AG.

Recombinant proteins. HEV-3, rat HEV capsid proteins, and their truncated variants were synthesized in yeast in Vilnius University Life Sciences Center Institute of Biotechnology Department of Eucaryotic Gene Engineering as described previously (Simanavicius et al., 2018b).

Generation of monoclonal antibodies was carried out as described previously (Simanavicius et al., 2018b). All experiments using laboratory mice were performed under controlled laboratory conditions according to Lithuanian and European legislation (license No. LT 59-902, permission No. 184 for the breeding of experimental mice; permission No. 209 for the generation of polyclonal and monoclonal antibodies issued by the State Food and Veterinary Service, Vilnius, Lithuania).

The MAb specificity and epitope mapping analysis were performed as described previously (Simanavicius et al., 2018b).

Immunofluorescence assay was performed as described previously (Simanavicius et al., 2018b, Grigas et al., 2020)

Investigation of HEV infections in wild rats was performed as described previously (Simanavicius et al., 2018a).

The analysis of pig blood samples for the presence of anti-HEV antibodies was performed using "HEV-Ab ELISA" (Axiom Diagnostic) and in-house indirect ELISA based on yeast-expressed HEV-3, rat HEV, and truncated rat HEV capsid proteins. Briefly, recombinant HEV capsid proteins were immobilized on PolySorp (Nunc) microtiter plates. Samples were diluted at 1:10. HRP-labeled anti-pig IgG $F(Ab')_2$ fragment was diluted 1:15000 and used as a secondary antibody.

The analysis of human blood samples for the presence of anti-HEV antibodies was performed using “Wantai HEV-IgM ELISA” and “Wantai HEV-IgG ELISA” (Beijing Wantai Biological Pharmacy) and in-house indirect ELISA based on yeast-expressed HEV-3, rat HEV, and truncated rat HEV capsid proteins. Briefly, recombinant HEV proteins were immobilized on PolySorp (Nunc) microtiter plates. Samples were diluted at 1:100. HRP-labeled anti-human IgG Fc fragment (SouthernBiotech) was diluted 1:10000 and used as a secondary antibody.

HEV antigen detection was performed using “Wantai HEV-Ag ELISA” and in-house capture ELISA based on the MAbs generated in this study. Briefly, MAb CPE4 was immobilized on MaxiSorp (Nunc) microtiter plates. Fifty µl of a sample was diluted with 20 µl of dilution buffer. For the detection, MAb CPD9 labeled with Alexa Fluor 555 (Invitrogen) was used. The labeling reaction was performed according to the manufacturer’s instructions. Fluorescence was measured using Varioskan Flash (Thermo Scientific).

3. RESULTS AND DISCUSSION

3.1. Generation and characterization of monoclonal antibodies against hepatitis E virus

Recombinant full-length HEV-3 and rat HEV capsid proteins expressed in yeast cells and self-assembled into VLPs (Fig. 3) were used as immunogens to produce MAbs. The immunogenicity of recombinant VLPs was evaluated by an indirect ELISA. Both

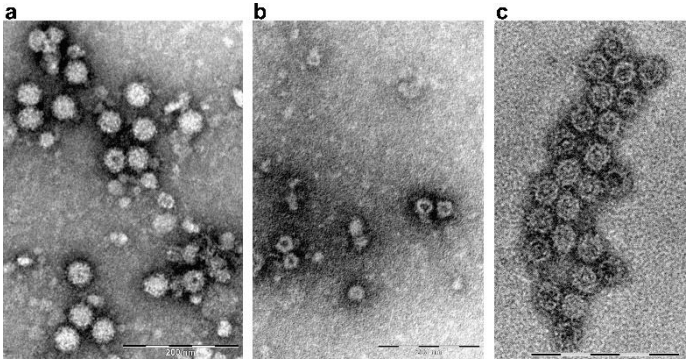


Figure 3. Electron micrograph of yeast-generated recombinant rat HEV capsid protein (1-645 aa) (a); HEV-3 (1-660 aa) (b); and rat HEV 112-608 aa fragment (c) in CsCl fractions (scale bar = 200 nm).

recombinant HEV capsid proteins were immunogenic as they induced high titers of antigen-specific IgG antibodies after the second immunization with either HEV-3 capsid protein (titer 1:9800) or rat HEV capsid protein (titer 1:4100). A fusion of spleen cells from the immunized mice with murine myeloma cells resulted in 11 and 7 hybridomas producing MAbs of IgG isotype against HEV-3 and rat HEV capsid proteins, respectively. The MAbs were tested in an indirect ELISA for their cross-reactivity with both antigens and divided into three groups based on cross-reactivity analysis results (Table 1). Six MAbs (2B2, CPE1, CPH7, CPE2,

CPF6, 5G5) raised against rat HEV capsid protein reacted exclusively with rat HEV capsid protein. Eight MAbs (CPB11, CPC7, CPH6, CPE9, CPD7, 4G4, 5F3, CPC9) raised against HEV-

Table 1. Characterization of the MAbs raised against HEV-3 and rat HEV capsid proteins.

MAbs specific to HEV-3 capsid protein					**MAbs specific to rat HEV capsid protein**			
MAb clone	**IgG subtype**	**ELISA** (HEV-3)	**WB** (HEV-3)	**IF** (HEV-3 strain 47832c)	**MAb clone**	**IgG subtype**	**ELISA** (rat HEV)	**WB** (rat HEV)
CPB11[a]	IgG2a	*2.32×10^{-10}*	-	+	**2B2**	IgG1	4.32×10^{-9}	-
CPC7[a]	IgG2a	*1.97×10^{-10}*	-	+	**CPE1**[d]	IgG1	4.19×10^{-10}	-
CPH6[a]	IgG2a	2.33×10^{-10}	-	+	**CPH7**[d]	IgG1	1.48×10^{-10}	-
CPE9[b]	IgG2a	7.28×10^{-10}	-	-	**CPE2**[e]	IgG1	2.57×10^{-10}	+
CPD7[b]	IgG2a	*2.32×10^{-11}*	-	-	**CPF6**[e]	IgG1	3.37×10^{-10}	+
4G4[c]	IgG1	*9.91×10^{-10}*	+	+	**5G5**	IgG1	7.93×10^{-10}	+
5F3[c]	IgG2b	*9.59×10^{-11}*	+	+				
CPC9	IgG1	*2.56×10^{-10}*	+	-				

MAbs cross-reactive with HEV-3 and rat HEV capsid proteins				
MAb clone	**IgG subtype**	**ELISA** (HEV-3/rat HEV)	**WB** (HEV-3/rat HEV)	**IF** (HEV-3 strain 47832c)
2E6[f]	IgG2a	*1.46×10^{-10}/3.15×10^{-10}*	-/-	+
CPE4[f]	IgG2a	*1.45×10^{-10}/3.38×10^{-10}*	-/-	+
CPD9	IgG2a	*2.32×10^{-11}/2.39×10^{-10}*	+/-	+
9C8	IgG1	8.24×10^{-11}/2.57×10^{-10}	+/+	+

MAb reactivity in ELISA. Apparent K_d values (M) determined by an indirect ELISA are indicated.
MAb reactivity pattern in immunofluorescence (IF) and Western blot (WB) test: -, negative; +, positive.
[a-f] groups of MAbs against overlapping epitopes. Identification of the groups was performed by a competitive ELISA.

3 capsid protein reacted only with HEV-3 capsid protein. Four MAbs (2E6, CPE4, CPD9, 9C8) demonstrated cross-reactivity with both proteins (Table 1). The reactivity of the MAbs was further investigated by Western blot to evaluate their capability to recognize linear epitopes in SDS-denatured HEV-3 and rat HEV capsid proteins (Fig. 4). Five of 8 MAbs reactive with HEV-3 capsid protein in ELISA did not react in Western blot indicating that they recognized conformational epitopes. Other 3 MAbs recognized linear epitopes as they were reactive in Western blot assay (Fig. 4c,

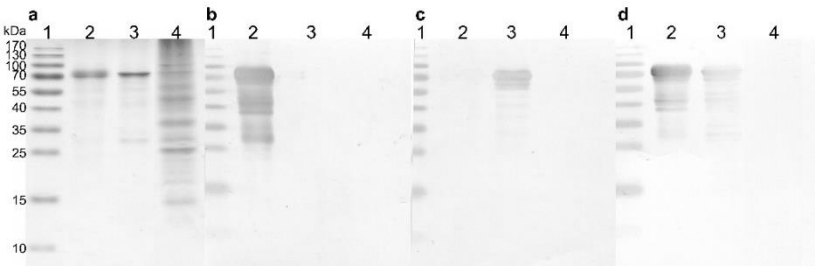


Figure 4. Analysis of the specificity of MAbs, representative for each of the three groups, by Western blot test. (a) PageBlue-stained SDS-PAGE; (b) Western blot analysis with the MAb CPE2 specific to the rat HEV capsid protein; (c) Western blot analysis with the MAb 4G4 specific to the HEV-3 capsid protein; (d) Western blot analysis with the cross-reactive MAb 9C8. Lane 1, PageRuler Prestained Protein Ladder (Thermo Fisher Scientific); lane 2, recombinant rat HEV capsid protein (0,5 μg protein per lane); lane 3, recombinant HEV-3 capsid protein (0,5 μg protein per lane); lane 4, lysate of pFX7-transformed S. cerevisiae cells.

lane 3, and Table 1). Three of 6 MAbs exclusively reactive with the rat HEV capsid protein recognized its conformational epitopes, and 3 recognized linear epitopes as they were reactive both in ELISA and Western blot test (Fig. 4b, lane 2 and Table 1). Two of the 4 cross-reactive MAbs recognized conformational structures of HEV-3 and rat HEV capsid proteins as they did not react with these antigens in Western blot (Table 1). The MAbs 9C8 and CPD9 showed different reactivities in the Western blot test: MAb 9C8 recognized linear epitopes in both HEV capsid proteins (Fig. 4d, lanes 2, 3) while MAb CPD9 recognized a linear epitope only in HEV-3 capsid protein (Table 1).

The affinity of the MAbs to HEV-3 and/or rat HEV capsid proteins was analyzed by an indirect ELISA and expressed as an apparent dissociation constant (K_d). The K_d values ranged from 9.91×10^{-10} to 2.32×10^{-11} M for the MAbs raised against the HEV-3

capsid protein and from 4.32×10^{-9} to 1.48×10^{-10} M for the MAbs raised against the rat HEV capsid protein (Table 1).

Mapping of MAb epitopes of HEV-3 and rat HEV capsid proteins was performed by two approaches. In the first step, a competitive and sandwich ELISAs with non-labeled and HRP-labeled MAbs were used. Based on the competitive ELISA results, all MAbs were divided into 6 groups (Table 1, groups denoted by superscript a-f). It was assumed that the MAbs belonging to a certain group compete

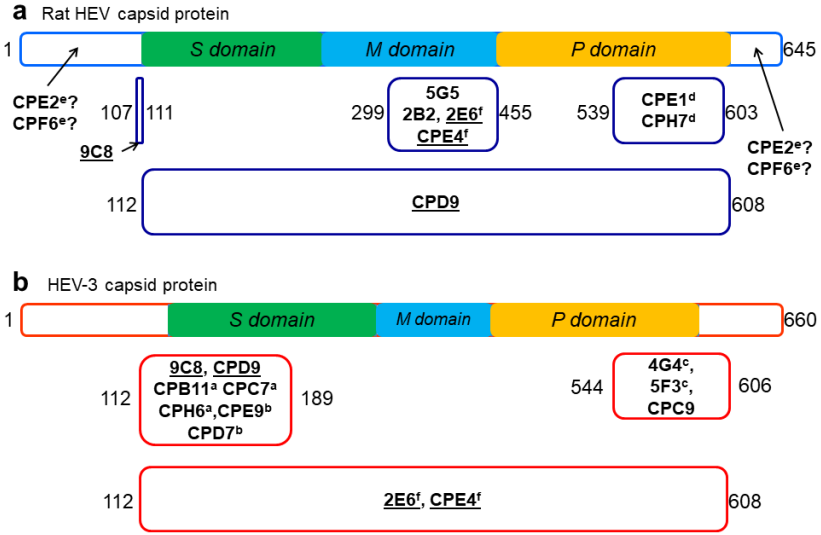


Figure 5. Schematic representation of the MAbs binding sites localized within rat HEV capsid protein (a) and HEV-3 capsid protein (b) are indicated. Cross-reactive MAbs are underlined. Approximate locations of HEV-3 capsid protein domains and putative rat HEV capsid protein domains are highlighted: S domain (green), M domain (blue), P domain (yellow). [a-f] groups of MAbs against overlapping epitopes. Identification of the groups was performed by a competitive and sandwich ELISAs (see Table 1).

for the same binding site on HEV capsid protein. Five MAbs remained ungrouped as they did not compete with any of the MAbs. In the second approach, the yeast-expressed N- and C-termini truncated HEV-3 and rat HEV capsid proteins and 6 additional overlapping fragments were generated in *E. coli* and used for localization of MAbs binding sites.

The localization of MAb epitopes using truncated capsid proteins and their overlapping fragments was performed by capture ELISA and Western blot test. Several MAbs binding sites along HEV-3 and

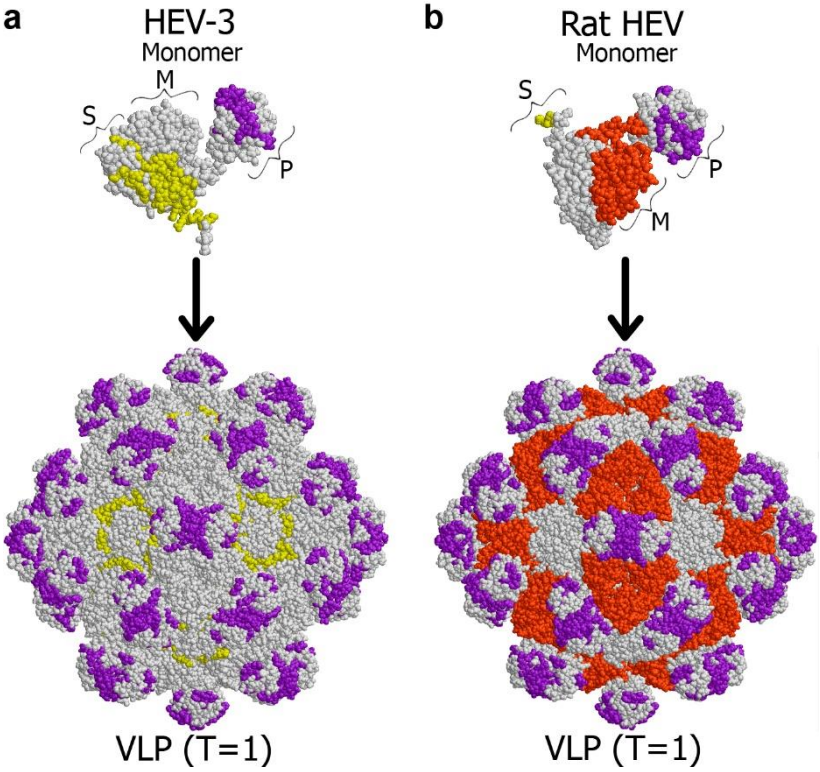


Figure 6. HEV-3 (a) and rat HEV (b) capsid protein monomer and VLP model. (a) aa 112-189 in yellow, aa 544-606 in violet; (b) aa 107-111 in yellow, aa 299-455 in red, aa 539-603 in violet.

rat HEV capsid proteins were identified (Fig. 5). The MAbs 5G5 and 2B2 recognized aa 299-455 sequence within the putative M domain of rat HEV capsid protein (Fig. 6b, marked in red). The putative M domain is located between the most protruded putative P domains in the VLP model. Antibodies could likely bind to these structures. The MAbs CPE1 and CPH7 were reactive with aa 539-603 sequence within the putative P domain of rat HEV capsid protein (Fig. 6b, marked in violet). The putative P domain is on top of the VLP surface thus antibodies could bind it the most easily. The MAbs CPB11, CPC7, CPH6, CPE9, and CPD7 recognized aa 112-189 of HEV-3 capsid protein S domain that is the most distant region from the VLP surface (Fig. 6a, marked in yellow). These antibodies may have arisen since full-length HEV-3 capsid protein was used for immunization. The MAbs 4G4, 5F3, and CPC9 are reactive to aa 544-606 sequence within the P domain of HEV-3 capsid protein (Fig. 6a, marked in violet). The cross-reactive epitope of the MAb 9C8 within capsid proteins of rat HEV and HEV-3 was determined most precisely as this MAb reacted with fragments comprising aa 107-456 of rat HEV capsid protein and aa 111-472 of HEV-3 capsid protein, but did not react with truncated rat HEV capsid protein comprising aa 112-608. This suggests that the MAb 9C8 recognizes an epitope comprising aa 107-111 within rat HEV capsid protein and an epitope comprising aa 112-189 within HEV-3 capsid protein. The alignment of aa sequences of HEV-1 to HEV-4 and rat HEV capsid proteins demonstrated that the putative epitope of the MAb 9C8 is formed by a 4 aa-long sequence N-TAPV-C common in both HEV-3 and rat HEV capsid proteins. Another cross-reactive MAb CPD9 recognized aa 112-189 of HEV-3 capsid protein S domain (Fig. 6b, marked in yellow) and aa 112-608 of rat HEV capsid protein. The cross-reactive MAbs 2E6 and CPE4 were reactive with aa 299-455 sequence within the putative M domain of rat HEV capsid protein (Fig. 6) and aa 112-608 sequence of HEV-3 capsid protein. The MAbs CPE2 and CPF6 did not react with any of the truncated capsid

proteins. Thus, binding sites of these two MAbs are located either at the N- or C-terminus of rat HEV capsid protein.

The epitopes recognized by the newly generated MAbs against yeast-expressed capsid proteins are located not only in the P domain but also in the S and M domains of HEV capsid proteins. This demonstrates the new reactivity pattern of HEV-specific MAbs as there are no published data on MAbs reactive with other parts of HEV capsid proteins than the P domain (Riddell et al., 2000, Schofield et al., 2000, Meng et al., 2001, Zhang et al., 2005, He et al., 2007, Takahashi et al., 2008, Zhang et al., 2009, Wei et al., 2014,

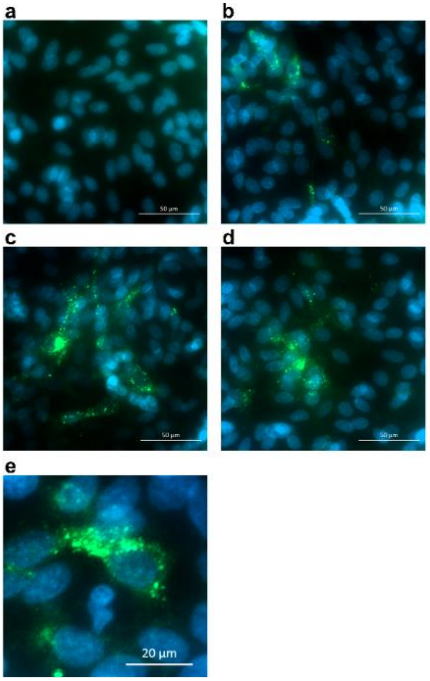


Figure 7. Analysis of the MAb reactivity with HEV-infected cells. HEV antigen appears green (FITC), cell nuclei blue (DAPI). (a) MAb CPE9 (negative); (b) MAb 5F3 (positive); (c) MAb CPD9 (positive); (d) MAb 2E6 (positive).

Gu et al., 2015, Tang et al., 2015, Kobayashi et al., 2016). Furthermore, our results on fine epitope mapping are in line with previous reports demonstrating that MAb binding sites, which recognize conformational epitopes, are located within the region of aa 112-608 of HEV capsid protein (Wei et al., 2014). In previous reports, neutralizing antibodies against HEV capsid protein were mapped to linear and conformational epitopes within the regions comprising aa 458-607 (Zhou et al., 2004), aa 578-607 (Schofield et al., 2000), and aa 452-617 (Meng et al., 2001).

The MAb ability to recognize a native virus is essential as only virus-reactive antibodies could be employed for HEV investigation. The MAbs were tested for their reactivity with HEV-3 strain 47832c-infected cells using an immunofluorescence assay. By this, 9 of 12 MAbs showed specific immunofluorescence characterized by granular cytoplasmatic staining of single cells or small groups of cells (Table 1). Examples of staining are presented in Figure 7. The MAb 5F3 ability to bind to a native HEV was also tested in immunofluorescence assay using cells infected with HEV strain isolated from a wild boar hunted in Lithuania. This MAb identified HEV-infected non-human primate kidney (Vero) and porcine kidney (PK-15) cells (Fig. 8).

The analysis of epitope mapping data and the immunofluorescence data indicates that the MAbs that recognize viral capsids in HEV-infected cells are directed to various epitopes. The MAbs CPB11, CPC7, CPH6, CPD9, and 9C8 are reactive with the S domain of HEV-3 capsid protein (Fig. 5 and 6a). The MAbs 5F3 and 4G4 are specific to the P domain (Fig. 6a). The P domain is located on top of the virus particle structure thus is easily accessible to the MAbs. Native HEV-reactive MAbs 2E6 and CPE4 binding sites are located in the putative M domain of rat HEV capsid protein. It is possible that in HEV-3 capsid protein these MAbs bind to the M domain as well. The M domain in both HEV VLPs is located on the surface between the protruding P domains (Fig. 6). In summary,

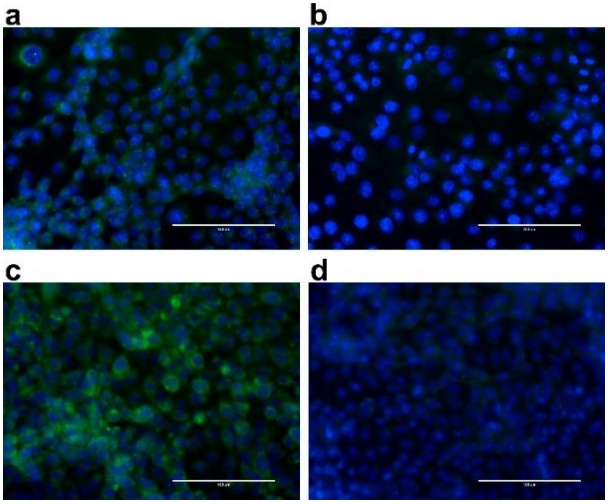


Figure 8. Analysis of the MAb 5F3 reactivity with HEV-infected cells. HEV antigen appears green (Alexa Fluor 488), cell nuclei blue (DAPI). HEV-infected PK -15 (a), Vero (c) cells; negative controls: PK-15 (b) and Vero (d). Scale bar: 100 μm (a-d).

ELISA, Western blot, immunofluorescence, and epitope mapping results reveal that a panel of MAbs recognizing a broad spectrum of binding sites on HEV-3 and rat HEV capsid proteins was generated. The identified MAbs binding sites are located in different domains of HEV capsid proteins, thus expanding the potential of the MAbs as useful tools for HEV detection and investigation. Moreover, the reactivity of the MAbs raised against yeast-expressed HEV-3 VLPs with HEV-infected cells indicates that recombinant VLPs resemble the antigenic structure of native viral capsids. The well-characterized MAbs described in our study might be used as tools for localization and visualization of HEV infection sites in infected tissues of various species by immunostaining techniques.

3.2.Serologic assays for antibody to HEV detection

Serologic HEV detection tests are designed to target antibodies to HEV. IgG antibodies are detected at the end of HEV infection or show the past infection. IgM antibodies are markers of the ongoing infection. When blood specimens are investigated, HEV proteins are used for the capture of HEV-specific antibodies. During the infection antibodies to HEV capsid protein are generated. Thus, yeast-expressed HEV-3, full-length, and truncated rat HEV capsid proteins were used to develop serologic HEV-specific antibody detection tests. In our study, wild rat, pig, and human samples were investigated. For every origin of the specimen serologic assays based on an indirect ELISA were designed. At first, wild rat samples were characterized by molecular methods to assess which rats were rat HEV RNR positive, and then later the presence of antibody to rat HEV was tested using newly developed ELISA. Pig and human samples were tested using commercially available serologic assays, then all samples were tested by newly developed ELISAs and the results were compared. In this section, in-house ELISAs for the detection of HEV-specific antibodies in rat, pig, and human samples are discussed and the results of HEV prevalence are described in further sections.

Serologic anti-rat HEV antibody tests are not available commercially. Thus, the goal to develop an ELISA for the detection of rat HEV capsid protein-specific antibodies was raised. Positive and negative controls are beneficial components of the test that makes interpretation of the results easier when no reference tests exist. For this purpose, polyclonal antibodies against rat HEV capsid protein were developed by immunizing laboratory rats. The rats were immunized once to raise both IgM and IgG antibodies so that polyclonal antibodies could be used as controls in both IgM and IgG anti-rat HEV detection tests. On day 13 after the immunization, the

anti-rat HEV antibody titer in rat blood serum was 1:70 for IgM and 1:1693 for IgG.

Indirect ELISA was developed for the detection of rat HEV-specific antibodies (Fig. 9). Yeast-expressed full-length and truncated rat HEV capsid proteins were tested as capture reagents. As a potential antibody source, chest cavity fluid from wild rats precipitated with ammonium sulfate was used. The amount of sample used was unified by the total protein concentration measured of the suspensions. Captured rat HEV capsid protein-specific antibodies were detected using either anti-rat IgM or IgG secondary antibodies labeled with HRP. It was observed that truncated rat HEV capsid protein is a more suitable reagent giving lower background noise (data not shown). Rat HEV seroprevalence is discussed in section 3.3.

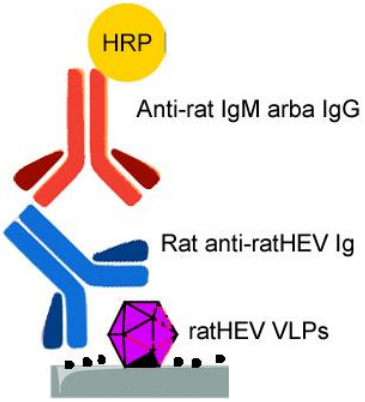


Figure 9. The scheme of an indirect ELISA used for the detection of rat HEV-specific antibodies in wild rat samples.

For the detection of anti-HEV pig and human IgG antibodies, an indirect ELISA was developed as well. As capture reagents, recombinant HEV-3, full-length, and truncated rat HEV capsid proteins were used. As an antibody source, pig or human blood serum specimens diluted 1:10 were used. Captured antibodies were

detected using HRP-labeled secondary antibodies to either pig IgG or human IgG. It was decided to use HEV-3 and rat HEV capsid proteins as both HEV variants are capable of infecting humans (Sridhar et al., 2020).

Furthermore, there are reports that in some individuals, antibodies from blood serum are more reactive with rat HEV capsid protein than HEV-1 or HEV-3 (Dremsek et al., 2012, Shimizu et al., 2016). Besides, it is thought that rat HEV could infect pigs (Pavio et al., 2017). It was hypothesized that using serologic tests it is possible to assess whether HEV-3 or rat HEV infection has occurred.

3.3.Studies on hepatitis E virus infection in wild rats

Norway rat and Black rat liver samples from Lithuania were analyzed for the presence of rat HEV and other hepeviruses using reverse transcription-quantitative PCR (RT-qPCR) specific for either rat HEV (Widén et al., 2014) or HEV-1-4 (Jothikumar et al., 2006). Nine of 109 (8.3%) wild rat samples were found to be positive for rat HEV RNA using rat HEV-specific RT-qPCR (Fig. 10). No positive samples were detected using HEV genotype 1-4 RT-qPCR. This finding is in line with previous investigations which demonstrated that Norway rats are not susceptible to HEV genotypes 1, 3, and 4 infections (Li et al., 2013a, Ryll et al., 2017). Furthermore, the current study results confirm rat HEV infections in Black rats from Europe as it was shown only once earlier (Ryll et al., 2017).

Rat HEV RNA-positive samples were analyzed by nested broad-spectrum reverse transcription PCR (NBS-RT-PCR) for confirmation. Only 6 of 9 rat HEV RT-qPCR positive samples were also positive in NBS-RT-PCR. It might be caused due to RNA degradation as the RT-qPCR target is smaller in length when

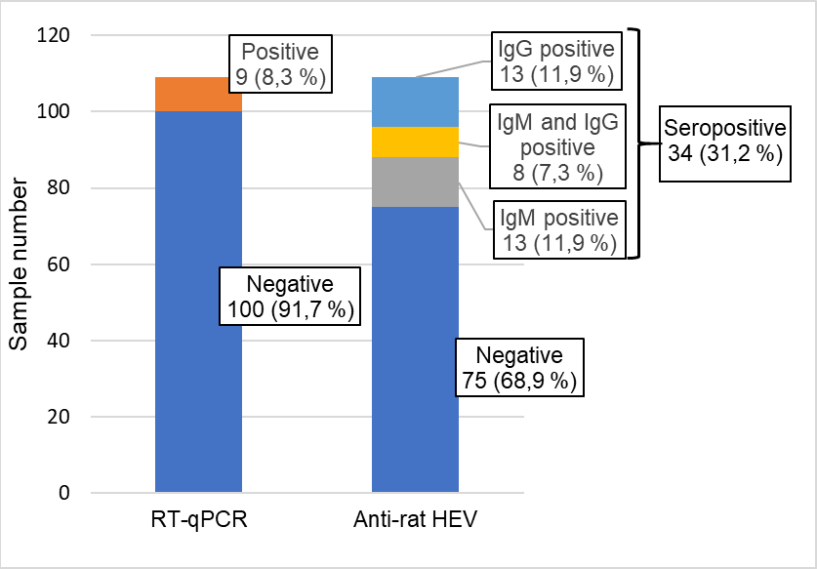


Figure 10. Results of molecular and serological screening of liver and blood samples of wild rats trapped in Lithuania (n=109).

compared to NBS-RT-PCR. The PCR products were subjected to sequencing. Multiple sequence alignment of the newly obtained rat HEV sequences revealed 98.7%-99.4% nucleotide sequence identity to HEV genotype C1 reference strain rat/R63/DEU/2009 (GenBank: GU345042) (Johne et al., 2010a). Phylogenetic analysis showed that all newly identified rat HEV sequences, independently of the rat species, cluster together with rat HEV sequences, species Orthohepevirus C, genotype C1 found in Germany (Fig. 11). This clustering pattern of HEV genomic sequences obtained from relatively close geographical regions was observed earlier and shows the circulation of rat HEV in local populations (Johne et al., 2012, Li et al., 2013b, Widén et al., 2014, Ryll et al., 2017).

In addition to molecular analysis, rat CCF samples were investigated by an indirect ELISA for the presence of anti-rat HEV IgM and IgG antibodies. Analysis of 109 CCF samples revealed a

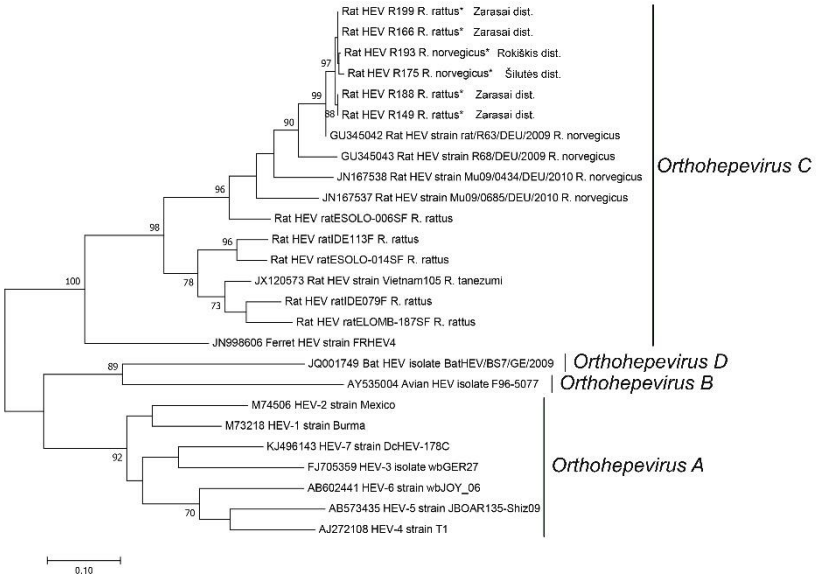


Figure 11. Phylogenetic tree showing the relation of HEV sequences obtained from wild rats trapped in Lithuania to various HEV sequences within the genus *Orthohepevirus*. The GenBank accession numbers, strains and hosts of the Orthohepevirus C HEV genotype C1 strains are indicated. The scale bar indicates phylogenetic distances in nucleotide substitutions per site. Bootstrap values equal and over 70% are indicated. An asterisk marks rat HEV sequences described in this study.

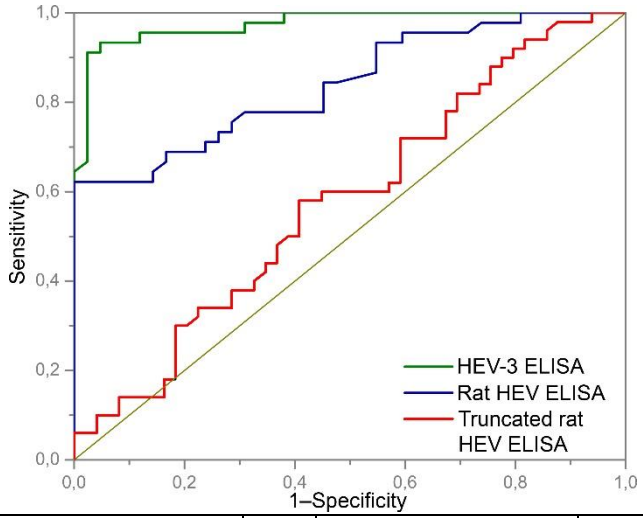
total of 34 samples (31.2%) positive for either IgM or IgG or both IgM and IgG antibodies against rat HEV (Fig. 10). The level of HEV seroprevalence demonstrated in this study is slightly higher than the 24.5% previously described in Germany (Johne et al., 2012). The observed high seroprevalence suggests a high rate of rat HEV circulation in the examined rat population. Thus, the Lithuanian wild rats' population may represent a promising object for future studies of rat HEV transmission routes. Comparing the serological and molecular investigation results did not show an association between

the presence of rat HEV RNA and rat HEV-specific antibodies. Only 4.6% of rats examined were positive for both rat HEV RNA and anti-rat HEV antibodies, while 26.6% and 3.7% of rats were only anti-rat HEV antibody positive and only rat HEV RNA positive, respectively. These results are in line with previous field and experimental studies suggesting that rat HEV infection in Norway rats is most likely non-persistent (Purcell et al., 2011, Johne et al., 2012). In contrast to Norway rats, there are still very limited data on the association of anti-HEV antibody and RNA detection in Black rats in Europe (Ryll et al., 2017). Our study revealed a similar pattern of rat HEV infection markers in Black rats as previously reported in Norway rats (Johne et al., 2012). This observation implies that the non-persistent rat HEV infection in individual rats is independent of rat species.

Summarizing, the current study demonstrated the presence of rat HEV infection in Black rats and Norway rats from Lithuania. Phylogenetic analysis of the newly identified rat HEV sequences showed a highly related clustering with genomic sequences of rat HEV sequences from Germany. Serological analysis using yeast-expressed rat HEV capsid protein revealed a high prevalence of rat HEV-specific antibodies in the rat population analyzed. The current investigation increases the knowledge of rat HEV geographical distribution in Europe.

3.4. Studies on hepatitis E virus infection in pigs

Food is the main source of HEV-3 infection in Europe. This virus genotype is a zoonotic virus as there are strains that infect both humans and pigs. HEV infections in pigs are asymptomatic (Pavio et al., 2017), but are symptomatic in humans. Therefore, seroepidemiological surveillance of HEV infection rate in pigs is very important.



Test	AUC	Standard Error	95% CI
HEV-3 ELISA	0.975	0.014	0.947–1.003
Rat HEV ELISA	0.840	0.042	0.757–0.923
Truncated rat HEV ELISA	0.578	0.058	0.465–0.690

Figure 12. ROC analysis of yeast-expressed HEV capsid proteins based ELISA tests targeting pig IgG anti-HEV.

A total of 99 pig blood serum samples were tested using "HEV-Ab ELISA" (Axiom) for the presence of total antibodies against HEV. The results showed that 50.51% of the samples were positive of anti-HEV antibodies. A previous study in Lithuania revealed 43.75% seroprevalence in pigs (Spancerniene et al., 2016). All pig blood serum samples were also tested using pig IgG antibody-targeted indirect ELISA based on recombinant yeast-expressed HEV capsid proteins. ROC analysis was performed to evaluate the diagnostic potential of the tests based on these proteins (Fig. 12). It was concluded that recombinant HEV-3 capsid protein-based ELISA (HEV-3 ELISA) was the most efficient as its AUC = 0.975 ± 0.014. Other recombinant proteins tested were of insufficient

performance in ELISA showing AUC = 0.840 ± 0.042 for full-length (Rat HEV ELISA) and AUC = 0.578 ± 0.058 for truncated (Truncated rat HEV ELISA) rat HEV capsid proteins. HEV-3 ELISA performance was likely better since a pig is a natural host of HEV-3 while rat HEV infection in pigs was not reported. However, HEV-3 ELISA was not as efficient as "HEV-Ab ELISA". This might be due to different antigens used and that HEV-3 ELISA targets IgG antibodies compared to total antibodies targeted in "HEV-Ab ELISA".

3.5. Studies on hepatitis E virus infection in humans

In Europe, HEV infections are the most dangerous for immunocompromised patients. People with chronic kidney diseases or kidney transplant recipients belong to the risk group of acquiring chronic hepatitis E. By performing a collaborative biomedical research "Evaluation of virologic and immunologic factors associated with altered kidney function and kidney transplant rejection" conducted by Vilnius University hospital Santaros clinics, a total of 228 human blood serum specimens were examined. The participants of this study were divided into 4 groups – kidney transplant recipients; chronic kidney disease patients for whom dialysis procedures are performed; chronic kidney disease patients for whom dialysis procedures are not performed; and healthy individuals (control group) (Fig. 13). Organ transplant recipients are at risk because they are under an immunosuppressant medicine regimen to prevent transplant rejection. Furthermore, HEV could be transmitted with an organ transplant (Kamar et al., 2017). Patients with chronic liver disease are at a higher risk of acquiring hepatitis E because their immune system is weak. These individuals could be divided into 2 groups based on whether dialysis procedures are performed. If dialysis is performed, a patient's disease condition is worse. Thus, patients under dialysis have more advanced chronic

kidney disease. Individuals in the control group are healthy people without chronic kidney or liver disease.

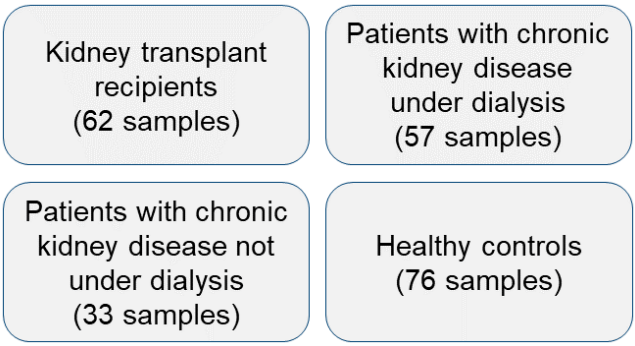


Figure 13. Research groups of patients for investigation of HEV infections.

First of all, blood serum samples were investigated using the most popular serologic assays for HEV-specific antibody detection according to previous reports: "Wantai HEV-IgM ELISA" and "Wantai HEV-IgG ELISA" that detect IgM and IgG antibodies to HEV, respectively. Only 1 sample was IgM anti-HEV positive. IgM to HEV is the first class of antibodies detected during HEV infection. The highest IgM level is observed during the acute phase of HEV infection and then decreases (Fig. 2). This result possibly shows that a patient was infected with HEV during the time of sampling. Anti-HEV IgG shows that HEV infection is in the chronic phase or has already passed. "Wantai HEV-IgG ELISA" test detected 34 positive samples (14.91%, CI 95% 10.84-20.15) out of 228 (Fig. 14). However, it could not be concluded that 14.91% of anti-HEV IgG prevalence was observed in Lithuania. A hypothesis could be raised that the HEV seroprevalence in Lithuania is 10.53% (CI 95% 5.19-19.66) as observed in the healthy control group. Similar seroprevalence was detected in Italy, the United Kingdom, Norway, Denmark, and Spain. However, as the control group consisted of

only 76 individuals, such group size could not be used for seroprevalence estimation in the general population. This part of our study aims to evaluate anti-HEV IgG seroprevalence in different risk groups.

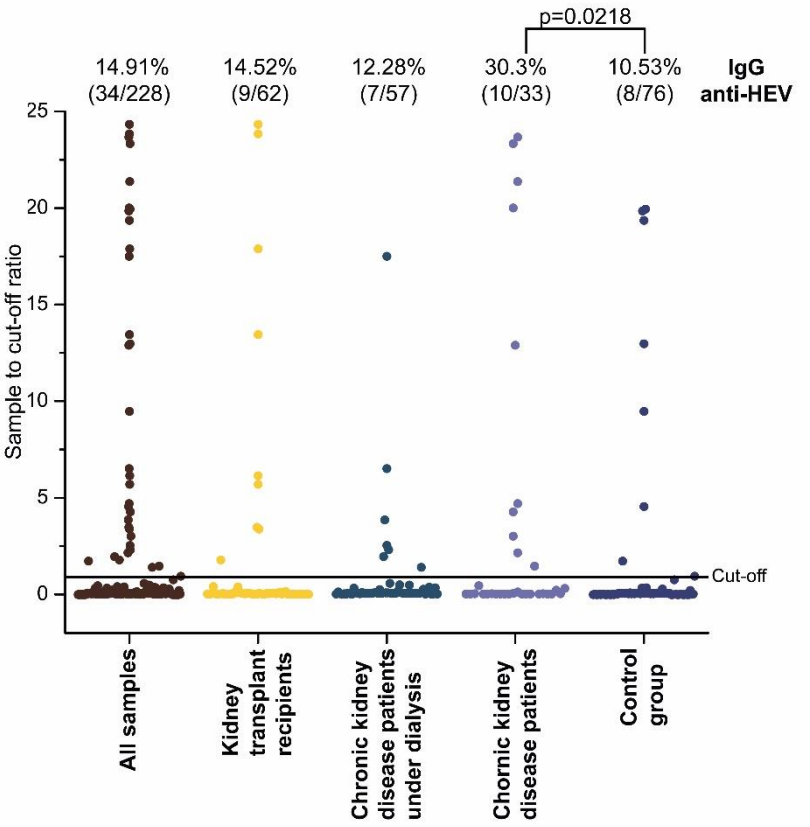
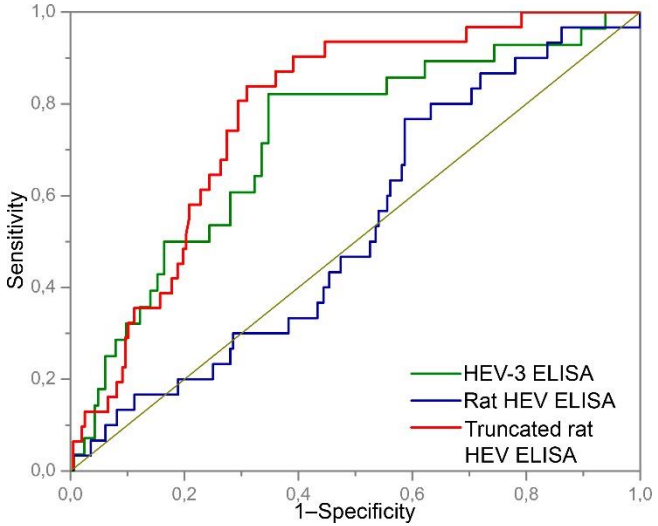


Figure 14. "Wantai HEV-IgG ELISA" results.

The highest anti-HEV IgG seroprevalence (30.3%, CI 95% 17.25-47.46) was detected in patients with chronic kidney disease, not under the dialysis group. In other groups the anti-HEV seroprevalence was lower – 12.28% (CI 95% 5.77-23.55) in patients

with chronic kidney disease under dialysis group and 14.52% (CI 95% 7.6-25.57) in kidney transplant recipients. Compared to the control group (10.53%), no significant difference was observed in kidney transplant recipients and patients under dialysis groups. Anti-HEV seroprevalence in patients with chronic kidney disease was significantly higher than in the control group (p=0.0218, Fig. 14). Chronic kidney disease patients are 3.7 times more likely to obtain HEV infection (OR=3.70 (CI 95% 1.30-10.49), p=0.014). It shows that chronic kidney disease is a risk factor for HEV infection. When comparing anti-HEV seroprevalence in groups by sex, age, immunosuppressants use and comorbidities no significant difference was observed.



Test	AUC	Standard Error	95% CI
HEV-3 ELISA	0.721	0.053	0.617–0.826
Rat HEV ELISA	0.53	0.062	0.409–0.652
Truncated rat HEV	0.777	0.053	0.673–0.881

Figure 15. ROC analysis of yeast-expressed HEV capsid proteins based ELISA tests targeting human IgG anti-HEV.

Serologic ELISA tests based on yeast-expressed HEV capsid proteins for the detection of anti-HEV IgG antibodies were developed and evaluated. HEV-3, full-length, and truncated rat HEV capsid proteins were used as reagents for HEV-specific IgG antibody capture in indirect ELISA. The efficiency of these tests was evaluated by ROC analysis using "Wantai HEV-IgG ELISA" as a reference test (Fig. 15). Surprisingly, truncated rat HEV capsid protein-based ELISA (Truncated rat HEV ELISA) was the most diagnostically efficient as its AUC is 0.777 ± 0.053. HEV-3 capsid protein-based test's (HEV-3 ELISA) AUC (0.721 ± 0.053) is almost the same. The worst performance was observed of full-length rat HEV capsid protein-based ELISA (Rat HEV ELISA, AUC = 0.530 ± 0.062). The results indicate that the in-house developed serologic tests based on yeast-expressed HEV capsid protein underperform compared to the reference test and are not suitable for diagnostics.

3.6.ELISA tests for hepatitis E virus antigen detection

Active HEV replication could be detected by HEV antigen presence in blood. Human and pig anti-HEV seropositive and rat HEV-positive samples were tested using "Wantai HEV-Ag ELISA" and in-house developed capture ELISA. Capture ELISA was developed using the MAbs generated in this study. This type of ELISA is that one MAb is used to capture the antigen and another MAb is used to detect an immunocomplex formed. When all possible MAbs pairs were evaluated to detect HEV antigen in capture ELISA, MAb CPE4 was chosen to capture HEV capsid protein and MAb CPD9 labeled with Alexa Fluor 555 was chosen to detect antigen captured by MAb CPE4 (Fig. 16). This two-epitope system could detect recombinant capsid protein of HEV-3 and rat HEV.

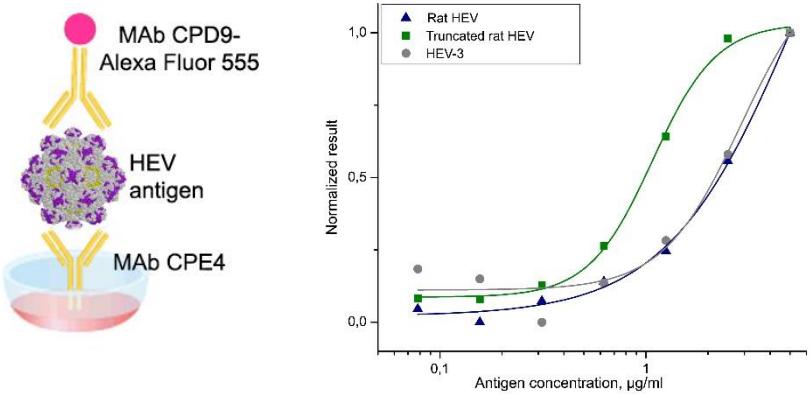


Figure 16. Scheme and specificity of the capture ELISA for HEV antigen detection.

"Wantai HEV-Ag ELISA" detected 2 HEV antigen-positive of 34 human seropositive samples tested (Table 2). The in-house capture ELISA detected 9 HEV antigen-positive samples out of 34 and 2 samples positive by "Wantai HEV-Ag ELISA" are among them. When pig blood samples were tested, "Wantai HEV-Ag ELISA" detected 11 HEV antigen-positive samples out of 72 while the in-house capture ELISA – 45 out of 72. However, only 2 of 11 HEV antigen-positive sample by "Wantai HEV-Ag ELISA" was also positive in capture ELISA. In rat samples, HEV antigen was

Table 2. The results of HEV antigen detection in the samples of different origin.

Sample type	**„Wantai HEV-Ag ELISA" positive (%)**	**Capture ELISA positive (%)**	**Positive by both tests (%)**
Human HEV seropositive	2/34 (5.88)	9/34 (26.47)	2/34 (5.88)
Pig HEV seropositive	11/72 (15.28)	45/72 (62.5)	2/72 (2.78)
Rat HEV positive	0/31 (0)	2/31 (6.45)	0/31 (0)

detected in 2 of 31 samples tested. No rat samples were positive by "Wantai HEV-Ag ELISA". Data obtained show that the agreement between the results by "Wantai HEV-Ag ELISA" and capture ELISA is low. This inconsistency could be caused by different test specificity when human and pig samples are tested. If the "Wantai HEV-Ag ELISA" test is considered as a reference test, capture ELISA would detect a lot of false-positive results. When evaluating the results of pig sample testing, some considerations on capture ELISA sensitivity might be raised as capture ELISA detects only 2 of 11 "Wantai HEV-Ag ELISA" positive samples. This inconsistency might be due to the specificity of the antibodies used in the tests. Recombinant HEV capsid proteins were used as analytes for both of the tests. "Wantai HEV-Ag ELISA" detected only HEV-3 capsid protein while capture ELISA is cross-specific to both HEV-3 and rat HEV capsid proteins. These obtained results demonstrate the different efficiency of the tests. Furthermore, the in-house capture ELISA detected some HEV antigen-positive rat samples. It might be concluded that "Wantai HEV-Ag ELISA" is not a suitable reference test for rat HEV antigen detection.

CONCLUSIONS

1. Yeast-expressed HEV-3 and rat HEV capsid proteins induce a strong immune response in mice. Immortalization of spleen cells isolated from immunized mice resulted in 18 stable hybridomas producing monoclonal antibodies of different specificities: 8 of them reactive with HEV-3 capsid protein, 6 - with rat HEV capsid protein, and 4 cross-reactive with HEV-3 and rat HEV capsid proteins. Nine out of 18 monoclonal antibodies are reactive with a native virus.

2. An antigenicity study of recombinant HEV capsid proteins revealed that the monoclonal antibodies bind to immunodominant regions located in all HEV capsid protein domains. Such antigenic structure of HEV-3 capsid protein was described for the first time.

3. The analysis of HEV infections in wild rats using the newly developed serologic assay demonstrated that 31.2% of the rats caught in Lithuania were positive for rat HEV-specific IgG, and 8.3% of the rats were positive for rat HEV RNA.

4. The evaluation of HEV infection rate in pigs revealed that 50.51% of pigs tested were positive for anti-HEV IgG, which is consistent with previous findings. The evaluation of ELISA tests based on yeast-expressed HEV capsid proteins showed that the most efficient serologic test in pigs was based on HEV-3 recombinant capsid protein.

5. The investigation of anti-HEV IgG prevalence in humans demonstrated that 14.91% of the individuals were positive for anti-HEV IgG. The comparison of anti-HEV seroprevalence in the study groups confirmed that chronic kidney disease is a risk factor for acquiring HEV infection.

6. Due to a low number of samples containing acute HEV infection markers, it was not possible to evaluate the in-house developed test based on monoclonal antibodies to HEV efficiency of detecting HEV-3 and rat HEV capsid proteins in rat HEV positive, pig and human HEV seropositive samples.

SANTRAUKA

Hepatitas E yra infekcinė liga, kurią sukelia hepatito E virusas (HEV). HEV-1 ir HEV-2 infekuoja tik žmones, o HEV-3 ir HEV-4 – žmones ir kitus žinduolius, pavyzdžiui, kiaules, šernus, elnius, triušius. Ekonomiškai silpnai išsivysčiusioms šalims būdingos HEV-1 ir HEV-2 infekcijos, kurios plinta per užterštą vandenį. Šių HEV genotipų infekcijos nustatomos ir Europoje, tačiau jos siejamos su kelionėmis. HEV-3 yra zoonotinis virusas, jis būdingas ekonomiškai išsivysčiusioms šalims. Šio viruso infekcijos pagrindinis šaltinis yra blogai termiškai apdoroti mėsos produktai. Neseniai buvo nustatytas žiurkių HEV kaip naujas hepatito E sukėlėjas. Serologinių testų žiurkių HEV infekcijų nustatymui nėra, o jų poreikis turėtų išaugti.

HEV infekcijos ir jos paplitimo tyrimai atliekami molekuliniais ir serologiniais metodais. Molekuliniai tyrimai remiasi atvirkštinės tranksripcijos PGR metodais, kurie yra standartizuoti įvairiems HEV genotipams. Serologiniai tyrimai atliekami naudojant įvairių gamintojų nustatymo sistemas, kurių rezultatai tiriant tuos pačius mėginius skiriasi, todėl yra poreikis kurti naujus įrankius ir sistemas HEV infekcijos diagnostikai ir tyrimams. Šiame darbe apibūdinti HEV antigenai, formuojantys virusą primenančias daleles, buvo pirmą kartą susintetinti mielėse. Mielėms sintetinant baltymus, jų vykdomos potransliacinės modifikacijos skiriasi nuo kitų eukariotinių ar prokariotinių ląstelių, todėl baltymai gali pasižymėti unikaliomis savybėmis.

Šiame darbe sukurti monokloniniai antikūnai (MAk) yra HEV tyrimams tinkami įrankiai, kurie papildo jau egzistuojančių priemonių arsenalą. Sukurti MAk pasižymi išskirtinėmis savybėmis. Visi anksčiau aprašyti antikūnai prieš HEV kapsidės baltymus jungėsi prie vieno iš trijų domenų, kuriame yra išsidėstę natūralios infekcijos metu susidarančių virusą neutralizuojančių antikūnų epitopai. Šiame darbe sukurtų ir apibūdintų MAk atpažįstamos sritys HEV kapsidės baltymuose yra išsidėsčiusios visuose domenuose.

Tokia antikūnų prieš HEV kolekcija aprašyta pirmą kartą. Tai suteikia galimybę modeliuoti ir išbandyti potencialiai našesnes ir plačiau pritaikomas HEV tyrimo metodikas. HEV biologijoje taip pat yra daug neatsakytų klausimų apie viruso replikaciją ir plitimą. Tokiems tyrimams galėtų pasitarnauti įvairiomis savybėmis ir specifiškumu pasižymintys antikūnai.

HEV paplitimo tyrimai Lietuvoje iki šiol yra riboti. Mokslininkai iš Lietuvos sveikatos mokslų universiteto yra atlikę HEV infekcijos paplitimo vertinimą kiaulių, šernų ir kitų laukinių kanopinių gyvūnų populiacijose (Spancerniene et al., 2018). Gauti rezultatai parodė, kad HEV infekcija yra dažna šių gyvūnų populiacijose Lietuvoje. Šiame darbe žiurkių, kiaulių ir žmonių tyrimams naudoti imunocheminiai metodai sukurti mielėse susintetintų HEV kapsidės baltymų ir monokloninių antikūnų pagrindu bei komercinės serologinės antikūnų prieš HEV nustatymo sistemos. Pirmą kartą buvo ištirtas HEV paplitimas žmonėse bei laukinėse žiurkėse. Laukinių žiurkių tyrimas reikšmingai papildo informaciją apie žiurkių HEV infekcijas šiaurės rytų Europoje. Atlikti tyrimai taip pat papildo žinias apie HEV infekcijos paplitimą kiaulių populiacijoje.

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• Grigas J, Simkute E, **Simanavicius M**, Pautienius A, Streimikyte–Mockeliune Z, Razukevicius D, Stankevicius A. Hepatitis E genotype 3 virus isolate from wild boar is capable of replication in non–human primate and swine kidney cells and mouse neuroblastoma cells. BMC Vet Res. 2020; 16:95. (Dissertation author performed immunofluorescence experiments using newly-developed MAbs against HEV).

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• **Simanavicius M**, Tamosiunas PL, Petraityte–Burneikiene R, Johne R, Zvirbliene A, Ulrich RG, Kucinskaite–Kodze I. Production and characterization of monoclonal antibodies against yeast–expressed hepatitis E virus capsid proteins. Poster. 6th European Congress of Virology (ECV2016), Hamburg, Germany, October 19-22, 2016 m.

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• **Simanavicius M**, Juskaite K, Verbickaite A, Jasiulionis M, Tamosiunas PL, Petraityte–Burneikiene R, Zvirbliene A, Ulrich RG, Kucinskaite–Kodze I. The evidence of hepatitis E virus infection in wild rats from Lithuania. Poster. 5th European Congress of Immunology (ECI2018), Amsterdam, The Netherlands, September 2-5, 2018.

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