



Review

Antiviral strategies for hepatitis E virus



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ABSTRACT

The hepatitis E virus is a common cause of acute hepatitis. Contrary to hepatitis B and C, hepatitis E is mostly a mild infection, although it has a high mortality in pregnant women and can evolve to chronicity in immunocompromised patients. Ribavirin and pegylated interferon- α are the only available therapies, but both have side effects that are not acceptable for prophylaxis or treatment of mild infections. In addition, these drugs cannot be used for all patient types (e.g. in case of pregnancy, specific organ transplants or co-morbidities) and in resource-poor settings. Hence there is an urgent need for better antiviral treatments that are efficacious and safe, also during pregnancy. In this review, a concise introduction to the virus and disease is provided, followed by a discussion of the available assay systems and potential molecular targets (viral proteins and host factors) for the development of inhibitors of HEV replication. Finally, directions for future research are presented.

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1. Introduction

Hepatitis E virus (HEV) is a feco-orally transmitted pathogen and one of the most common causes of acute hepatitis worldwide. Although the virus was already identified in 1983 (Balayan et al., 1983), many of its epidemiological and clinical features are becoming clear just recently. HEV is responsible for both acute outbreaks in developing countries and sporadic cases worldwide and is more and more being recognized as a problem in the transplant setting (Kamar et al., 2008a; Pischke et al., 2012). Although most infections are self-resolving, hepatitis E is characterized by a high mortality in pregnant women and can lead to chronic infections in immunocompromised patients. A vaccine has recently been approved for the Chinese, but not for other markets (Zhu et al., 2010). Current therapeutic options (i.e. ribavirin and interferon- α) have severe side effects and treatment failure has been reported. It may be important to have potent and safe inhibitors of HEV replication at hand to rapidly contain outbreaks and to treat immunodeficient patients.

In this review, a general introduction to HEV is presented, followed by an overview of different potential antiviral targets and currently available methods for drug testing. Directions for developing antivirals against the HEV will be suggested.

2. Hepatitis E virus

2.1. Genome structure

HEV has a capped positive-sense single-stranded RNA genome that comprises a 5' untranslated region (UTR), open reading frames (ORF) 1–3 and a 3' UTR followed by a polyA-tract (see Fig. 1). ORF1 encodes the non-structural proteins and contains methyltransferase, protease, macrodomain, helicase and RNA-dependent RNA polymerase (RdRp)-encoding sequences (Koonin et al., 1992). Between the protease and the macrodomain, a hypervariable region containing a proline-rich hinge was found (Smith et al., 2012). The overlapping ORFs 2 and 3 are translated from a single subgenomic RNA into the structural proteins (Graff et al., 2006). ORF2 encodes the 660-aa capsid protein that consists of a shell domain (S), a middle domain (M, also called P1) and a protruding domain (P, also called P2) (Guu et al., 2009; Yamashita et al., 2009). The protein translated from ORF3 is 114 aa in length, is phosphorylated (Zafrullah et al., 1997) and unique to HEV and closely related viruses.

2.2. Replication cycle

Although understudied, the HEV replication cycle seems typical for a single-stranded RNA virus of positive polarity (see Fig. 2). The virus particle first binds to heparan sulfate proteoglycans (HSPGs) on the host cell membrane (Kalia et al., 2009), transfers to its (unknown) cellular membrane receptor(s) and the resulting complex is internalized through clathrin-mediated endocytosis (Kapur et al., 2012). Consequently, the capped viral genome is released from the virion during the uncoating process and directly translated by the host cell ribosomal machinery. The non-structural proteins thus generated allow for the replication of the viral genome (probably in specific viral replication complexes) and the production of subgenomic RNA that is translated into the structural proteins from ORF2 and ORF3. Full-length RNA progeny is assembled with ORF2 capsid protein into viral particles of 27–30 nm (Balayan et al., 1983) that are consequently released from the cell in a non-lytic fashion. During release, HEV particles probably acquire a lipid bilayer (envelope) and associated ORF3 protein that are removed later by bile salts and enteric proteases respectively (Takahashi et al., 2010; Yamada et al., 2009).

2.3. Genetic diversity

HEV is classified into the *Hepevirus* genus in the family of the *Hepeviridae* (ICTV). At least 4 genotypes are currently recognized (Smith et al., 2013); genotypes 1 and 2 solely infect humans while genotypes 3 and 4 are zoonotic agents with their main reservoir in domestic pigs (Feagins et al., 2007; Meng et al., 1997; Tamada et al., 2004). Two additional genotypes have been proposed for HEV isolates from Japanese wild boars (Smith et al., 2013). Several viruses that are more or less related to HEV have been isolated from different species, including chickens (avian HEV, Haqshenas et al., 2001), rabbits (Zhao et al., 2009), rats (John et al., 2010), bats (Drexler et al., 2012), ferrets (Raj et al., 2012) and trout (Batts et al., 2011) (Fig. 3). The exact classification of these viruses remains to be determined (Smith et al., 2013) and future discovery of additional hepeviruses seems likely.

3. Hepatitis E

3.1. Epidemiology of disease

HEV genotypes 1 and 2 only infect humans and are endemic in many developing regions where they tend to cause large-scale

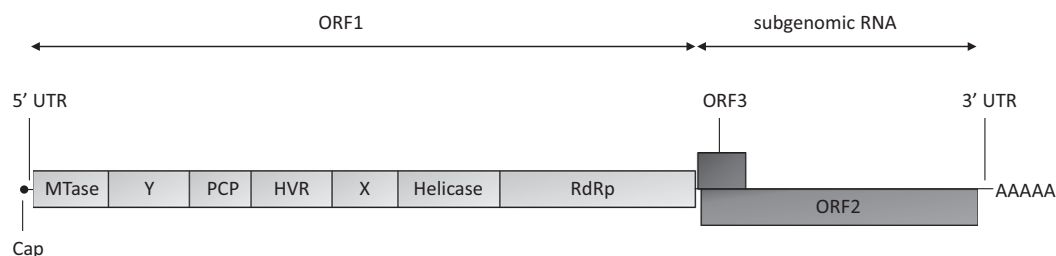


Fig. 1. Organization of the HEV genome. Non-structural proteins are translated from ORF1 while the ORF2 and ORF3 structural proteins are translated from a single subgenomic RNA. UTR, untranslated region; Y, Y-domain; PCP, papain-like cysteine protease; HVR, hypervariable region; X, macro domain; RdRp, RNA-dependent RNA polymerase.

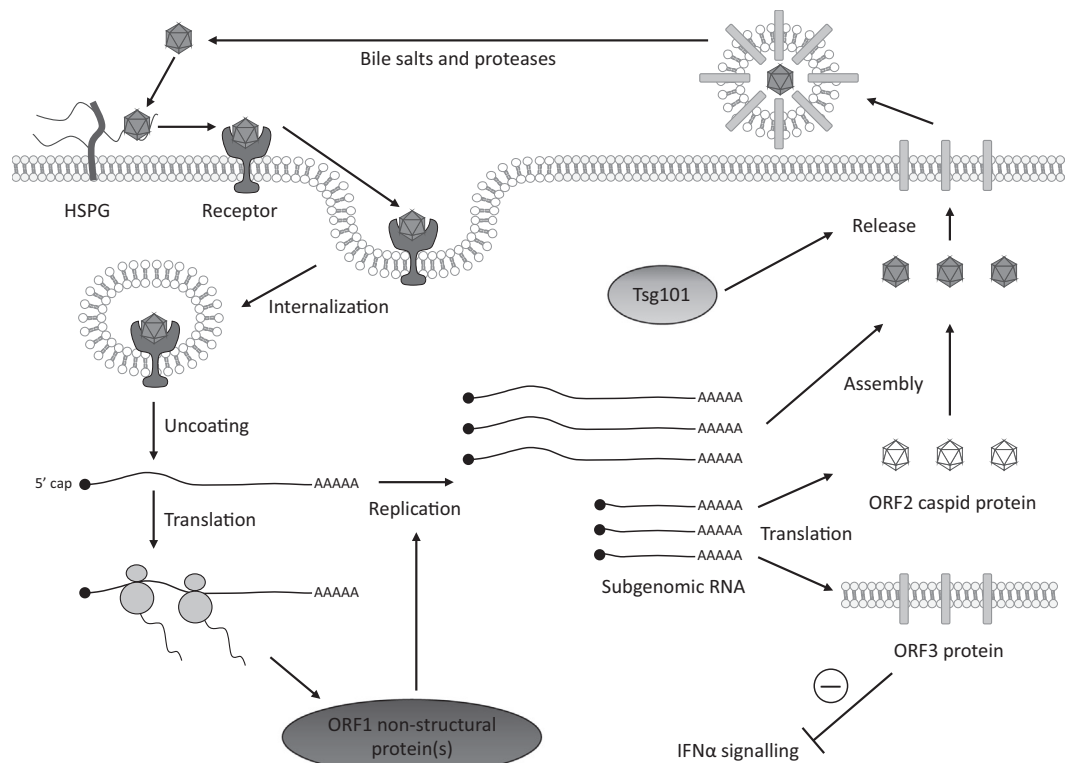


Fig. 2. HEV replication cycle. HEV particles first bind to heparan sulphate proteoglycans (HSPG) and are consequently transferred to an unknown functional receptor, thus mediating cellular uptake through clathrin-mediated endocytosis. Following uncoating, the viral RNA genome is released into the cytoplasm and translated into non-structural proteins. These proteins form a replication complex that produces new full-length and subgenomic viral RNA. The latter is translated into capsid protein (ORF2) and the membrane associated ORF3 protein that is known to interfere with interferon- α signaling. Viral RNA is packaged into capsid protein and released from the cell with help of the host factor Tumor susceptibility gene 101 (Tsg101). The released particles are associated with lipids and ORF3 proteins; both are consequently removed through the bile acids and digestive proteases respectively.

water-borne outbreaks (Corwin et al., 1996; Naik et al., 1992). Genotype 1 is mainly found in Asia and Africa (Sugitani et al., 2009; Teshale et al., 2010), while genotype 2 has been isolated from Mexico and Africa, but seems less common (Maila et al., 2004; Velázquez et al., 1990) (see Fig. 4). It is estimated that infections with these genotypes result in 70,000 deaths annually (Rein et al., 2012). Genotypes 3 and 4 on the other hand are zoonotic agents with their main reservoir in domestic pigs (Colson et al., 2010; Li et al., 2005; Takahashi et al., 2004). Genotype 3 has a worldwide distribution, while genotype 4 is mostly found in Asia (Miyashita et al., 2012; Wang et al., 2012). However, genotype 4 has also been isolated from European pigs and patients recently (Hakze-van der Honing et al., 2011; Jebbaoui et al., 2013). Genotype 3 and 4 infections often result from consumption of undercooked pig or deer meat and may thus cause sporadic cases of hepatitis E. Although exact epidemiological data are missing, these infections seem a lot more common than initially thought (Dalton et al., 2008; Ijaz et al., 2009; Versluis et al., 2013).

3.2. Clinical syndromes

Acute hepatitis E is characterized by jaundice, fatigue, gastrointestinal discomfort, etc. and is clinically indistinguishable from for instance hepatitis A (Khuroo, 1980), although many infections remain asymptomatic. Most cases of hepatitis E resolve spontaneously, but in some patients the infection may progress to fulminant hepatitis. Overall mortality rates are estimated between 0.5% and 4% (e.g. Khuroo, 1980; Teshale et al., 2010).

One particularly intriguing characteristic is that hepatitis E is often lethal in pregnant women with mortality rates of up to 20–25% (Khuroo et al., 1981; Tsega et al., 1993), although this has only

been observed for genotype 1 infections. Increased frequencies of obstetric complications and stillbirths have also been reported (Patra et al., 2007). The underlying mechanisms of this phenomenon (hormonal, immunological, genetical factors, etc.) are unclear to date.

A more recent finding is that HEV can establish chronic infections in immunocompromised subjects such as transplant and HIV-infected patients (Colson et al., 2009; Dalton et al., 2009; Kamar et al., 2008b). This has only been reported for genotype 3 thus far. Such chronic infections are rarely symptomatic and are sometimes mistaken for drug-induced liver injury (Davern et al., 2011). Chronic hepatitis E may lead to cirrhosis in 10% of patients (Kamar et al., 2011) and can ultimately result in death.

Occasional extrahepatic manifestations have also been reported; these are mostly neurological complications such as Guillain-Barré syndrome and neuralgic amyotrophy (Fong and Illahi, 2009; Kamar et al., 2010b; Sood et al., 2000) or nephrological manifestations such as glomerulonephritis (Kamar et al., 2012).

As evident from the discussion above, hepatitis E is profoundly different from the well-known disease courses of hepatitis B and C. While a considerable portion of patients infected with the hepatitis B virus (HBV) and especially the hepatitis C virus (HCV) evolves to chronicity, spontaneous recovery is the rule for hepatitis E where chronic disease has thus far only been reported in immunocompromised patients. The high rate of mortality in pregnant women is also unique to hepatitis E and no similar phenomena have been reported for hepatitis A, B or C.

3.3. Diagnostic methods

The most frequently used tests for diagnosing hepatitis E are detection of IgM and IgG antibodies by enzyme-linked immunosor-

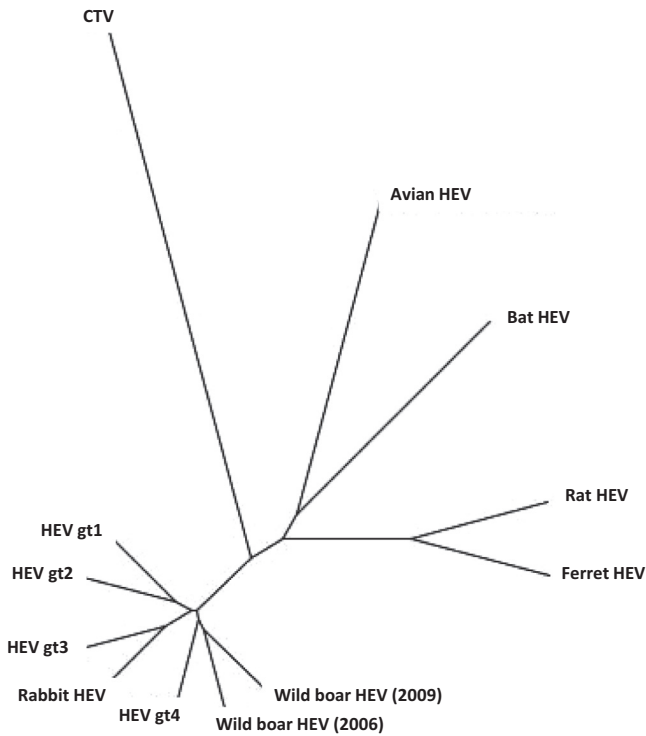


Fig. 3. HEV species and genotypes. The phylogenetic relationship of different human HEV genotypes and the reported animal strains was plotted with the Virus Pathogen Database and Analysis Resource (ViPR, Pickett et al., 2012) based on following sequences: HEV gt1 (GenBank accession No. M80581), HEV gt2 (M74506), HEV gt3 (HQ389543), rabbit HEV (FJ906895), HEV gt4 (AB220973), wild boar HEV 2006 (AB602441), wild boar HEV 2009 (AB573435), ferret HEV (JN998606), rat HEV (GU345043), bat HEV (JQ001749), avian HEV (AY535004), CTV (HQ731075). CTV, cutthroat trout virus; gt, genotype.

bent assays (ELISA) and detection of viral RNA by (quantitative) reverse transcription PCR (RT-PCR). Presence of IgM antibodies indicates a recent infection, while IgG antibodies appear later

and persist afterwards, at least for some time, indicating a past exposure. The current ELISAs suffer from large variability between assays (Bendall et al., 2010; Drobeniuc et al., 2010; Mast et al., 1998) and therefore require urgent optimization and validation. Acute infection is usually confirmed by (quantitative) RT-PCR (e.g. Jothikumar et al., 2006), although these assays should be standardized as well to increase reliability.

Since the incubation and symptomatic periods for acute hepatitis E are only a few weeks, the successful use of antiviral drugs for acute cases of hepatitis E would depend on the availability of a rapid and inexpensive diagnostic test. Such test should also be easy to employ in resource-poor settings.

4. Current control strategies

4.1. Vaccines

Vaccination would be an effective strategy to prevent HEV infection. Recently, such a HEV vaccine based on a recombinant truncated capsid protein was approved in China (Hecolin®, Zhu et al., 2010). It remains unclear whether the manufacturer intends to obtain market approval in other countries as well. Another recombinant vaccine successfully completed phase 2 clinical trials in Nepal (Shrestha et al., 2007), but further development was halted. Such vaccines may be useful as a prophylactic measure in high-risk patients such as immunocompromised patients and pregnant women. This would require specific clinical trials to demonstrate protective efficacy in these populations; to the best of our knowledge, such studies have not yet been reported for Hecolin®. However, the organization of large-scale vaccination campaigns against HEV may not be particularly cost-effective given the mostly benign course of infection in developing countries and the relatively low incidence in the developed world.

4.2. Other prophylactic measures

Since HEV is transmitted feco-orally, sanitary measures are probably the most effective strategy available to prevent viral

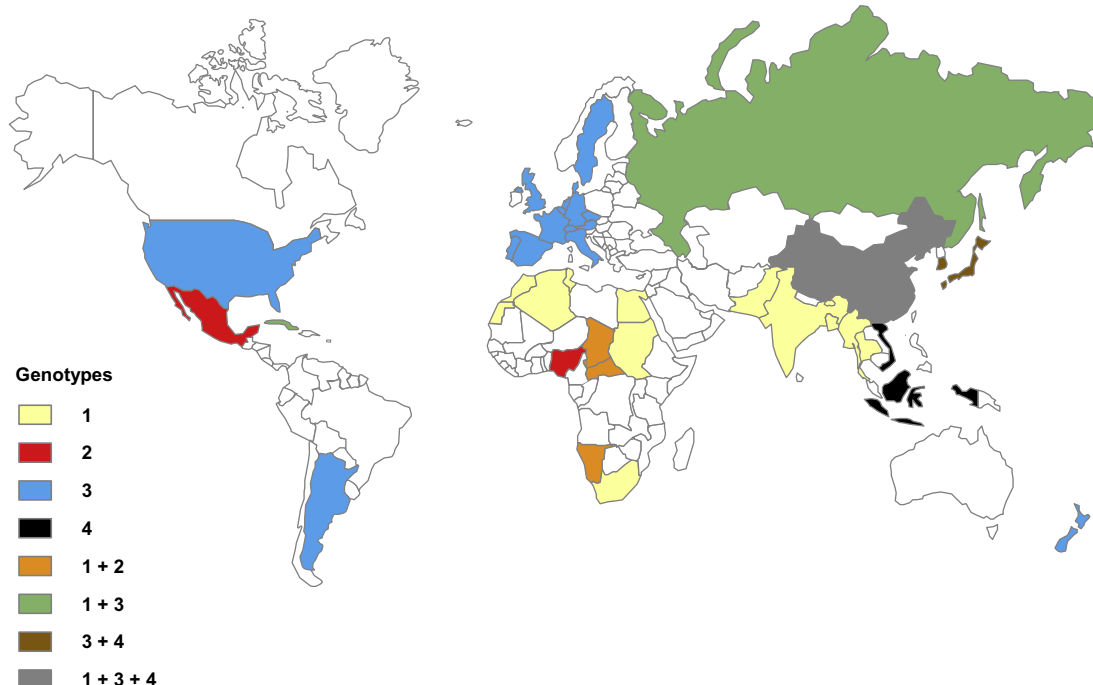


Fig. 4. Geographic distribution of HEV genotypes 1–4. Countries with confirmed human infections are color-coded according to the detected genotype. This is probably an incomplete picture as there are no data available for many countries and genotyping is not always performed and/or reported.

spread. This is especially the case for genotypes 1 and 2 that often occur in large water-borne outbreaks. Availability of clean drinking water and proper disposal of waste water would be effective control strategies. Genotypes 3 and 4 are mostly spread through contaminated food stuffs, such as the infamous French figatellu sausage made from uncooked pig liver (Berto et al., 2013a; Colson et al., 2010). Thorough heating effectively kills the virus and prevents infection (Feagins et al., 2008a). Although ineffectively, HEV can also be transmitted via blood transfusion (Boxall et al., 2006; Colson et al., 2007), suggesting that screening of donor blood may be warranted. In addition, vertical transmission of HEV has been reported (Khuroo et al., 1995), indicating that extra caution is required in pregnant women.

5. Why do we need antiviral drugs against hepatitis E virus?

5.1. Current standard of care

Chronic HEV infections in transplant patients can occasionally be resolved by decreasing immunosuppression, leading to spontaneous clearance in about 30% of the patients (Kamar et al., 2011). When this approach is unsuccessful and for other types of patients, there are generally 2 possibilities: the use of pegylated interferon-alpha (PEG-IFN α) or ribavirin. PEG-IFN α has been used successfully in liver transplant, kidney transplant and leukemia patients with chronic hepatitis E (Alric et al., 2010; Haagsma et al., 2010; Kamar et al., 2010a,c). However, ribavirin seems to be the drug of choice in most cases with successful treatments being reported for multiple types of transplant patients (Kamar et al., 2010d; Mallet et al., 2010; Pischke et al., 2012, 2013), but also for HIV and leukemia patients with chronic hepatitis E (Giordani et al., 2013; Neukam et al., 2013). Acute hepatitis E seems to respond to ribavirin treatment as well (Gerolami et al., 2011; Pischke et al., 2013). In addition, the anti-HEV activity of both drugs has been confirmed *in vitro* (Debing et al., 2014). Nevertheless, they need to be administered for at least 3 months and are known for their severe adverse effects. For PEG-IFN α for instance, this includes influenza-like symptoms and neuropsychiatric side effects (Manns et al., 2006). Ribavirin on the other hand may induce severe anemia necessitating dose reductions (Kamar et al., 2010d; Pischke et al., 2012). These reductions may in turn lead to treatment failure (Pischke et al., 2013). One may deem such severe side effects acceptable in light of the severity of chronic hepatitis E (as is the case for chronic hepatitis C as well), but this is certainly not true when these drugs would be used for prophylaxis or to treat mild infections. In addition, both ribavirin and PEG-IFN α are contra-indicated in pregnant women and other specific patient populations, leaving no available treatment options for these people. Also when considering acute infections during large outbreaks in developing countries, PEG-IFN α is not an option due to the fact that parenteral administration is needed and the required follow-up of hematological parameters during ribavirin treatment is difficult to implement in such settings.

5.2. Future therapy for hepatitis E

For the reasons stated above, safe and effective treatments for hepatitis E are urgently required. In case of acute infections, antiviral drugs would be useful to shorten the period of illness and prevent progression to fulminant hepatic failure. Such application would require rapid diagnosis of hepatitis E (see also Section 3.3). Another possibility would be to use antivirals to halt viral spread during epidemics and outbreaks. The fact that these outbreaks occur rather often in refugee camps (e.g. Ahmed et al., 2013; Centers for Disease Control and Prevention, 2013) may represent an opportunity for clinical trials testing the prophylactic potential of HEV

inhibitors if such studies would be appropriate from an ethical point-of-view.

In pregnant women, such drugs could be life-saving for both mother and child, on the condition that they are devoid of teratogenic and significant adverse effects. Ideally, these women should be screened regularly with a rapid and sensitive HEV test and treated as soon as possible. This may be difficult to implement in developing countries where genotype 1 is endemic, so an approved HEV inhibitor should be sufficiently potent to arrest an established (fulminant) infection. Modulation of the hormonal system may be another interesting therapeutic option (see suggestions in Section 7.6), although the development of such strategy would require more detailed insights into disease pathogenesis.

For chronically infected patients, rapid clearance of HEV is required before irreversible progression to cirrhosis has occurred. Ideally, these drugs should be applicable in a diverse range of immunocompromised patients without overt toxicity. Since ribavirin treatment has a rather good efficacy in chronically HEV-infected patients, a combination regimen with another HEV-inhibitor could be envisaged (as is currently being explored for HCV: ribavirin + direct-acting antiviral(s), (e.g. Osinusi et al., 2013; Zeuzem et al., 2013). This may allow decreasing the ribavirin dosage, thus reducing and hopefully avoiding anemia and other side effects. In addition, we observed a slight synergistic effect *in vitro* for the combination of ribavirin and interferon- α (Debing et al., 2014), suggesting that such a combination regimen may be worth considering in the clinical setting. Successful combination therapy has already been reported for a chronically HEV-infected HIV patient (Dalton et al., 2011b).

Developing antiviral drugs for the treatment of HEV infections will commercially not be as rewarding as for instance the development of HCV inhibitors (Debing et al., 2013a). Therefore, it appears opportune to consider other strategies than the classical discovery-development of completely new chemical entities as well, for instance off-label use of drugs with robust anti-HEV activity that are registered for unrelated indications when such molecules would be identified.

6. Currently available methods for drug testing

6.1. *In vitro* testing

Efficient cell culture systems for HEV have only recently become available. The Okamoto research group reported on both genotype 3 and 4 strains that replicate in PLC/PRF/5 (hepatoma) and/or A549 (lung adenocarcinoma) cell lines (Takahashi et al., 2010; Tanaka et al., 2007, 2009). The PLC/PRF/5 cells were also employed in a 3D cell culture system for HEV (Berto et al., 2013b). On the other hand, the NIH research group of Emerson derived a passage 6 virus of the Kernow-C1 strain that replicates in Huh7 and especially HepG2/C3A hepatoma cells (Shukla et al., 2011, 2012). This strain is derived from a chronically infected patient and contains an inserted human ribosomal S17 RNA fragment in the hypervariable region (Shukla et al., 2011). Detailed analyses indicated that this fragment and several additional mutations contributed to the improved *in vitro* replication. Although the replication capacity of this strain is sufficient for virus yield assays (e.g. Debing et al., 2014), overall growth kinetics are still rather slow. No cytopathic effect (CPE) has been described for any of these HEV strains, precluding the use of standard virology techniques such as CPE reduction assays and plaque assays. In addition, genotype 1 replicates only poorly in cell culture (Nguyen et al., 2013) while it probably accounts for most of the human infections worldwide.

Several replicon constructs have also been established in which the 5' part of the structural proteins is replaced by a GFP, neomycin

resistance or luciferase gene (Graff et al., 2006; Shukla et al., 2012). The Kernow-C1 p6/luc reporter replicon proved especially useful in our hands as transient transfection into Huh7 cells allows for a 3-day antiviral assay to be performed (Debing et al., 2014). For large-scale cell-based screenings with such replicons, a selectable reporter replicon would be highly convenient.

Another possible strategy to identify novel inhibitors of HEV replication is to use a surrogate virus. We recently reported that the cutthroat trout virus (CTV) may serve as such a surrogate (Debing et al., 2013b). CTV was isolated in 1988 from spawning trout in the western USA (Hedrick et al., 1991) and recent studies revealed that the virus belongs to its own genus in the *Hepeviridae* family (Smith et al., 2013). It has a remarkable similarity to HEV, especially when considering the helicase and polymerase sequences (Batts et al., 2011; Debing et al., 2013b). CTV replicates readily in cell culture allowing for RT-qPCR-based virus yield assays to be performed (Debing et al., 2013b). In addition, its *in vitro* replication kinetics can be further improved through repeated passaging (our unpublished results). In addition, CTV is a cytopathic virus. The CPE induced in CHSE-214 cells is rather limited, but we observed that nearly complete CPE can be attained in other piscine cell lines (our unpublished results). The avian HEV has also been successfully employed as a surrogate model (e.g. Kenney et al., 2012; Pudupakam et al., 2011).

In conclusion, recent developments have considerably expanded the number of assays and tools available for HEV antiviral studies. However, the possibilities are still rather limited and the field would benefit from for instance HEV strains with improved replication kinetics, enzymatic assays for the different non-structural proteins and selectable reporter replicons.

6.2. Evaluation in laboratory animals

An ideal animal model for HEV would be a small inexpensive animal (preferably rodent) that can be infected with human HEV or at least a closely related virus and that develops acute hepatitis with a clear increase in liver markers and with high viral titers. Mortality is not required, although a lethal phenotype is highly useful for antiviral drug testing. An animal model that mimics the hormone-dependent effects observed in pregnant women would be a plus.

Several animal models are available for *in vivo* HEV studies (see Table 1 for an overview), but they all have certain limitations. For instance, chicken and trout can be used as experimental animal models when infected with avian HEV and CTV, respectively (Bilham et al., 2005; Hedrick et al., 1994). Since both viruses are only surrogates for human HEV, further validation of experimental results would be required in another model. Genuine HEV infections can be performed in pigs (Feagins et al., 2008b; Halbur et al., 2001; Sanford et al., 2011) and non-human primates such as macaques and chimpanzees (Emerson et al., 2001; Meng et al., 1998; Purcell et al., 2003; Yu et al., 2010). However, ethical concerns as well as practical issues may make such studies complicated. Rodents may present a more accessible model organism. For instance, laboratory rats can be experimentally infected with rat HEV, but contradicting reports have been published for the human HEV genotypes (Li et al., 2013a,b; Purcell et al., 2011; Zhu et al., 2013). Successful infection of Balb/c nude but not C57BL/6 mice with HEV was reported (Huang et al., 2009; Li et al., 2008), while rabbits can be infected with rabbit HEV and certain genotype 4 strains (Cheng et al., 2012). Finally, infection of Mongolian gerbils with genotype 4 has also been reported (Li et al., 2009). Due to the large variability between published results, a robust model that has been evaluated by multiple laboratories has not yet been reported. In addition, none of these models resulted in a lethal phenotype. For the predominant genotype 1 HEV, primates are even the only available

model at this time. Promising strategies towards a robust animal model may be infection of immunodeficient rats or mice with rat HEV, discovery studies for a murine HEV species, HEV infection of mice with chimeric human or porcine livers (Meuleman et al., 2005) or establishment of transgenic mice with a human HEV receptor (when identified), as was done successfully for poliovirus (Ren et al., 1990) and HCV (Dorner et al., 2011).

6.3. Clinical trials

When a HEV inhibitor would be shown to be successful in a (surrogate) animal model and would have an excellent safety and pharmacokinetic profile, clinical studies would be the next stage in development. For these studies, one could consider treating chronically infected patients, possibly in a combination regimen with ribavirin. However, these patients may have multiple co-morbidities and there could be confounding factors such as concomitant drug use leading to drug-drug interactions. Since hepatitis E has a rather long incubation period and clinical symptoms are often apparent for several weeks, clinical studies could possibly be performed in an outbreak setting as well; in this way, both prophylactic and therapeutic efficacy may be assessed.

7. Potential drug targets

Below we present a selection of viral and host proteins may be attractive targets for antiviral therapy (see also Table 2).

7.1. Inhibitors of HEV entry

The entry of a virus particle into the host cell is a multi-step process that involves attachment of the virus to its receptor on the cellular membrane, internalization of the virion-receptor complex and subsequent uncoating of the viral capsid releasing the RNA genome into the cytoplasm. The functional HEV receptor is still unknown to date, although there are indications that genotype 1 may use a different receptor(s) than genotype 3 (Nguyen et al., 2013). It was also found that HEV attaches to HSPGs, more specifically to 6-O-sulfated syndecan-1, which may account for its liver tropism (Kalia et al., 2009). It is hypothesized that HSPGs act as a highly abundant initial attachment receptor that binds HEV with relatively low affinity. In a second step, the virion probably transfers to a high-affinity functional receptor and the resulting complex is internalized. Structure determination of a HEV-like particle combined with mutation and cell binding studies have identified a receptor binding site on top of the P2 dimer that protrudes from the 2-fold axis of symmetry (Yamashita et al., 2009). Interestingly, this site is homologous to the norovirus receptor-binding site that attaches to histo-blood group trisaccharides (Guu et al., 2009). In addition, another sialic acid-binding site was found in the P1 region of the capsid protein (Guu et al., 2009).

Despite the limited information that is available on HEV attachment and entry, several potentially interesting antiviral strategies can be derived from these data. First, the homology between the putative HEV and norovirus receptor-binding sites suggests that norovirus attachment inhibitors could possibly be interesting leads for further development into anti-HEV drugs. No confirmed norovirus entry inhibitors have been described to date, but two potentially interesting prototype molecules were designed through NMR binding studies (Rademacher et al., 2011) and in a similar way, citrate was found to compete with histo-blood group saccharides for norovirus binding (Hansman et al., 2012), suggesting a promising role for glycomimetics. A second and possibly overlapping approach is to search for inhibitors of HEV attachment to HSPGs. Heparan sulfate and related molecules inhibit the

Table 1

Overview of reported animal models for HEV and surrogate viruses with reported pathology (gt, genotype; IFN, interferon; ALT, alanine transaminase).

Animal model	Virus species	Characteristics	References
Chicken	Avian HEV	Seroconversion, virus shedding, gross hepatic lesions, splenomegaly	Billam et al. (2005)
Trout	Cutthroat trout virus	Avirulent, only detected during spawning, IFN induction	Hedrick et al. (1994)
Pig	HEV gt 3 and 4	Seroconversion, virus shedding, histopathological damage	Feagins et al. (2008a,b), Halbur et al. (2001) and Sanford et al. (2011)
Macaque (rhesus, cynomolgus), chimpanzee	HEV gt 1–4	Seroconversion, virus shedding, histopathological damage	Emerson et al. (2001), Meng et al. (1998), Purcell et al. (2003) and Yu et al. (2010)
Rat (Wistar)	Rat HEV HEV gt 1, 3 or 4	Seroconversion, virus shedding, no alterations in ALT Not infectable	Li et al. (2013b) Li et al. (2013b)
(Nude)	Rat HEV	Seroconversion, limited virus shedding, higher titers than in immunocompetent rats	Li et al. (2013b) and Purcell et al. (2011)
(Sprague–Dawley)	HEV gt 3 Rat HEV	Not infectable Seroconversion, limited virus shedding, histopathological damage, no alterations in ALT	Li et al. (2013a) Purcell et al. (2011)
	HEV gt 1, 2 or 3 HEV gt 4	Not infectable (after intravenous injection) Seroconversion, virus shedding (after intrahepatic RNA inoculation)	Purcell et al. (2011) Zhu et al. (2013)
Mouse (Balb/c nude)	HEV gt 4	Seroconversion, virus shedding, histopathological liver damage	Huang et al. (2009)
(C57BL/6)	HEV gt 1, 3 and 4	Not infectable	Li et al. (2008)
Rabbit	Rabbit HEV HEV gt 4	Seroconversion, virus shedding Seroconversion, virus shedding, symptomatic hepatitis (only for H4-NJ703 strain)	Cheng et al. (2012) Cheng et al. (2012)
Mongolian gerbil	HEV gt 4	Virus shedding, slight histopathological liver changes	Li et al. (2009)

Table 2

HEV-encoded proteins and their (putative) functions.

HEV-encoded proteins	Confirmed functions	Crystal structure?	References
ORF1	Non-structural proteins		
Methyltransferase	Guanylttransferase, guanine-7-methyltransferase	No	Magden et al. (2001)
Papain-like cysteine protease	Proteolytic processing of ORF1? Deubiquitinating activity	No	Karpe and Lole (2011), Parvez (2013), Perttilä et al. (2013) and Sehgal et al. (2006)
Hypervariable region	Influences efficiency of viral RNA replication, host/cell type specificity?	No	Pudupakam et al. (2011) and Shukla et al. (2012)
Macrodomain	Poly-ADP-ribose-binding, weak ADP-ribose 1''-phosphohydrolase activity	No	Egloff et al. (2006)
Helicase	5'–3' RNA unwinding, NTPase, RNA 5'-triphosphatase	No	Karpe and Lole (2010a,b)
RdRp	RNA-dependent RNA polymerase activity	No	Agrawal et al. (2001) and Rehman et al. (2008)
ORF2	Capsid protein	Yes (HEV-like particle)	Guu et al. (2009) and Yamashita et al. (2009)
ORF3	Interaction with multiple cellular proteins, including Tsg101, hemopexin, ... Suppression of interferon- α signaling Particle egress	No	Geng et al. (2013), Ratra et al. (2008), Surjit et al. (2006), Dong et al. (2012) and Yamada et al. (2009)

attachment of multiple viruses, both *in vitro* and *in vivo* (e.g. Ali et al., 2012; Bugatti et al., 2007; Lee et al., 2006). However, the clinical applicability of such molecules remains questionable and would require complete abolishment of any anticoagulative properties. Pentosan polysulfate is such an oligosaccharide derivative that is approved and marketed for pain relief in interstitial cystitis (Davis et al., 2008). Given that this drug inhibits entry of multiple viruses (Baba et al., 1988; García-Villalón and Gil-Fernández, 1991), it may be worthwhile to test it as a therapeutic option for hepatitis E, although it still has some anticoagulant properties (Scully et al., 1983). Thirdly, it has been shown that a truncated ORF2 protein is capable of preventing HEV infection *in vitro* (He et al., 2008). Through further truncation of this protein or otherwise mimicking the receptor-binding region of HEV, it would theoretically be possible to prevent virions from binding to their cellular receptor. To illustrate the feasibility of such an approach,

we refer to Myrcludex-B which is a lipopeptide derived from the HBV envelope protein and efficiently inhibits HBV entry *in vitro* and *in vivo* (Volz et al., 2013).

The strategies outlined above illustrate that the lacking of an identified receptor is an important hurdle for the identification and study of attachment, entry and uncoating inhibitors for HEV. Now that efficient cell culture systems are available (e.g. Shukla et al., 2012), this hiatus can and should be addressed.

7.2. RNA-dependent RNA polymerase inhibitors

Following release of the viral RNA in the cytoplasm and initial translation, the RNA genome is replicated by an RNA-dependent RNA polymerase. Sequence alignments predicted an RdRp-encoding sequence at the 3' end of ORF1 (see Fig. 1, Koonin et al., 1992) that belongs to the alphavirus-supergroup of RdRp's

(Koonin, 1991). The polymerase activity was later confirmed by detection of newly synthesized negative-sense viral RNA *in vitro* (Agrawal et al., 2001; Rehman et al., 2008). The RdRp interacts with stem loops in the 3' end of the viral genome and with the polyA-tail (Agrawal et al., 2001) and co-localizes with the endoplasmic reticulum (Rehman et al., 2008). However, further details about the HEV RdRp structure and function are missing.

As evidenced by the success of nucleoside and nucleotide inhibitors of HIV and herpesviruses, viral polymerases are attractive targets for antiviral therapy. Also the RdRp of RNA viruses has been targeted successfully by such molecules, consider for instance the nucleoside analogs that were developed against HCV, e.g. sofosbuvir is highly active, safe and has a high barrier to resistance. Sofosbuvir has successfully completed phase 3 clinical trials (Lawitz et al., 2013) and may receive market approval in the near future. 2'-C-methylcytidine (2'CMC) is a related molecule and was the first nucleoside analog that entered clinical studies for the treatment of HCV infections. It has been shown to *in vitro* inhibit the replication of a number of other positive-sense single-stranded RNA viruses (Julander et al., 2010; Rocha-Pereira et al., 2013). However, neither 2'CMC nor a number of its analogs exhibited significant activity in the transient HEV replicon assay (as described in Debing et al., 2014), unpublished results). Also only moderate antiviral activity of 2'CMC was observed against CTV (Debing et al., 2013b). Despite these negative results, other nucleos(t)ide analogs may possibly be interesting inhibitors of HEV replication. Moreover, research efforts could be directed towards developing an RdRp inhibitor that targets both HEV and the distantly related alphavirus family. Such a broad-spectrum inhibitor would provide a much-needed treatment option for multiple infections by neglected viruses such as the Chikungunya virus, Sindbis virus and the equine encephalitis viruses. Elucidation of the HEV RdRp crystal structure and development of a convenient enzymatic assay would allow to jumpstart this specific domain of HEV antiviral research.

7.3. Methyltransferase inhibitors

The HEV genome has a 5' cap structure which is a crucial requirement for infectivity (Emerson et al., 2001). Capping is a multistep process involving hydrolysis of the γ -phosphate from the terminal nucleotide, subsequent conjugation to GMP resulting in a 5'-ppp-5' triphosphate bond and finally methylation of the newly added guanosine. The 5' proportion of the ORF1 sequence was predicted to encode a viral methyltransferase (Koonin et al., 1992) and indeed, the expressed protein displays guanylyltransferase and guanine-7-methyltransferase activities (Magden et al., 2001). The missing RNA triphosphatase function may be fulfilled by the HEV helicase (Karpe and Lole, 2010a).

Since HEV is heavily dependent on the capping of its RNA genome for infectivity, the viral methyltransferase seems an ideal target for antiviral therapy, all the more since these enzymatic activities are virus-specific (Magden et al., 2001) and thus may allow for development of selective inhibitors. Successful targeting of viral methyltransferases has been described for instance for dengue virus (Barral et al., 2013) and West Nile virus (Chen et al., 2013). Moreover, a highly efficient inhibitor of Chikungunya virus replication that selects for resistance mutations in nsP1 (viral methyltransferase) was recently identified in our laboratory (unpublished results; Delang L., Leyssen P. & Neyts J.); indicating that inhibition of this transferase may be a valuable strategy to inhibit the replication of RNA viruses, including HEV. Solving the crystal structure and development of robust enzymatic assays would be significant advances.

7.4. Helicase inhibitors

RNA viruses require the RNA-unwinding activity of a virally encoded helicase enzyme for replication; the energy needed for this process is provided by the hydrolysis of NTPs (Kwong et al., 2005). As for the RdRp and methyltransferase, the NTPase and RNA unwinding activities of the predicted HEV helicase domain have been confirmed *in vitro* (Karpe and Lole, 2010b). This helicase belongs to the 5'–3' unwinding SF-1 helicase superfamily, which also comprises the alphavirus and coronavirus (CoV) helicases. Potent inhibitors have been identified for the SARS-CoV nsP13 helicase (Adedeji et al., 2012; Tanner et al., 2005), suggesting that it may also be possible to develop inhibitors of the HEV helicase. An important caveat is that such inhibitors need to be highly specific for viral helicases without affecting host helicase activity to avoid toxicity. Examples from the herpesvirus field (helicase-primease inhibitors such as letermovir which has successfully finished phase 2 clinical studies (Marschall et al., 2012)) show that such selectivity can be attained.

7.5. Targeting other viral proteins

Besides the above mentioned enzymes, the HEV genome encodes several other proteins of which the function and significance have not been elucidated completely, like the macro domain, the viral protease and the ORF3 protein. Given that more information would become available, they may constitute suitable targets for antiviral therapy.

The macrodomain, formerly known as the X-domain, is located just upstream of the helicase gene (Fig. 1, Koonin et al., 1992). Its name is derived from the homologous non-histone domain of the macroH2A histone (Egloff et al., 2006). The exact function of the HEV macrodomain is unclear, although it strongly binds poly(-ADP-ribose) (Egloff et al., 2006), an important post-translational protein modification involved in cell survival and apoptosis, among other functions (Schreiber et al., 2006). Since it has been suggested that the biological function of viral macrodomains may be unrelated to that of their human homologs (Neuvonen and Ahola, 2009), they could represent attractive targets for inhibition. However, this would require a more profound understanding of the function and activity of this domain.

Secondly, a papain-like cysteine protease domain has been predicted in the HEV genome sequence (Koonin et al., 1992), but it remains unclear whether this protease is fully functional. Consequently, one of the enigmas of HEV molecular biology is whether ORF1 is processed into discrete functional units or not: several publications reported processing of ORF1 to some extent (Panda et al., 2000; Parvez, 2013; Ropp et al., 2000; Sehgal et al., 2006), while others found no processing whatsoever (Ansari et al., 2000; Perttilä et al., 2013; Suppiah et al., 2011). On the other hand, deubiquitination activity has been reported for the methyltransferase-protease fusion domain which may be involved in combating cellular antiviral responses (Karpe and Lole, 2011) and a recent mutational study suggested a glycine-triad as the protease substrate sequence (Parvez, 2013). Although viral proteases in general are interesting targets for antiviral therapy (as exemplified in the fields of HIV and HCV), more insight into the activity, function and structure of the HEV protease would be essential before focusing on protease inhibitors.

ORF3 encodes a 114 aa phosphoprotein (Graff et al., 2006) that is exclusively found in hepeviruses. ORF3 protein is dispensable for *in vitro* infection of cultured cells (Emerson et al., 2006), but required for infection and seroconversion of rhesus macaques (Graff et al., 2005). A number of functions have been proposed and several binding partners have been identified (Holla et al., 2013; Ratra et al., 2008), but in general, ORF3 protein seems to optimize the

host cell for HEV replication by increasing cell survival (Chandra et al., 2010, 2008; Moin et al., 2007) and suppressing interferon- α signaling (Dong et al., 2012). In addition, ORF3 protein has been implicated in virion egress (Yamada et al., 2009) and disruption of blood coagulation (Geng et al., 2013). It should be noted that most of these results have been obtained in artificial systems, i.e. overexpression of ORF3 protein in cultured cells instead of genuine infection with HEV. In order to be targeted for antiviral development, more insight into the ORF3 structure and function is required. Since the protein has multiple cellular binding partners, it may be interesting to look for inhibitors of these protein–protein interactions (see also Section 7.6). Such an approach may yield very specific inhibitors of HEV replication with low toxicity and a high barrier to resistance, especially given the overlap with ORF2 which is expected to substantially limit the number of viable mutations. The feasibility of identifying protein–protein interaction inhibitors as antiviral molecules was proven for the interaction between LEDGF/p75 and the HIV integrase for instance (Christ et al., 2010).

7.6. Host factors

Since many direct-acting antiviral agents are prone to the emergence and selection of resistant viruses, there is an increasing interest in targeting host factors that are co-opted by viruses for their own replication. Such host factor-targeting antivirals should have an increased barrier to resistance and may potentially have broad-spectrum activity. Multiple binding partners have been identified for HEV structural and non-structural proteins. The functional significance of many of these interactions remains unclear to date, but some of the host factors may be well-suited for antiviral targeting. Here, we discuss a few host factors that we believe have the most potential at being druggable.

First, tumor susceptibility gene 101 (Tsg101) is a central mediator in the endosomal protein sorting pathway (Surjit et al., 2006). HEV ORF3 protein has a PSAP-motif through which it associates with Tsg101, thus increasing secretion of the immunosuppressive α 1-microglobulin and locally suppressing the host immune response (Surjit et al., 2006). In addition, Tsg101 is required for virion release by interacting with the ORF3 protein PSAP-motif (Kenney et al., 2012; Nagashima et al., 2011a,b). The involvement of Tsg101 in virion release is not limited to HEV; it was also found to be essential for HIV and Ebola virus budding (Garrus et al., 2001; Martin-Serrano et al., 2001) and is being explored as a target for antiviral therapy (Chen et al., 2010). For instance, cyclic peptides blocking the interaction between Tsg101 and the PTAP motif of the HIV Gag-protein were found to block HIV budding (Tavassoli et al., 2008). Moreover, Tsg101 is expressed on the cell membrane during both HIV and influenza virus infection and targeting by antibodies blocks virus production (Bonavia et al., 2010; Diaz et al., 2010). Similar questions can be posed for HEV: does HEV induce expression of Tsg101 at the cell membrane? Can the interaction between Tsg101 and ORF3 be blocked with a small molecule or (cyclic) peptide? Potentially, antiviral research on HEV could piggy-back on the ongoing efforts for HIV and influenza virus and thus advance rapidly.

Another interesting finding is that the proteasome inhibitor MG132 inhibits HEV replication, indicating that HEV likely requires an active ubiquitin–proteasome system for infection (Karpe and Meng, 2012). Similar findings have been reported for influenza virus (Haasbach et al., 2011), HIV (Schubert et al., 2000) and vaccinia virus (Satheshkumar et al., 2009). This suggests that targeting the proteasome system may be an interesting strategy for identifying broad-spectrum antiviral drugs. Proteasome inhibitors such as bortezomib and carfilzomib are currently being used in the treatment of multiple myeloma, based on the increased susceptibility

of cancer cells compared to non-malignant cells (Hideshima et al., 2001). However, it is unknown whether such a selectivity can also be attained for virus-infected cells versus healthy cells to avoid severe side effects.

A similar analysis may apply to heat shock protein 90 (hsp90) as a cellular target for anti-HEV drugs and antiviral therapy in general. Hsp90 is required for intracellular trafficking of HEV particles and inhibition of hsp90 with geldanamycin blocks HEV infection (Zheng et al., 2010). Many viruses depend on hsp90 and their replication can be blocked by hsp90 inhibitors (Basha et al., 2005; Geller et al., 2013; Sun et al., 2013). Such inhibitors are currently being developed as anticancer therapies (Jhaveri and Modi, 2012 for an overview), but the question remains if sufficient selectivity can be attained for safe antiviral therapy. An alternative strategy may be to specifically inhibit the protein–protein interaction between the HEV capsid and hsp90, thus leaving hsp90 enzymatic activity undisturbed.

Finally, there are multiple indications that HEV replication may be influenced by sex hormones. First, mortality increases dramatically in pregnant women infected with genotype 1 (up to 25%, Khuroo et al., 1981; Tsega et al., 1993). The underlying mechanism is still unclear, but it is known that low progesterone receptor mRNA levels and mutations in the progesterone receptor gene (PROGINS) are associated with poor pregnancy outcome (fulminant hepatic failure, fetal and maternal death) during HEV infection (Bose et al., 2011). On the other hand, autochthonous HEV infections in developed countries seem to primarily affect males over 50 (Dalton et al., 2011a; Davern et al., 2011; Mansuy et al., 2009). We reported that the replication of the CTV, a HEV surrogate virus, can be directly influenced by different concentrations of progesterone, testosterone and 17 β -estradiol (Debing et al., 2013b), suggesting the possibility of a similar phenomenon in HEV infection. Several potential strategies can be derived from the limited information that is available. Since decreased progesterone receptor activity is associated with poor pregnancy outcome in hepatitis E (Bose et al., 2011) and high concentrations of progesterone inhibit CTV replication (Debing et al., 2013b), one may consider progesterone supplementation in pregnant women with hepatitis E. Such supplementation has been approved by the FDA for the prevention of preterm birth (Norwitz and Caughey, 2011). Similarly, testosterone levels decrease with age and low testosterone concentrations facilitate CTV replication (Debing et al., 2013b), suggesting that testosterone supplementation may be beneficial in elderly men (chronically) infected with HEV. A third possibility may be the use of selective estrogen receptor modulators (SERMs), such as tamoxifen and raloxifene. SERMs were found to inhibit HCV replication *in vitro* (Murakami et al., 2013; Watashi et al., 2007) and raloxifene improved the efficacy of pegylated interferon + ribavirin in menopausal women chronically infected with HCV (Furusyo et al., 2012). It would be interesting to look into the effects of SERMs on HEV or CTV replication as they may represent another possible treatment option for hepatitis E (but not during pregnancy). To fully explore the potential treatment of hepatitis E through modulation of the hormonal system, a robust animal model displaying hormone-dependent virus replication would be much appreciated.

8. Directions for future research

Antiviral drugs for the treatment of hepatitis E are required for both acute and chronic infections. Such drugs should have an increased potency, efficacy and safety compared to the currently used ribavirin and PEG-IFN α , thus allowing for prophylactic use, containment of outbreaks and preferably treatment of pregnant

women and other high-risk patients as well. It would also be interesting to further characterize the mechanism of action of ribavirin and PEG-IFN α in hepatitis E. As evident from the discussion above, HEV is an understudied pathogen and most of the suggested antiviral strategies require a more elaborate fundamental knowledge of the molecular virology. In addition, better model systems should be developed, both *in vitro* and *in vivo*, for proper antiviral research to be conducted.

HEV is an extremely interesting pathogen that deserves more profound study. In the last decade, much effort has gone to the HCV antiviral field. As many (candidate) drugs are currently in advanced clinical development, awaiting market approval or even on the market, HEV research could potentially benefit from research groups switching their main focus from HCV to HEV.

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References

- Adedeji, A.O., Singh, K., Calcaterra, N.E., DeDiego, M.L., Enjuanes, L., Weiss, S., Sarafianos, S.G., 2012. Severe acute respiratory syndrome coronavirus replication inhibitor that interferes with the nucleic acid unwinding of the viral helicase. *Antimicrob. Agents Chemother.* 56, 4718–4728.
- Agrawal, S., Gupta, D., Panda, S.K., 2001. The 3' end of hepatitis E virus (HEV) genome binds specifically to the viral RNA-dependent RNA polymerase (RdRp). *Virology* 282, 87–101.
- Ahmed, J.A., Moturi, E., Spiegel, P., Schilperoord, M., Burton, W., Kassim, N.H., Mohamed, A., Ochieng, M., Nderitu, L., Navarro-Colorado, C., Burke, H., Cookson, S., Handzel, T., Waiboci, L.W., Montgomery, J.M., Teshale, E., Marano, N., 2013. Hepatitis E outbreak, Dadaab refugee camp, Kenya, 2012. *Emerg. Infect. Dis.* 19, 1010–1012.
- Ali, M.M., Karasneh, G.A., Jardine, M.J., Tiwari, V., Shukla, D., 2012. A 3-O-sulfated heparan sulfate binding peptide preferentially targets herpes simplex virus 2-infected cells. *J. Virol.* 86, 6434–6443.
- Alric, L., Bonnet, D., Laurent, G., Kamar, N., Izopet, J., 2010. Chronic hepatitis E virus infection: successful virologic response to pegylated interferon-alpha therapy. *Ann. Intern. Med.* 153, 135–136.
- Ansari, I.H., Nanda, S.K., Durgapal, H., Agrawal, S., Mohanty, S.K., Gupta, D., Jameel, S., Panda, S.K., 2000. Cloning, sequencing, and expression of the hepatitis E virus (HEV) nonstructural open reading frame 1 (ORF1). *J. Med. Virol.* 60, 275–283.
- Baba, M., Snoeck, R., Pauwels, R., de Clercq, E., 1988. Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and human immunodeficiency virus. *Antimicrob. Agents Chemother.* 32, 1742–1745.
- Balayán, M.S., Andjaparidze, A.G., Savinskaya, S.S., Ketiladze, E.S., Braginsky, D.M., Savinov, A.P., Poleschuk, V.F., 1983. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology* 20, 23–31.
- Barral, K., Sallamand, C., Petzold, C., Coutard, B., Collet, A., Thillier, Y., Zimmermann, J., Vasseur, J.-J., Canard, B., Rohayem, J., Debart, F., Decroly, E., 2013. Development of specific dengue virus 2'-O- and N7-methyltransferase assays for antiviral drug screening. *Antiviral Res.* 99, 292–300.
- Basha, W., Kitagawa, R., Uhara, M., Imazu, H., Uechi, K., Tanaka, J., 2005. Geldanamycin, a potent and specific inhibitor of Hsp90, inhibits gene expression and replication of human cytomegalovirus. *Antiviral Chem. Chemother.* 16, 135–146.
- Batts, W., Yun, S., Hedrick, R., Winton, J., 2011. A novel member of the family Hepeviridae from cutthroat trout (*Oncorhynchus clarkii*). *Virus Res.* 158, 116–123.
- Bendall, R., Ellis, V., Ijaz, S., Ali, R., Dalton, H., 2010. A comparison of two commercially available anti-HEV IgG kits and a re-evaluation of anti-HEV IgG seroprevalence data in developed countries. *J. Med. Virol.* 82, 799–805.
- Berto, A., Grierson, S., Hakze-van der Honing, R., Martelli, F., Johne, R., Reetz, J., Ulrich, R.G., Pavio, N., Van der Poel, W.H.M., Banks, M., 2013a. Hepatitis E virus in pork liver sausage, France. *Emerg. Infect. Dis.* 19, 264–266.
- Berto, A., Van der Poel, W.H.M., Hakze-van der Honing, R., Martelli, F., La Ragione, R.M., Inglese, N., Collins, J., Grierson, S., Johne, R., Reetz, J., Dastjerdi, A., Banks, M., 2013b. Replication of hepatitis E virus in three-dimensional cell culture. *J. Virol. Methods* 187, 327–332.
- Billam, P., Huang, F.F., Sun, Z.F., Pierson, F.W., Duncan, R.B., Elvinger, F., Guenette, D.K., Toth, T.E., Meng, X.J., 2005. Systematic pathogenesis and replication of avian hepatitis E virus in specific-pathogen-free adult chickens. *J. Virol.* 79, 3429–3437.
- Bonavia, A., Diaz, L.S., Santos, D., Cassella, J., Fesseha, Z., Bamba, D., Sui, B., Li, W.-B., Duan, R., Chen, L.-M., Donis, R.O., Goldblatt, M., Kinch, M.S., 2010. Antibody targeting of TSG101 on influenza-infected cells. *Virus Adapt. Treat.* 2010, 147–157.
- Bose, P.D., Das, B.C., Kumar, A., Gondal, R., Kumar, D., Kar, P., 2011. High viral load and deregulation of the progesterone receptor signaling pathway: association with hepatitis E-related poor pregnancy outcome. *J. Hepatol.* 54, 1107–1113.
- Boxall, E., Herborn, A., Kochethu, G., Pratt, G., Adams, D., Ijaz, S., Teo, C.-G., 2006. Transfusion-transmitted hepatitis E in a “nonhyperendemic” country. *Transfus. Med. (Oxford, England)* 16, 79–83.
- Bugatti, A., Urbinati, C., Ravelli, C., De Clercq, E., Liekens, S., Rusnati, M., 2007. Heparin-mimicking sulfonic acid polymers as multitarget inhibitors of human immunodeficiency virus type 1 Tat and gp120 proteins. *Antimicrob. Agents Chemother.* 51, 2337–2345.
- Centers for Disease Control and Prevention (CDC), 2013. Investigation of hepatitis E outbreak among refugees – Upper Nile, South Sudan, 2012–2013. *MMWR Morb. Mortal. Wkly. Rep.* 62, 581–586.
- Chandra, V., Kar-Roy, A., Kumari, S., Mayor, S., Jameel, S., 2008. The hepatitis E virus ORF3 protein modulates epidermal growth factor receptor trafficking, STAT3 translocation, and the acute-phase response. *J. Virol.* 82, 7100–7110.
- Chandra, V., Kalia, M., Hajela, K., Jameel, S., 2010. The ORF3 protein of hepatitis E virus delays degradation of activated growth factor receptors by interacting with CIN85 and blocking formation of the Cbl-CIN85 complex. *J. Virol.* 84, 3857–3867.
- Chen, H., Liu, X., Li, Z., Zhan, P., De Clercq, E., 2010. TSG101: a novel anti-HIV-1 drug target. *Curr. Med. Chem.* 17, 750–758.
- Chen, H., Liu, L., Jones, S.A., Banavali, N., Kass, J., Li, Z., Zhang, J., Kramer, L.D., Ghosh, A.K., Li, H., 2013. Selective inhibition of the West Nile virus methyltransferase by nucleoside analogs. *Antiviral Res.* 97, 232–239.
- Cheng, X., Wang, S., Dai, X., Shi, C., Wen, Y., Zhu, M., Zhan, S., Meng, J., 2012. Rabbit as a novel animal model for hepatitis E virus infection and vaccine evaluation. *PLoS One* 7, e51616.
- Christ, F., Voet, A., Marchand, A., Nicolet, S., Desimie, B.A., Marchand, D., Bardiot, D., Van der Veken, N.J., Van Remoortel, B., Strelkov, S.V., De Maeyer, M., Chaltin, P., Debyser, Z., 2010. Rational design of small-molecule inhibitors of the LEDGF/p75-integrase interaction and HIV replication. *Nat. Chem. Biol.* 6, 442–448.
- Colson, P., Coze, C., Gallian, P., Henry, M., De Micco, P., Tamalet, C., 2007. Transfusion-associated hepatitis E, France. *Emerg. Infect. Dis.* 13, 648–649.
- Colson, P., Kaba, M., Moreau, J., Brouqui, P., 2009. Hepatitis E in an HIV-infected patient. *J. Clin. Virol.* 45, 269–271.
- Colson, P., Borentain, P., Queyriaux, B., Kaba, M., Moal, V., Gallian, P., Heyries, L., Raoult, D., Gerolami, R., 2010. Pig liver sausage as a source of hepatitis E virus transmission to humans. *J. Infect. Dis.* 202, 825–834.
- Corwin, A.L., Khien, H.B., Clayson, E.T., Pham, K.S., Vo, T.T., Vu, T.Y., Cao, T.T., Vaughn, D., Mervin, J., Richie, T.L., Putri, M.P., He, J., Graham, R., Wignall, F.S., Hyams, K.C., 1996. A waterborne outbreak of hepatitis E virus transmission in southwestern Vietnam. *Am. J. Trop. Med. Hyg.* 54, 559–562.
- Dalton, H.R., Bendall, R., Ijaz, S., Banks, M., 2008. Hepatitis E: an emerging infection in developed countries. *Lancet Infect. Dis.* 8, 698–709.
- Dalton, H.R., Bendall, R.P., Keane, F.E., Tedder, R.S., Ijaz, S., 2009. Persistent carriage of hepatitis E virus in patients with HIV infection. *N. Engl. J. Med.* 361, 1025–1027.
- Dalton, H.R., Bendall, R.P., Rashid, M., Ellis, V., Ali, R., Ramnarace, R., Stableforth, W., Headdon, W., Abbott, R., McLaughlin, C., Froment, E., Hall, K.J., Michell, N.P., Thatcher, P., Henley, W.E., 2011a. Host risk factors and autochthonous hepatitis E infection. *Eur. J. Gastroenterol. Hepatol.* 23, 1200–1205.
- Dalton, H.R., Keane, F.E., Bendall, R., Mathew, J., Ijaz, S., 2011b. Treatment of chronic hepatitis E in a patient with HIV infection. *Ann. Intern. Med.* 155, 479–480.
- Davern, T.J., Chalasani, N., Fontana, R.J., Hayashi, P.H., Protiva, P., Kleiner, D.E., Engle, R.E., Nguyen, H., Emerson, S.U., Purcell, R.H., Tillmann, H.L., Gu, J., Serrano, J., Hoofnagle, J.H., Drug-Induced Liver Injury Network (DILIN), 2011. Acute hepatitis E infection accounts for some cases of suspected drug-induced liver injury. *Gastroenterology* 141 (1665–1672), e1–e9.
- Davis, E.L., El Khoudary, S.R., Talbot, E.O., Davis, J., Regan, L.J., 2008. Safety and efficacy of the use of intravesical and oral pentosan polysulfate sodium for interstitial cystitis: a randomized double-blind clinical trial. *J. Urol.* 179, 177–185.
- Debing, Y., Emerson, S.U., Wang, Y., Pan, Q., Balzarini, J., Dallmeier, K., Neyts, J., 2014. Ribavirin inhibits *in vitro* hepatitis E virus replication through depletion of cellular GTP pools and is moderately synergistic with alpha interferon. *Antimicrob. Agents Chemother.* 58, 267–273.
- Debing, Y., Jochmans, D., Neyts, J., 2013a. Intervention strategies for emerging viruses: use of antivirals. *Curr. Opin. Virol.* 3, 217–224.
- Debing, Y., Winton, J., Neyts, J., Dallmeier, K., 2013b. Cutthroat trout virus as a surrogate *in vitro* infection model for testing inhibitors of hepatitis E virus replication. *Antiviral Res.* 100, 98–101.
- Diaz, L., Mao, H., Zhou, Y., Kohli, M., Cassella, J., Santos, D., Fesseha, Z., Weng, K., Chen, H., Bamba, D., Marks, J.D., Goldblatt, M., Kinch, M., 2010. TSG101 exposure on the surface of HIV-1 infected cells: implications for monoclonal antibody therapy for HIV/AIDS. *Am. J. Transl. Res.* 2, 368–380.
- Dong, C., Zafrullah, M., Mixson-Hayden, T., Dai, X., Liang, J., Meng, J., Kamili, S., 2012. Suppression of interferon- α signaling by hepatitis E virus. *Hepatology (Baltimore, Md.)* 55, 1324–1332.
- Dorner, M., Horwitz, J.A., Robbins, J.B., Barry, W.T., Feng, Q., Mu, K., Jones, C.T., Schoggins, J.W., Catanese, M.T., Burton, D.R., Law, M., Rice, C.M., Ploss, A., 2011. A genetically humanized mouse model for hepatitis C virus infection. *Nature* 474, 208–211.

- Drexler, J.F., Seelen, A., Corman, V.M., Fumie Tateno, A., Cottontail, V., Melim Zerbini, R., Gloza-Rausch, F., Klose, S.M., Adu-Sarkodie, Y., Oppong, S.K., Kalko, E.K.V., Osterman, A., Rasche, A., Adam, A., Müller, M.A., Ulrich, R.G., Leroy, E.M., Lukashev, A.N., Drosten, C., 2012. Bats worldwide carry hepatitis E virus-related viruses that form a putative novel genus within the family Hepeviridae. *J. Virol.* 86, 9134–9147.
- Drobeniuc, J., Meng, J., Reuter, G., Greene-Montfort, T., Khudyakova, N., Dimitrova, Z., Kamili, S., Teo, C.-G., 2010. Serologic assays specific to immunoglobulin M antibodies against hepatitis E virus: pangenotypic evaluation of performances. *Clin. Infect. Dis.* 51, e24–e27.
- Egloff, M.-P., Malet, H., Putics, A., Heinonen, M., Dutartre, H., Frangeul, A., Gruez, A., Campanacci, V., Cambillau, C., Ziebuhr, J., Ahola, T., Canard, B., 2006. Structural and functional basis for ADP-ribose and poly(ADP-ribose) binding by viral macro domains. *J. Virol.* 80, 8493–8502.
- Emerson, S.U., Zhang, M., Meng, X.J., Nguyen, H., St Claire, M., Govindarajan, S., Huang, Y.K., Purcell, R.H., 2001. Recombinant hepatitis E virus genomes infectious for primates: importance of capping and discovery of a cis-reactive element. *Proc. Natl. Acad. Sci. USA* 98, 15270–15275.
- Emerson, S.U., Nguyen, H., Torian, U., Purcell, R.H., 2006. ORF3 protein of hepatitis E virus is not required for replication, virion assembly, or infection of hepatoma cells in vitro. *J. Virol.* 80, 10457–10464.
- Feagins, A.R., Opriessnig, T., Guenette, D.K., Halbur, P.G., Meng, X.-J., 2007. Detection and characterization of infectious Hepatitis E virus from commercial pig livers sold in local grocery stores in the USA. *J. Gen. Virol.* 88, 912–917.
- Feagins, A.R., Opriessnig, T., Guenette, D.K., Halbur, P.G., Meng, X.J., 2008a. Inactivation of infectious hepatitis E virus present in commercial pig livers sold in local grocery stores in the United States. *Int. J. Food Microbiol.* 123, 32–37.
- Feagins, A.R., Opriessnig, T., Huang, Y.W., Halbur, P.G., Meng, X.J., 2008b. Cross-species infection of specific-pathogen-free pigs by a genotype 4 strain of human hepatitis E virus. *J. Med. Virol.* 80, 1379–1386.
- Fong, F., Illahi, M., 2009. Neuralgic amyotrophy associated with hepatitis E virus. *Clin. Neurol. Neurosurg.* 111, 193–195.
- Furusyo, N., Ogawa, E., Sudoh, M., Murata, M., Ihara, T., Hayashi, T., Ikezaki, H., Hiramane, S., Mukae, H., Toyoda, K., Tanai, H., Okada, K., Kainuma, M., Kajiwar, E., Hayashi, J., 2012. Raloxifene hydrochloride is an adjuvant antiviral treatment of postmenopausal women with chronic hepatitis C: a randomized trial. *J. Hepatol.* 57, 1186–1192.
- García-Villalón, D., Gil-Fernández, C., 1991. Antiviral activity of sulfated polysaccharides against African swine fever virus. *Antiviral Res.* 15, 139–148.
- Garrus, J.E., von Schwedler, U.K., Pornillos, O.W., Morham, S.G., Zavitz, K.H., Wang, H.E., Wettstein, D.A., Stray, K.M., Côté, M., Rich, R.L., Myszk, D.G., Sundquist, W.L., 2001. Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* 107, 55–65.
- Geller, R., Andino, R., Frydman, J., 2013. Hsp90 inhibitors exhibit resistance-free antiviral activity against respiratory syncytial virus. *PLoS One* 8, e56762.
- Geng, Y., Yang, J., Huang, W., Harrison, T.J., Zhou, Y., Wen, Z., Wang, Y., 2013. Virus host protein interaction network analysis reveals that the HEV ORF3 protein may interrupt the blood coagulation process. *PLoS One* 8, e56320.
- Gerolami, R., Borentain, P., Raissouni, F., Motte, A., Solas, C., Colson, P., 2011. Treatment of severe acute hepatitis E by ribavirin. *J. Clin. Virol.* 52, 60–62.
- Giordani, M.T., Fabris, P., Brunetti, E., Gobler, S., Romanò, L., 2013. Hepatitis E and lymphocytic leukemia in man, Italy. *Emerg. Infect. Dis.* 19, 2054–2056.
- Graff, J., Nguyen, H., Yu, C., Elkins, W.R., St Claire, M., Purcell, R.H., Emerson, S.U., 2005. The open reading frame 3 gene of hepatitis E virus contains a cis-reactive element and encodes a protein required for infection of macaques. *J. Virol.* 79, 6680–6689.
- Graff, J., Torian, U., Nguyen, H., Emerson, S.U., 2006. A bicistronic subgenomic mRNA encodes both the ORF2 and ORF3 proteins of hepatitis E virus. *J. Virol.* 80, 5919–5926.
- Guu, T.S.Y., Liu, Z., Ye, Q., Mata, D.A., Li, K., Yin, C., Zhang, J., Tao, Y.J., 2009. Structure of the hepatitis E virus-like particle suggests mechanisms for virus assembly and receptor binding. *Proc. Natl. Acad. Sci. USA* 106, 12992–12997.
- Haagsma, E.B., Riezebos-Brilman, A., van den Berg, A.P., Porte, R.J., Niesters, H.G.M., 2010. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. *Liver Transpl.* 16, 474–477.
- Haasbach, E., Pauli, E.-K., Spranger, R., Mitzner, D., Schubert, U., Kircheis, R., Planz, O., 2011. Antiviral activity of the proteasome inhibitor VL-01 against influenza A viruses. *Antiviral Res.* 91, 304–313.
- Hakze-van der Honing, R.W., van Coillie, E., Antonis, A.F.G., van der Poel, W.H.M., 2011. First isolation of hepatitis E virus genotype 4 in Europe through swine surveillance in the Netherlands and Belgium. *PLoS One* 6, e22673.
- Halbur, P.G., Kasornrondkub, C., Gilbert, C., Guenette, D., Potters, M.B., Purcell, R.H., Emerson, S.U., Toth, T.E., Meng, X.J., 2001. Comparative pathogenesis of infection of pigs with hepatitis E viruses recovered from a pig and a human. *J. Clin. Microbiol.* 39, 918–923.
- Hansman, G.S., Shahzad-Ul-Hussan, S., McLellan, J.S., Chuang, G.-Y., Georgiev, I., Shimoi, T., Katayama, K., Bewley, C.A., Kwong, P.D., 2012. Structural basis for norovirus inhibition and fucose mimicry by citrate. *J. Virol.* 86, 284–292.
- Haqshenas, G., Shivaprasad, H.L., Woolcock, P.R., Read, D.H., Meng, X.J., 2001. Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. *J. Gen. Virol.* 82, 2449–2462.
- He, S., Miao, J., Zheng, Z., Wu, T., Xie, M., Tang, M., Zhang, J., Ng, M.-H., Xia, N., 2008. Putative receptor-binding sites of hepatitis E virus. *J. Gen. Virol.* 89, 245–249.
- Hedrick, R.P., Yun, S., Wingfield, W.H., 1991. A small RNA virus isolated from Salmonid Fishes in California, USA. *Can. J. Fish Aquat. Sci.* 48, 99–104.
- Hedrick, R.P., LaPatra, S.E., Yun, S., Lauda, K.A., Jones, G.R., Congleton, J.L., de Kinkelin, P., 1994. Induction of protection from infectious hematopoietic necrosis virus in rainbow trout *Oncorhynchus mykiss* by pre-exposure to the avirulent cutthroat trout virus (CTV). *Dis. Aquat. Organ.* 20, 111–118.
- Hideshima, T., Richardson, P., Chauhan, D., Palombella, V.J., Elliott, P.J., Adams, J., Anderson, K.C., 2001. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. *Cancer Res.* 61, 3071–3076.
- Holla, R.P., Ahmad, I., Ahmad, Z., Jameel, S., 2013. Molecular virology of hepatitis E virus. *Semin. Liver Dis.* 33, 3–14.
- Huang, F., Zhang, W., Gong, G., Yuan, C., Yan, Y., Yang, S., Cui, L., Zhu, J., Yang, Z., Hua, X., 2009. Experimental infection of Balb/c nude mice with Hepatitis E virus. *BMC Infect. Dis.* 9, 93.
- ICTV, <http://www.ictvonline.org/virusTaxonomy.asp>.
- Ijaz, S., Vyse, A.J., Morgan, D., Pebody, R.G., Tedder, R.S., Brown, D., 2009. Indigenous hepatitis E virus infection in England: more common than it seems. *J. Clin. Virol.* 44, 272–276.
- Jebbloui, A., Haim-Boukobza, S., Marchadier, E., Mokhtari, C., Roque-Afonso, A.-M., 2013. Genotype 4 hepatitis e virus in france: an autochthonous infection with a more severe presentation. *Clin. Infect. Dis.* 57, e122–e126.
- Jhaveri, K., Modi, S., 2012. HSP90 inhibitors for cancer therapy and overcoming drug resistance. *Adv. Pharmacol. (San Diego, Calif.)* 65, 471–517.
- Johne, R., Heckel, G., Plenge-Bönig, A., Kindler, E., Maresch, C., Reetz, J., Schielke, A., Ulrich, R.G., 2010. Novel hepatitis E virus genotype in Norway rats, Germany. *Emerg. Infect. Dis.* 16, 1452–1455.
- Jothikumar, N., Cromeans, T.L., Robertson, B.H., Meng, X.J., Hill, V.R., 2006. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *J. Virol. Methods* 131, 65–71.
- Julander, J.G., Jha, A.K., Choi, J.-A., Jung, K.-H., Smeets, D.F., Morrey, J.D., Chu, C.K., 2010. Efficacy of 2'-C-methylcytidine against yellow fever virus in cell culture and in a hamster model. *Antiviral Res.* 86, 261–267.
- Kalia, M., Chandra, V., Rahman, S.A., Sehgal, D., Jameel, S., 2009. Heparan sulfate proteoglycans are required for cellular binding of the hepatitis E virus ORF2 capsid protein and for viral infection. *J. Virol.* 83, 12714–12724.
- Kamar, N., Mansuy, J.-M., Cointault, O., Selves, J., Abravanel, F., Danjoux, M., Otal, P., Esposito, L., Durand, D., Izopet, J., Rostaing, L., 2008a. Hepatitis E virus-related cirrhosis in kidney- and kidney-pancreas-transplant recipients. *Am. J. Transplant.* 8, 1744–1748.
- Kamar, N., Selves, J., Mansuy, J.-M., Ouezzani, L., Péron, J.-M., Guitard, J., Cointault, O., Esposito, L., Abravanel, F., Danjoux, M., Durand, D., Vinel, J.-P., Izopet, J., Rostaing, L., 2008b. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N. Engl. J. Med.* 358, 811–817.
- Kamar, N., Abravanel, F., Garrouste, C., Cardeau-Desangles, I., Mansuy, J.M., Weclawiak, H., Izopet, J., Rostaing, L., 2010a. Three-month pegylated interferon-alpha-2a therapy for chronic hepatitis E virus infection in a haemodialysis patient. *Nephrol. Dial. Transplant.* 25, 2792–2795.
- Kamar, N., Izopet, J., Cintas, P., Garrouste, C., Uro-Coste, E., Cointault, O., Rostaing, L., 2010b. Hepatitis E virus-induced neurological symptoms in a kidney-transplant patient with chronic hepatitis. *Am. J. Transplant.* 10, 1321–1324.
- Kamar, N., Rostaing, L., Abravanel, F., Garrouste, C., Esposito, L., Cardeau-Desangles, I., Mansuy, J.M., Selves, J., Peron, J.M., Otal, P., Muscare, F., Izopet, J., 2010c. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. *Clin. Infect. Dis.* 50, e30–33.
- Kamar, N., Rostaing, L., Abravanel, F., Garrouste, C., Lhomme, S., Esposito, L., Basse, G., Cointault, O., Ribes, D., Nogier, M.B., Alric, L., Peron, J.M., Izopet, J., 2010d. Ribavirin therapy inhibits viral replication on patients with chronic hepatitis E virus infection. *Gastroenterology* 139, 1612–1618.
- Kamar, N., Garrouste, C., Haagsma, E.B., Garrigue, V., Pischke, S., Chauvet, C., Dumortier, J., Cnnesson, A., Cassuto-Viguier, E., Thervet, E., Conti, F., Lebray, P., Dalton, H.R., Santella, R., Kanaan, N., Essig, M., Mousson, C., Radenne, S., Roque-Afonso, A.M., Izopet, J., Rostaing, L., 2011. Factors associated with chronic hepatitis in patients with hepatitis E virus infection who have received solid organ transplants. *Gastroenterology* 140, 1481–1489.
- Kamar, N., Weclawiak, H., Guilbeau-Frugier, C., Legrand-Abravanel, F., Cointault, O., Ribes, D., Esposito, L., Cardeau-Desangles, I., Guitard, J., Sallusto, F., Muscare, F., Peron, J.M., Alric, L., Izopet, J., Rostaing, L., 2012. Hepatitis E virus and the kidney in solid-organ transplant patients. *Transplantation* 93, 617–623.
- Kapur, N., Thakral, D., Durgapal, H., Panda, S.K., 2012. Hepatitis E virus enters liver cells through receptor-dependent clathrin-mediated endocytosis. *J. Viral Hepat.* 19, 436–448.
- Karpe, Y.A., Lole, K.S., 2010a. RNA 5'-triphosphatase activity of the hepatitis E virus helicase domain. *J. Virol.* 84, 9637–9641.
- Karpe, Y.A., Lole, K.S., 2010b. NTPase and 5'-3' RNA duplex-unwinding activities of the hepatitis E virus helicase domain. *J. Virol.* 84, 3595–3602.
- Karpe, Y.A., Lole, K.S., 2011. Deubiquitination activity associated with hepatitis E virus putative papain-like cysteine protease. *J. Gen. Virol.* 92, 2088–2092.
- Karpe, Y.A., Meng, X.-J., 2012. Hepatitis E virus replication requires an active ubiquitin-proteasome system. *J. Virol.* 86, 5948–5952.
- Kenney, S.P., Pudupakam, R.S., Huang, Y.-W., Pierson, F.W., LeRoith, T., Meng, X.-J., 2012. The PSAP motif within the ORF3 protein of an avian strain of the hepatitis E virus is not critical for viral infectivity in vivo but plays a role in virus release. *J. Virol.* 86, 5637–5646.

- Khuroo, M.S., 1980. Study of an epidemic of non-A, non-B hepatitis. Possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. *Am. J. Med.* 68, 818–824.
- Khuroo, M.S., Teli, M.R., Skidmore, S., Sofi, M.A., Khuroo, M.I., 1981. Incidence and severity of viral hepatitis in pregnancy. *Am. J. Med.* 70, 252–255.
- Khuroo, M.S., Kamili, S., Jameel, S., 1995. Vertical transmission of hepatitis E virus. *Lancet* 345, 1025–1026.
- Koonin, E.V., 1991. The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA viruses. *J. Gen. Virol.* 72 (Pt. 9), 2197–2206.
- Koonin, E.V., Gorbalenya, A.E., Purdy, M.A., Rozanov, M.N., Reyes, G.R., Bradley, D.W., 1992. Computer-assisted assignment of functional domains in the nonstructural polyprotein of hepatitis E virus: delineation of an additional group of positive-strand RNA plant and animal viruses. *Proc. Natl. Acad. Sci. USA* 89, 8259–8263.
- Kwong, A.D., Rao, B.G., Jeang, K.-T., 2005. Viral and cellular RNA helicases as antiviral targets. *Nat. Rev. Drug Discov.* 4, 845–853.
- Lawitz, E., Mangia, A., Wyles, D., Rodriguez-Torres, M., Hassanein, T., Gordon, S.C., Schultz, M., Davis, M.N., Kayali, Z., Reddy, K.R., Jacobson, I.M., Kowdley, K.V., Nyberg, L., Subramanian, G.M., Hyland, R.H., Arterburn, S., Jiang, D., McNally, J., Brainard, D., Symonds, W.T., McHutchison, J.G., Sheikh, A.M., Younossi, Z., Gane, E.J., 2013. Sofosbuvir for previously untreated chronic hepatitis C infection. *N. Engl. J. Med.* 368, 1878–1887.
- Lee, E., Pavy, M., Young, N., Freeman, C., Lobigs, M., 2006. Antiviral effect of the heparan sulfate mimetic, PI-88, against dengue and encephalitic flaviviruses. *Antiviral Res.* 69, 31–38.
- Li, T.-C., Chijiwa, K., Sera, N., Ishibashi, T., Etoh, Y., Shinohara, Y., Kurata, Y., Ishida, M., Sakamoto, S., Takeda, N., Miyamura, T., 2005. Hepatitis E virus transmission from wild boar meat. *Emerg. Infect. Dis.* 11, 1958–1960.
- Li, T.-C., Suzuki, Y., Ami, Y., Tsunemitsu, H., Miyamura, T., Takeda, N., 2008. Mice are not susceptible to hepatitis E virus infection. *J. Vet. Med. Sci.* 70, 1359–1362.
- Li, W., Sun, Q., She, R., Wang, D., Duan, X., Yin, J., Ding, Y., 2009. Experimental infection of Mongolian gerbils by a genotype 4 strain of swine hepatitis E virus. *J. Med. Virol.* 81, 1591–1596.
- Li, T.-C., Ami, Y., Suzuki, Y., Takeda, N., Takaji, W., 2013a. No evidence for hepatitis E virus genotype 3 susceptibility in rats. *Emerg. Infect. Dis.* 19, 1343–1345.
- Li, T.-C., Yoshizaki, S., Ami, Y., Suzuki, Y., Yasuda, S.P., Yoshimatsu, K., Arikawa, J., Takeda, N., Wakita, T., 2013b. Susceptibility of laboratory rats against genotypes 1, 3, 4, and rat hepatitis E viruses. *Vet. Microbiol.* 163, 54–61.
- Magden, J., Takeda, N., Li, T., Auvinen, P., Ahola, T., Miyamura, T., Merits, A., Kääriäinen, L., 2001. Virus-specific mRNA capping enzyme encoded by hepatitis E virus. *J. Virol.* 75, 6249–6255.
- Maila, H.T., Bowyer, S.M., Swanepoel, R., 2004. Identification of a new strain of hepatitis E virus from an outbreak in Namibia in 1995. *J. Gen. Virol.* 85, 89–95.
- Mallet, V., Nicand, E., Sultanik, P., Chakvetadze, C., Tessé, S., Thervet, E., Mouthon, L., Sogni, P., Pol, S., 2010. Brief communication: case reports of ribavirin treatment for chronic hepatitis E. *Ann. Intern. Med.* 153, 85–89.
- Manns, M.P., Wedemeyer, H., Cornberg, M., 2006. Treating viral hepatitis C: efficacy, side effects, and complications. *Gut* 55, 1350–1359.
- Mansuy, J.M., Abravanel, F., Miedouge, M., Mengelle, C., Merviel, C., Dubois, M., Kamar, N., Rostaing, L., Alric, L., Moreau, J., Peron, J.M., Izopet, J., 2009. Acute hepatitis E in south-west France over a 5-year period. *J. Clin. Virol.* 44, 74–77.
- Marschall, M., Stammering, T., Urban, A., Wildum, S., Ruebsamen-Schaeff, H., Zimmermann, H., Lischka, P., 2012. In vitro evaluation of the activities of the novel anticytomegalovirus compound AIC246 (Ietermovir) against herpesviruses and other human pathogenic viruses. *Antimicrob. Agents Chemother.* 56, 1135–1137.
- Martin-Serrano, J., Zang, T., Bieniasz, P.D., 2001. HIV-1 and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nat. Med.* 7, 1313–1319.
- Mast, E.E., Alter, M.J., Holland, P.V., Purcell, R.H., 1998. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E virus antibody serum panel evaluation group. *Hepatology (Baltimore, Md.)* 27, 857–861.
- Meng, X.J., Purcell, R.H., Halbur, P.G., Lehman, J.R., Webb, D.M., Tsareva, T.S., Haynes, J.S., Thacker, B.J., Emerson, S.U., 1997. A novel virus in swine is closely related to the human hepatitis E virus. *Proc. Natl. Acad. Sci. USA* 94, 9860–9865.
- Meng, X.J., Halbur, P.G., Shapiro, M.S., Govindarajan, S., Bruna, J.D., Mushahwar, I.K., Purcell, R.H., Emerson, S.U., 1998. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *J. Virol.* 72, 9714–9721.
- Meuleman, P., Libbrecht, L., De Vos, R., de Hemptinne, B., Gevaert, K., Vandekerckhove, J., Roskams, T., Leroux-Roels, G., 2005. Morphological and biochemical characterization of a human liver in a uPA-SCID mouse chimera. *Hepatology (Baltimore, Md.)* 41, 847–856.
- Miyashita, K., Kang, J.-H., Saga, A., Takahashi, K., Shimamura, T., Yasumoto, A., Fukushima, H., Sogabe, S., Konishi, K., Uchida, T., Fujinaga, A., Matsui, T., Sakurai, Y., Tsuji, K., Maguchi, H., Taniguchi, M., Abe, N., Fazle Akbar, S.M., Arai, M., Mishiro, S., 2012. Three cases of acute or fulminant hepatitis E caused by ingestion of pork meat and entrails in Hokkaido, Japan: zoonotic food-borne transmission of hepatitis E virus and public health concerns. *Hepatol. Res.* 42, 870–878.
- Moin, S.M., Panteva, M., Jameel, S., 2007. The hepatitis E virus Orf3 protein protects cells from mitochondrial depolarization and death. *J. Biol. Chem.* 282, 21124–21133.
- Murakami, Y., Fukasawa, M., Kaneko, Y., Suzuki, T., Wakita, T., Fukazawa, H., 2013. Selective estrogen receptor modulators inhibit hepatitis C virus infection at multiple steps of the virus life cycle. *Microbes Infect.* 15, 45–55.
- Nagashima, S., Takahashi, M., Jirintai, S., Tanaka, T., Nishizawa, T., Yasuda, J., Okamoto, H., 2011a. Tumour susceptibility gene 101 and the vacuolar protein sorting pathway are required for the release of hepatitis E virions. *J. Gen. Virol.* 92, 2838–2848.
- Nagashima, S., Takahashi, M., Jirintai, Tanaka, T., Yamada, K., Nishizawa, T., Okamoto, H., 2011b. A PSAP motif in the ORF3 protein of hepatitis E virus is necessary for virion release from infected cells. *J. Gen. Virol.* 92, 269–278.
- Naik, S.R., Aggarwal, R., Salunke, P.N., Mehrotra, N.N., 1992. A large waterborne viral hepatitis E epidemic in Kanpur, India. *Bull. World Health Organ.* 70, 597–604.
- Neukam, K., Barreiro, P., Macías, J., Avellón, A., Cifuentes, C., Martín-Carbonero, L., Echevarría, J.M., Vargas, J., Soriano, V., Pineda, J.A., 2013. Chronic hepatitis E in HIV patients: rapid progression to cirrhosis and response to oral ribavirin. *Clin. Infect. Dis.* 57, 465–468.
- Neuvonen, M., Ahola, T., 2009. Differential activities of cellular and viral macro domain proteins in binding of ADP-ribose metabolites. *J. Mol. Biol.* 385, 212–225.
- Nguyen, H.T., Shukla, P., Torian, U., Faulk, K., Emerson, S.U., 2013. HEV genotype 1 infection of swine kidney cells *in vitro* is inhibited at multiple levels. *J. Virol.*
- Norwitz, E.R., Caughey, A.B., 2011. Progesterone supplementation and the prevention of preterm birth. *Rev. Obstet. Gynecol.* 4, 60–72.
- Osinusi, A., Meissner, E.G., Lee, Y.-J., Bon, D., Heytens, L., Nelson, A., Sneller, M., Kohli, A., Barrett, L., Proschan, M., Herrmann, E., Shivakumar, B., Gu, W., Kwan, R., Teferi, G., Talwani, R., Silk, R., Kotb, C., Wroblewski, S., Fishbein, D., Dewar, R., Highbarger, H., Zhang, X., Kleiner, D., Wood, B.J., Chavez, J., Symonds, W.T., Subramanian, M., McHutchison, J., Polis, M.A., Fauci, A.S., Masur, H., Kottlil, S., 2013. Sofosbuvir and ribavirin for hepatitis C genotype 1 in patients with unfavorable treatment characteristics: a randomized clinical trial. *JAMA* 310, 804–811.
- Panda, S.K., Ansari, I.H., Durgapal, H., Agrawal, S., Jameel, S., 2000. The *in vitro*-synthesized RNA from a cDNA clone of hepatitis E virus is infectious. *J. Virol.* 74, 2430–2437.
- Parvez, M.K., 2013. Molecular characterization of hepatitis E virus ORF1 gene supports a papain-like cysteine protease (PCP)-domain activity. *Virus Res.* 178, 553–556.
- Patra, S., Kumar, A., Trivedi, S.S., Puri, M., Sarin, S.K., 2007. Maternal and fetal outcomes in pregnant women with acute hepatitis E virus infection. *Ann. Intern. Med.* 147, 28–33.
- Perttälä, J., Spuul, P., Ahola, T., 2013. Early secretory pathway localization and lack of processing for hepatitis E virus replication protein pORF1. *J. Gen. Virol.* 94, 807–816.
- Pickett, B.E., Sadat, E.L., Zhang, Y., Noronha, J.M., Squires, R.B., Hunt, V., Liu, M., Kumar, S., Zaremba, S., Gu, Z., Zhou, L., Larson, C.N., Dietrich, J., Klem, E.B., Scheuermann, R.H., 2012. ViPR: an open bioinformatics database and analysis resource for virology research. *Nucleic Acids Res.* 40, D593–598.
- Pischke, S., Stiefel, P., Franz, B., Bremer, B., Suneetha, P.V., Heim, A., Ganzenmueller, T., Schlue, J., Horn-Wichmann, R., Raupach, R., Darnedde, M., Scheibner, Y., Taubert, R., Haverich, A., Manns, M.P., Wedemeyer, H., Bara, C.L., 2012. Chronic hepatitis E in heart transplant recipients. *Am. J. Transplant.* 12, 3128–3133.
- Pischke, S., Hardtke, S., Bode, U., Birkner, S., Chatzikyriakou, C., Kauffmann, W., Bara, C.L., Gottlieb, J., Wenzel, J., Manns, M.P., Wedemeyer, H., 2013. Ribavirin treatment of acute and chronic hepatitis E: a single-centre experience. *Liver Int.* 33, 722–726.
- Pudupakam, R.S., Kenney, S.P., Córdoba, L., Huang, Y.-W., Dryman, B.A., Leroith, T., Pierson, F.W., Meng, X.-J., 2011. Mutational analysis of the hypervariable region of hepatitis E virus reveals its involvement in the efficiency of viral RNA replication. *J. Virol.* 85, 10031–10040.
- Purcell, R.H., Nguyen, H., Shapiro, M., Engle, R.E., Govindarajan, S., Blackwelder, W.C., Wong, D.C., Prieels, J.P., Emerson, S.U., 2003. Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. *Vaccine* 21, 2607–2615.
- Purcell, R.H., Engle, R.E., Rood, M.P., Kabrane-Lazizi, Y., Nguyen, H.T., Govindarajan, S., St. Claire, M., Emerson, S.U., 2011. Hepatitis E virus in rats, Los Angeles, California, USA. *Emerg. Infect. Dis.* 17, 2216–2222.
- Rademacher, C., Guirard, J., Kitov, P.I., Fiege, B., Dalton, K.P., Parra, F., Bundle, D.R., Peters, T., 2011. Targeting norovirus infection-multipotent entry inhibitor design based on NMR experiments. *Chemistry* 17, 7442–7453.
- Raj, V.S., Smits, S.L., Pas, S.D., Provacia, L.B.V., Moorman-Roest, H., Osterhaus, A.D.M.E., Haagmans, B.L., 2012. Novel hepatitis E virus in ferrets, The Netherlands. *Emerg. Infect. Dis.* 18, 1369–1370.
- Ratra, R., Kar-Roy, A., Lal, S.K., 2008. The ORF3 protein of hepatitis E virus interacts with hemopexin by means of its 26 amino acid N-terminal hydrophobic domain II. *Biochemistry (Mosc.)* 47, 1957–1969.
- Rehman, S., Kapur, N., Durgapal, H., Panda, S.K., 2008. Subcellular localization of hepatitis E virus (HEV) replicase. *Virology* 370, 77–92.
- Rein, D.B., Stevens, G.A., Theaker, J., Wittenborn, J.S., Wiersma, S.T., 2012. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology (Baltimore, Md.)* 55, 988–997.
- Ren, R.B., Costantini, F., Gorgacz, E.J., Lee, J.J., Racaniello, V.R., 1990. Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. *Cell* 63, 353–362.
- Rocha-Pereira, J., Jochmans, D., Debing, Y., Verbeken, E., Nascimento, M.S.J., Neyts, J., 2013. The viral polymerase inhibitor 2'-C-methylcytidine inhibits Norwalk virus replication and protects against norovirus-induced diarrhea and mortality in a mouse model. *J. Virol.* 87, 11798–11805.
- Ropp, S.L., Tam, A.W., Beames, B., Purdy, M., Frey, T.K., 2000. Expression of the hepatitis E virus ORF1. *Arch. Virol.* 145, 1321–1337.

- Sanford, B.J., Dryman, B.A., Huang, Y.-W., Feagins, A.R., Leroith, T., Meng, X.-J., 2011. Prior infection of pigs with a genotype 3 swine hepatitis E virus (HEV) protects against subsequent challenges with homologous and heterologous genotypes 3 and 4 human HEV. *Virus Res.* 159, 17–22.
- Satheshkumar, P.S., Anton, L.C., Sanz, P., Moss, B., 2009. Inhibition of the ubiquitin-proteasome system prevents vaccinia virus DNA replication and expression of intermediate and late genes. *J. Virol.* 83, 2469–2479.
- Schreiber, V., Dantzer, F., Ame, J.-C., de Murcia, G., 2006. Poly(ADP-ribose): novel functions for an old molecule. *Nat. Rev. Mol. Cell Biol.* 7, 517–528.
- Schubert, U., Ott, D.E., Chertova, E.N., Welker, R., Tessmer, U., Princiotta, M.F., Bennink, J.R., Krausslich, H.G., Yewdell, J.W., 2000. Proteasome inhibition interferes with gag polyprotein processing, release, and maturation of HIV-1 and HIV-2. *Proc. Natl. Acad. Sci. USA* 97, 13057–13062.
- Scully, M.F., Weerasinghe, K.M., Ellis, V., Djazaeri, B., Kakkar, V.V., 1983. Anticoagulant and antiheparin activities of a pentosan polysulphate. *Thromb. Res.* 31, 87–97.
- Sehgal, D., Thomas, S., Chakraborty, M., Jameel, S., 2006. Expression and processing of the Hepatitis E virus ORF1 nonstructural polyprotein. *Virol. J.* 3, 38.
- Shrestha, M.P., Scott, R.M., Joshi, D.M., Mammen Jr, M.P., Thapa, G.B., Thapa, N., Myint, K.S.A., Fourneau, M., Kuschner, R.A., Shrestha, S.K., David, M.P., Seriwatana, J., Vaughn, D.W., Safary, A., Endy, T.P., Innis, B.L., 2007. Safety and efficacy of a recombinant hepatitis E vaccine. *N. Engl. J. Med.* 356, 895–903.
- Shukla, P., Nguyen, H.T., Torian, U., Engle, R.E., Faulk, K., Dalton, H.R., Bendall, R.P., Keane, F.E., Purcell, R.H., Emerson, S.U., 2011. Cross-species infections of cultured cells by hepatitis E virus and discovery of an infectious virus-host recombinant. *Proc. Natl. Acad. Sci. USA* 108, 2438–2443.
- Shukla, P., Nguyen, H.T., Faulk, K., Mather, K., Torian, U., Engle, R.E., Emerson, S.U., 2012. Adaptation of a genotype 3 hepatitis E virus to efficient growth in cell culture depends on an inserted human gene segment acquired by recombination. *J. Virol.* 86, 5697–5707.
- Smith, D.B., Vanek, J., Ramalingam, S., Johannessen, I., Templeton, K., Simmonds, P., 2012. Evolution of the hepatitis E virus hypervariable region. *J. Gen. Virol.* 93, 2408–2418.
- Smith, D.B., Purdy, M.A., Simmonds, P., 2013. Genetic variability and the classification of hepatitis E virus. *J. Virol.* 87, 4161–4169.
- Sood, A., Midha, V., Sood, N., 2000. Guillain-Barré syndrome with acute hepatitis E. *Am. J. Gastroenterol.* 95, 3667–3668.
- Sugitani, M., Tamura, A., Shimizu, Y.K., Sheikh, A., Kinukawa, N., Shimizu, K., Moriama, M., Komiya, K., Li, T.-C., Takeda, N., Arakawa, Y., Suzuki, K., Ishaque, S.M., Roy, P.K., Raihan, A.S.M.A., Hasan, M., 2009. Detection of hepatitis E virus RNA and genotype in Bangladesh. *J. Gastroenterol. Hepatol.* 24, 599–604.
- Sun, X., Bristol, J.A., Iwahori, S., Hagemeyer, S.R., Meng, Q., Barlow, E.A., Fingerroth, J.D., Tarakanova, V.L., Kalejta, R.F., Kenney, S.C., 2013. Hsp90 inhibitor 17-DMAG decreases expression of conserved herpesvirus protein kinases and reduces virus production in Epstein-Barr virus-infected cells. *J. Virol.* 87, 10126–10138.
- Suppiah, S., Zhou, Y., Frey, T.K., 2011. Lack of processing of the expressed ORF1 gene product of hepatitis E virus. *Virol. J.* 8, 245.
- Surjit, M., Oberoi, R., Kumar, R., Lal, S.K., 2006. Enhanced alpha1 microglobulin secretion from Hepatitis E virus ORF3-expressing human hepatoma cells is mediated by the tumor susceptibility gene 101. *J. Biol. Chem.* 281, 8135–8142.
- Takahashi, K., Kitajima, N., Abe, N., Mishiro, S., 2004. Complete or near-complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. *Virology* 330, 501–505.
- Takahashi, M., Tanaka, T., Takahashi, H., Hoshino, Y., Nagashima, S., Jirintai, Mizuo, H., Yazaki, Y., Takagi, T., Azuma, M., Kusano, E., Isoda, N., Sugano, K., Okamoto, H., 2010. Hepatitis E Virus (HEV) strains in serum samples can replicate efficiently in cultured cells despite the coexistence of HEV antibodies: characterization of HEV virions in blood circulation. *J. Clin. Microbiol.* 48, 1112–1125.
- Tamada, Y., Yano, K., Yatsushashi, H., Inoue, O., Mawatari, F., Ishibashi, H., 2004. Consumption of wild boar linked to cases of hepatitis E. *J. Hepatol.* 40, 869–870.
- Tanaka, T., Takahashi, M., Kusano, E., Okamoto, H., 2007. Development and evaluation of an efficient cell-culture system for Hepatitis E virus. *J. Gen. Virol.* 88, 903–911.
- Tanaka, T., Takahashi, M., Takahashi, H., Ichiyama, K., Hoshino, Y., Nagashima, S., Mizuo, H., Okamoto, H., 2009. Development and characterization of a genotype 4 hepatitis E virus cell culture system using a HE-JF5/15F strain recovered from a fulminant hepatitis patient. *J. Clin. Microbiol.* 47, 1906–1910.
- Tanner, J.A., Zheng, B.-J., Zhou, J., Watt, R.M., Jiang, J.-Q., Wong, K.-L., Lin, Y.-P., Lu, L.-Y., He, M.-L., Kung, H.-F., Kesel, A.J., Huang, J.-D., 2005. The adamantane-derived bananins are potent inhibitors of the helicase activities and replication of SARS coronavirus. *Chem. Biol.* 12, 303–311.
- Tavassoli, A., Lu, Q., Gam, J., Pan, H., Benkovic, S.J., Cohen, S.N., 2008. Inhibition of HIV budding by a genetically selected cyclic peptide targeting the Gag-TSG101 interaction. *ACS Chem. Biol.* 3, 757–764.
- Teshale, E.H., Howard, C.M., Grytdal, S.P., Handzel, T.R., Barry, V., Kamili, S., Drobeniuc, J., Okware, S., Downing, R., Tapper, J.W., Bakamutumaho, B., Teo, C.G., Ward, J.W., Holmberg, S.D., Hu, D.J., 2010. Hepatitis E epidemic, Uganda. *Emerg. Infect. Dis.* 16, 126–129.
- Tsege, E., Krawczynski, K., Hansson, B.G., Nordenfelt, E., 1993. Hepatitis E virus infection in pregnancy in Ethiopia. *Ethiop. Med. J.* 31, 173–181.
- Velázquez, O., Stettler, H.C., Avila, C., Ornelas, G., Alvarez, C., Hadler, S.C., Bradley, D.W., Sepúlveda, J., 1990. Epidemic transmission of enterically transmitted non-A, non-B hepatitis in Mexico, 1986–1987. *JAMA* 263, 3281–3285.
- Versluis, J., Pas, S.D., Agteresch, H.J., de Man, R.A., Maaskant, J., Schipper, M.E.I., Osterhaus, A.D.M.E., Cornelissen, J.J., van der Eijk, A.A., 2013. Hepatitis E virus: an underestimated opportunistic pathogen in recipients of allogeneic hematopoietic stem cell transplantation. *Blood* 122, 1079–1086.
- Volz, T., Allweiss, L., M Barek, M.B., Warlich, M., Lohse, A.W., Pollok, J.M., Alexandrov, A., Urban, S., Petersen, J., Lütgehetmann, M., Dandri, M., 2013. The entry inhibitor Myrcludex-B efficiently blocks intrahepatic virus spreading in humanized mice previously infected with hepatitis B virus. *J. Hepatol.* 58, 861–867.
- Wang, H., He, Y., Shen, Q., Wang, X., Yang, S., Cui, L., Ren, L., Sun, G., Hua, X., Shao, S., Zhang, W., 2012. Complete genome sequence of the genotype 4 hepatitis E virus strain prevalent in swine in Jiangsu Province, China, reveals a close relationship with that from the human population in this area. *J. Virol.* 86, 8334–8335.
- Watanashi, K., Inoue, D., Hijikata, M., Goto, K., Aly, H.H., Shimotohno, K., 2007. Anti-hepatitis C virus activity of tamoxifen reveals the functional association of estrogen receptor with viral RNA polymerase NS5B. *J. Biol. Chem.* 282, 32765–32772.
- Yamada, K., Takahashi, M., Hoshino, Y., Takahashi, H., Ichiyama, K., Nagashima, S., Tanaka, T., Okamoto, H., 2009. ORF3 protein of hepatitis E virus is essential for virion release from infected cells. *J. Gen. Virol.* 90, 1880–1891.
- Yamashita, T., Mori, Y., Miyazaki, N., Cheng, R.H., Yoshimura, M., Unno, H., Shima, R., Moriishi, K., Tsukihara, T., Li, T.C., Takeda, N., Miyamura, T., Matsuura, Y., 2009. Biological and immunological characteristics of hepatitis E virus-like particles based on the crystal structure. *Proc. Natl. Acad. Sci. USA* 106, 12986–12991.
- Yu, C., Boon, D., McDonald, S.L., Myers, T.G., Tomioka, K., Nguyen, H., Engle, R.E., Govindarajan, S., Emerson, S.U., Purcell, R.H., 2010. Pathogenesis of hepatitis E virus and hepatitis C virus in chimpanzees: similarities and differences. *J. Virol.* 84, 11264–11278.
- Zafrullah, M., Ozdener, M.H., Panda, S.K., Jameel, S., 1997. The ORF3 protein of hepatitis E virus is a phosphoprotein that associates with the cytoskeleton. *J. Virol.* 71, 9045–9053.
- Zeuzem, S., Asselah, T., Angus, P., Zarski, J.-P., Larrey, D., Müllhaupt, B., Gane, E., Schuchmann, M., Lohse, A.W., Pol, S., Bronowicki, J.-P., Roberts, S., Arasteh, K., Zoulim, F., Heim, M., Stern, J.O., Nehmiz, G., Kukulj, G., Böcher, W.O., Mensa, F.J., 2013. Faldaprevir (BI 207127) and ribavirin oral therapy for treatment-naïve HCV genotype 1: SOUND-C1 final results. *Antivir. Ther.*
- Zhao, C., Ma, Z., Harrison, T.J., Feng, R., Zhang, C., Qiao, Z., Fan, J., Ma, H., Li, M., Song, A., Wang, Y., 2009. A novel genotype of hepatitis E virus prevalent among farmed rabbits in China. *J. Med. Virol.* 81, 1371–1379.
- Zheng, Z.-Z., Miao, J., Zhao, M., Tang, M., Yeo, A.E.T., Yu, H., Zhang, J., Xia, N.-S., 2010. Role of heat-shock protein 90 in hepatitis E virus capsid trafficking. *J. Gen. Virol.* 91, 1728–1736.
- Zhu, F.-C., Zhang, J., Zhang, X.-F., Zhou, C., Wang, Z.-Z., Huang, S.-J., Wang, H., Yang, C.-L., Jiang, H.-M., Cai, J.-P., Wang, Y.-J., Ai, X., Hu, Y.-M., Tang, Q., Yao, X., Yan, Q., Xian, Y.-L., Wu, T., Li, Y.-M., Miao, J., Ng, M.-H., Shih, J.W.-K., Xia, N.-S., 2010. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet* 376, 895–902.
- Zhu, Y., Yu, X., Zhang, Y., Ni, Y., Si, F., Yu, R., Dong, S., Huang, Y., Li, Z., 2013. Infectivity of a genotype 4 hepatitis E virus cDNA clone by intrahepatic inoculation of laboratory rats. *Vet. Microbiol.* 166, 405–411.