Hepatitis E virus: a review

P. Vasickova[1,2], I. Psikal[2], P. Kralik[2], F. Widen[3], Z. Hubalek[4,5], I. Pavlik[2]

[1]University of Veterinary and Pharmaceutical Sciences Brno, Czech Republic
[2]Veterinary Research Institute, Brno, Czech Republic
[3]The National Veterinary Institute, Uppsala, Sweden
[4]Faculty of Science, Masaryk University, Brno, Czech Republic
[5]Institute of Vertebrate Biology of the Academy of Sciences of the Czech Republic, Brno, Czech Republic

ABSTRACT: The hepatitis E virus (HEV), the causative agent of hepatitis E, is a non-enveloped RNA virus. The HEV genome is formed by a non-segmented positive-sense RNA chain. The 3´end of the chain is polyadenylated and the 5´end is structurally characterised by the so called "capping". According to currently accepted taxonomy, HEV is classified in the genus *Hepevirus*, the only member of the Hepeviridae family. HE is usually transmitted via the faecal-oral route due to the fact that drinking water or water for industrial purposes is contaminated due to poor sanitation. This spread of HEV has been reported in developing countries of Asia, Africa, South and Central America. However, cases in countries with the sporadic occurrence of HEV have been associated with travelling to countries with an increased risk of infection (developing countries in Asia, Africa and America). HEV infections have subsequently been described in people who have not travelled to endemic countries. Further studies of the HEV suggested other routes of transmission and a zoonotic potential of the virus (pigs and deer as the potential source of human infection).

Keywords: risk assessment; food safety; foodborne viral outbreaks; zoonoses; pigs

Contents

Partially supported by the Ministry of Agriculture of the Czech Republic (Grant No. MZE-0002716201), Grants No. FOOD-CT-2005-007081 (PathogenCombat) and Cost Action 929 ENVIRONET of Brussels, EC.

1. Introduction

Hepatitis E virus (HEV), the etiological agent of hepatitis E (HE), was described for the first time using electron microscopy in 1983 as a spherical viral particle being 27 to 30 nm in size. The virus originated from the stool of a volunteer orally infected with faeces from suspect cases of non-A and non-B hepatitis (Balayan et al., 1983). The HEV genome comprises a non-segmented positive-sense RNA chain and the virus is non-enveloped (Acha and Szyfres, 2003). HEV was suggested to be classified in the Picornaviridae family (Balayan et al., 1983). However, later studies showed that it does not belong to members of this family. Between 1988 and 1998, HEV was tentatively classified in the Caliciviridae family, based on virion morphology. This classification was also rejected after a phylogeny analysis of the HEV genome, and HEV was newly classified as an independent genus *HEV-like virus,* unassigned to any family (Berke and Matson, 2000; Acha and Szyfres, 2003).

At present, HEV is the only member of the *Hepevirus* genus, Hepeviridae family (Emerson et al., 2004; http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs_hepev.htm). Phylogenetically, HEV shows the highest, but limited, similarity with the Rubella virus: *Alphavirus* genus, Togaviridae family and with the Necrotic yellow vein virus of sugar beet: *Furovirus* genus, Togaviridae family (Berke and Matson, 2000).

HEV causes acute sporadic and epidemic viral hepatitis worldwide. HEV infections are spread mainly by the faecal-oral route and large epidemics due to this virus are often associated with contaminated water (Ashbolt, 2004; Koopmans and Duizer, 2004; Vasickova et al., 2005). There is also a possibility of zoonotic transmission of the virus. Seroepidemiological studies revealed that anti-HEV antibodies are present in numerous animal species including pigs, rodents, chickens, dogs, cows, sheep and goats from developing and industrialised countries (Tien et al., 1997; Favorov et al., 1998; Arankalle et al., 2001). In addition, an isolate of HEV from a pig, designated as swine HEV, has been identified and shown to be closely related to two human isolates of HEV (US-1 and US-2) identified in the United States (Meng et al., 1997). Similar findings have also been reported in pigs from Taiwan with a different strain of swine HEV (Hsieh et al., 1999). Experimental cross species infection has been demonstrated: swine HEV has been shown to infect rhesus monkeys and chimpanzees and the US-2 strain of human HEV has been shown to infect pigs (Meng et al., 1998a; Halbur et al., 2001).

With regard to this knowledge, HEV may be viewed as a new emerging pathogen with a zoonotic potential. Accordingly, the purpose of the review article is to summarise current knowledge, which may serve for the identification and more detailed characterisation of the virus not only in the Czech Republic, but also in other member states of the European Union.

2. HE in humans

2.1. Clinical manifestations

HEV infections are spread by the faecal-oral route. However, the mechanism allowing the virus

to reach the site of primary multiplication has not been fully clarified yet. Replication of viral particles takes place in intestinal mucosa cells, but primary in the cytoplasm of hepatocytes. Virions are transported with bile from liver tissue to the intestine (Williams et al., 2001). Aggarwal et al. (2000) analysed faeces collected from 20 patients affected by acute HE, 35 days after appearance of the first signs of the disease. They did not detect HEV ribonucleic acid (RNA) in any of the samples, but HEV RNA was present in the blood serum 29 days after the appearance of the first symptoms. Viremia was detected in one patient after 45 days. The incubation time ranges between 3 to 8 weeks; symptoms of the disease appeared in macaques two weeks after experimental intravenous infection (Li et al., 1994; Tsarev et al., 1994, as quoted by Anderson and Shrestha, 2002).

Various clinical manifestations of the disease have been observed, from more frequent subclinical forms to fulminant forms of hepatitis. HEV infection is most often seen in children, young to middle aged adults (15 to 40 years old) and might be serious in pregnant women. In most cases, the signs and symptoms of the disease include moderately severe hepatitis with concurrent signs of influenza-like symptoms, abdominal pain, tenderness, nausea, vomiting and fever in the first (preicteric) phase of 1 to 10 days. The second (icteric) phase (15 to 40 days) with concurrent jaundice and dark urine is followed by viremia, liver enzyme elevations, antibody seroconversion and clearing of the virus. Clinically, HE is typically a self-limiting disease without progression to chronic illness. HE is mostly asymptomatic in children but fulminant hepatitis occurs more frequently during pregnancy with a mortality rate of 20% among pregnant women in the thrird trimester, and can also cause premature births. The reason for the high mortality of pregnant women is not fully determined (Hussaini et al., 1997; Bednar et al., 1999; Worm et al., 2002; Emerson and Purcell, 2003).

2.2. Immune response of an infected organism

With the onset of symptoms, antibodies against HEV, IgM and subsequently IgG class appear in the titre sera of infected patients. The IgM sharply declines at the beginning of convalescence; these antibodies may be detected for the following two to three months only (Chauhan et al., 1993). In contrast, IgG antibodies usually persist in the organisms of infected people for several years, in 47% of patients for more than 14 years (Clayson et al., 1995).

2.3. Gross lesions in liver tissue

Specific changes in the morphology of liver tissue have been observed in HE patients. Liver biopsy specimens have shown either non-specific inflammatory or prominent canalicular bile stasis with a pseudoglandular arrangement of hepatocytes around distended bile canaliculi (cholestatic form). Histopathological changes gradually resolve over 3 to 6 months (Anderson and Shrestha, 2002).

3. HE in animals

Serological studies of HEV in endemic and non-endemic regions have shown that anti-HEV antibodies are present in domestic and wild animal species. That gives evidence that these animals have been in contact with HEV or an antigenically related agent. A reservoir may exist in these animals and constitute a risk for spreading to the human population (Arankalle et al., 1994; Favorov et al., 1998; Arankalle et al., 2001).

3.1. Domestic animals

3.1.1. Domestic pigs

Anti-HEV antibodies were detected in pigs worldwide (Table 1). It has been demonstrated that HEV, in most cases, causes only subclinical infections in pigs, *Sus scrofa domesticus* (Meng et al., 1998b; Halbur et al., 2001; Kasorndorkbua et al., 2002). Halbur et al. (2001) performed experimental *intra venam* inoculation of specific-pathogen-free (SPF) pigs with US viral strains of HE identified in humans (US-2) and pigs (swUS-1). Clinical signs or elevated levels of hepatic enzymes or bilirubin were not observed. Liver tissue from a group of pigs infected with swine HEV showed gross lesions, and histopathology revealed mild hepatitis. Damage to this tissue was less severe than in the group of pigs infected with a human strain. HE was diagnosed in animals experimentally infected with Asian human HEV (Balayan et al., 1990).

Table 1. Prevalence of the IgG antibody to hepatitis E virus in serum of domestic pigs (*Sus scrofa domesticus*) detected by enzyme immunoassay according to the geographic region

Country	Number of			References
	tested	positive	%	
Argentina	97	22	22.7	Munne et al., 2006
Brazil	357	227	63.6	Vitral et al., 2005
Canada	712	129	18.1	Meng et al., 1999
Canada	998	594	59.5	Yoo et al., 2001
China	82	22	25.0	Meng et al., 1999
China	419	330	78.8	Wang et al., 2002
Great Britain	256	219	85.5	Banks et al., 2004a
India	284	122	43.0	Arankalle et al., 2002
Indonesia	99	71	72.0	Wibawa et al., 2004
Korea	140	57	40.7	Meng et al., 1999
Mexico	125	8	6.0	Cooper et al., 2005
New Zealand	72	54	75.0	Garkavenko et al., 2001
Spain	60	15	25.0	Pina et al., 2000
Sweden	204	118	58.0	Banks et al., 2004a
Taiwan	275	102	37.1	Hsieh et al., 1999
Thailand	76	10	13.0	Cooper et al., 2005
Thailand	75	23	30.7	Meng et al., 1999
The Netherlands	34	8	23.5	Banks et al., 2004a
USA	293	202	68.9	Meng et al., 1997
USA	84	29	34.5	Withers et al., 2002
Total	4 742	2 362	49.8	

3.1.2. Poultry

Anti-HEV antibodies have been detected in 44% of examined chicken in Vietnam (Tien et al., 1997) and in 20% of chicken in Brazil (Vitral et al., 2005). Among 70 tested flocks of hens in the USA, 54 (71%) gave seropositive responses for IgG to HEV (Huang et al., 2002a). Avian HEV was identified and characterised in bile samples from chickens showing hepatitis-splenomegaly syndrome (HS; Haqshenas et al., 2001). HEV infection was most likely a factor (however, not the only one) essential for the clinical development of HS syndrome. Regressive ovaries, red fluid in the abdomen, enlarged liver and spleen and up to 20% drop in egg production may be observed in infected poultry (Billam et al., 2005).

3.1.3. Dogs and cats

Anti-HEV antibodies were detected in 27% of tested dog serum samples in Vietnam (Tien et al., 1997) and 7% of animals in Brazil (Vitral et al., 2005). Among 135 domestic cats tested in Japan, HEV antibodies were detected in 44 (33%) of them (Okamoto et al., 2004).

3.1.4. Other domestic animals

Serological screenings showed the presence of antibodies against HEV in 29% to 62% of cows in Somalia, Tajikistan and Turkmenistan (endemic regions), in 42% to 67% of sheep and goats in Turkmenistan and in about 12% of cows in the

Table 2. Prevalence of the IgG antibody to hepatitis E virus detected by enzyme immunoassay in serum of rodents according to the species and to the geographic region

Country	Animal species	Number of			References
		tested	positive	%	
Brazil	*Nectomys* sp.	4	2	50.0	Vitral et al., 2005
India	*Rattus rattus rufescens*	58	9	18.0	Arankalle et al., 2001
India	*Bandicota bengalensis*	22	12	54.5	Arankalle et al., 2001
India	*Rattus norvegicus*	32	0	0	Arankalle et al., 2001
India	*Rattus rattus andamanensis*	55	2	3.6	Arankalle et al., 2001
India	*Mus musculus*	12	0	0	Arankalle et al., 2001
India	*Mus* spp.	25	0	0	Arankalle et al., 2001
Japan	*Rattus norvegicus*	362	114	31.5	Hirano et al., 2003
Japan	*Rattus rattus*	90	12	13.3	Hirano et al., 2003
Japan	*Mus caroli*[1]	41	0	0	Hirano et al., 2003
Japan	*Apodemus* sp.	12	0	0	Hirano et al., 2003
Japan	*Mus musculus*	2	0	0	Hirano et al., 2003
USA	*Rattus rattus*	113	102	90.3	Kabrane-Lazizi et al., 1999a
USA	*Rattus norvegicus*	108	83	76.9	Kabrane-Lazizi et al., 1999a
USA	*Rattus exulans*	18	15	83.3	Kabrane-Lazizi et al., 1999a
Total		954	351	36.8	

[1] Okinawa mice

Ukraine in non-endemic geographic areas (Favorov et al., 1998). Among calf serum samples from various regions in China, only 6.3% (12/160) were positive against HEV; specific antibodies were not detected in goats from the same areas (Wang et al., 2002). Vitral et al. (2005) detected antibodies against HEV in Brazil in one of 70 (1.4%) tested cows; IgG was not found in any of the 12 sheep and five goats tested.

3.2. Wild animals

3.2.1. Rodents

Serological surveys showed the presence of antibodies against HEV in several species of rodents (Table 2), especially rats (*Rattus norvegicus, R. rattus*) in Brazil (Vitral et al., 2005), Japan (Hirano et al., 2003) and the USA (Kabrane-Lazizi et al., 1999a; Favorov et al., 2000).

3.2.2. Artiodactyla

Among 117 serum samples from Sika deer (*Cervus nippon yesoensis, C. n. centralis* and *C. n. nippon*) and 35 serum samples from wild boars (*S. scrofa leucomystax)* tested in Japan, antibodies to HEV were detected in 2% and 9%, respectively (Sonoda et al., 2004). Takahashi et al. (2004), who examined wild boar (*S. scrofa leukomystax*) and Sika deer (*C. n. nippon*) in Japan, detected HEV RNA in both species.

3.2.3. Carnivores

There is an interesting report on the detection of antibodies against HEV in wild mongooses (*Herpestes javanicus*; Li et al., 2006b) and HEV infection in wild mongooses (*Herpestes* sp.) in Japan (Nakamura et al., 2006). Possible virological and epidemiological consequences are still unclear.

4. Characteristic features and prevalence of HEV in various animal species

Anti-HEV antibodies were detected in many animal species. However, the HEV genome has been detected only in humans, pigs, rodents, Sika deer and mongoose. Fully or partly sequenced genomes of these animal strains have been compared and assigned into four genotypes: I, II, III and IV (Schlauder and Mushahwar, 2001; Takahashi et al., 2004; Nakamura et al., 2006). Newly discovered avian HEV is genetically related to, but distinct from, other known HEV strains. Whether avian HEV represents a fifth of the HEV genotype or belongs to a separate genus remains to be determined (Huang et al., 2002a).

4.1. HEV genome

HEV has a single-stranded positive-sense RNA genome of 7.2 kb. It consists of a short 5´ nontranslated region (27 to 35 nucleotides in length) followed by three partially overlapping forward open reading frames (ORFs, from 5´end ORF1, ORF3, ORF2). The 3´ nontranslated region is 65 to 74 nucleotides in length, terminated with a poly(A) end with 150 to 200 nucleotides in length. The 5´ end of the RNA is modified by m^7G capping (Kabrane-Lazizi et al., 1999b). The HEV genome structure in all human and animal strains is comparable. The following data concerning numbers of nucleotides in respective ORFs HEV correspond to the Burma HEV strain: genotype I, GeneBank accession number M73218 (Tam et al., 1991, as quoted by Schlauder and Mushahwar, 2001).

4.1.1. Open reading frame 1 (ORF1)

ORF1 begins at the 5´end of the viral genome following the 27 to 35 nucleotide long non-coding region. This gene consists of 5 079 nucleotides and codes for a non-structural protein (polyprotein) with a length of 1 693 amino acids. ORF1 polyprotein participates in the replication of viral particles and the modification of structural protein(s) (Worm et al., 2002; Emerson and Purcell, 2003). Computer analysis of ORF1 according to Koonin et al. (1992) revealed a few putative amino acid domains (from N to C end):

(i) methyltransferase
(ii) domain of an unknown role, designated Y (*Rubella virus*-like)
(iii) papain-like protease (found in alphaviruses and *Rubella virus*)
(iv) "proline-rich hinge" domain
(v) X domain of an unknown role (*Rubella virus*-like)
(vi) NTP-binding sequence associated with helicase activity (that supports RNA unfolding essential for genome replication and transcription)
(vii) RNA-dependent RNA polymerase (identified in clone ET1.1)

It is not clear whether this gene-encoded polyprotein is split following translation, which would result in the formation of two or more independent enzymes, or whether it exists as an integrated unit. Due to the fact that the 5´ non-coding genome region is only 27 to 35 nucleotides long; translation is obviously initiated by capping. The cap on the 5´ end was detected by both direct (by binding of anti-cap antibodies) and indirect (pyrophosphatase-dependent elongation reactions) methods (Kabrane-Lazizi et al., 1999b; Zhang et al., 2001). Capping enzyme (that might be present at the sites of putative protease) activity was demonstratively confirmed by the expression of the first 979 amino acids in ORF1 (Magden et al., 2001). A hypervariable proline-rich hinge domain was found between the X domain and the domain of papain-like protease. A very low similarity of nucleotides and amino acids between geographically different strains exists (Worm et al., 2002).

4.1.2. Open reading frame 2 (ORF2)

The ORF2 segment of the HEV genome comprises of 1 980 nucleotides before a 65 nucleotide long polyadenylated terminal sequence at the 3´end of the ribonucleic chain. It is separated from ORF1 with nucleotides. This gene encodes the main structural (if not the only) virion capsid protein. It is a glycoprotein of 71–88 kDa (660 amino acids; saccharide component lies at the N end) with a potential signal region (it contains high arginin and lysine concentrations) for the endoplasmic reticulum. The protein is synthesised as a precursor, which is then, due to the signal sequence, changed into a mature protein and glycosylated at three possible sites. The presence of extra saccharide is more usual for surface proteins of enveloped vi-

ruses than for capsid proteins of non-enveloped viruses (Zafrullah et al., 1999).

HEV capsid protein was used to form virus-like particles (VLPs) by means of baculovirus expression of the ORF2 gene inside insect cells (Li et al., 1997, as quoted by Xing et al., 1999). These particles have been characterised by means of cryo-electron microscopy and by three-dimensional reconstruction of the structure. ORF2 protein (between 112 to 607 amino acids) formed 30 homodimers in a form of icosahedra; smaller in size in comparison with fresh virions (Xing et al., 1999). It should be emphasised that the size and modification of infectious viral particle proteins has not been determined yet. Phylogenetic affinity of HEV to the Rubella virus (genus *Alphavirus*), with characteristic glycoprotein protrusions on the envelope (Emerson and Purcell, 2003) is noteworthy.

4.1.3. Open reading frame 3 (ORF3)

The last and smallest open reading frame, ORF3, is found between ORF1 and ORF2. ORF3 overlaps the ORF1 segment by one nucleotide near its 5´end and at the 3´end overlaps ORF2 by 328 nucleotides (Wang et al., 2000). It should be noted that ORF3 does not overlap ORF1 in HEV-T1. The start codon is 28 nucleotides behind the ORF1 stop codon. The genotype IV forms a protein of 114 amino acids; the protein size in the other HEV types is 123 amino acids. The genotype group IV lacks the first 9 of 32 amino acids, which code for the hydrophobic domain associated with cytoskeleton binding. It is not known whether the ORF3 protein is a part of a virion, or whether it is a non-structural protein. The protein is non-glycosylated, but phosphorylated *ex vivo* in serin-80, which is a residuum, conserved in genotype groups I and III, but not in groups II and IV. Function of this protein remains obscure (Zafrullah et al., 1997; Emerson and Purcell, 2003).

4.2. Genotypes and prevalence of human HEV strains

The HEV genome has only been completely mapped in a few HEV strains, while most of them have been sequenced only partly. Based on all available data, phylogenetic analysis and comparison of sequences have been performed: these have revealed that there are four major genotypes of HEV: I, II, III and IV (Table 3). Homology of members of the same genotype is presumed not to be less than 81% (Schlauder and Mushahwar, 2001). The phylogenetic analysis divided HEV genotype I into five subtypes, genotype II into 2 subtypes, whereas genotypes III and IV were divided into 10 and 7 subtypes, respectively (Lu et al., 2006). A consensus for HEV classification does not yet exist.

4.3. Swine HEV and its prevalence

HEV from clinical samples of pigs kept in the USA was identified and described for the first time in 1997 (Meng et al., 1997). Subsequently, HEV strains have been detected in other countries with a high production of pork all over the world (Table 3).

A comparison of segments of sequenced genomes and subsequent classification of these strains in HEV genotypes showed their affiliation with genotype III (Meng et al., 1997; Garkavenko et al., 2001; Okamoto et al., 2001; Huang et al., 2002b; Yazaki et al., 2003; Banks et al., 2004a; Lu et al., 2004; Cooper et al., 2005) and genotype IV (Arankalle et al., 2002; Wang et al., 2002; Wu et al., 2002; Yazaki et al., 2003). Swine HEV strains are usually most homologous with human HEV from the same geographic areas (Hsieh et al., 1999; Pina et al., 2000; Wang et al., 2002; Huang et al., 2002b; Yazaki et al., 2003), with the exceptions of swine HEV from Mexico and Thailand where human HEV has been classified as genotype I and II, swine HEV as genotype III (Cooper et al., 2005). Human strains of HEV from Kyrgyzstan were classified as genotype I and swine strains as genotype III (Lu et al., 2004), whereas human HEV from India as genotype I and that of swine as genotype IV (Arankalle et al., 2002).

4.4. Avian HEV and "big liver and spleen disease virus" (BLSV)

Haqshenas et al. (2001) identified nucleic acid and viral particles of HEV in bile samples from chickens affected by HS in the USA. The primers originally intended for the detection of "big liver and spleen disease virus" (BLSV) were used for the detection of viral RNA. This virus mainly affects commer-

Table 3. Genotypic designation for isolates of human and swine hepatitis E virus strains according to the countries

Genotype	Country	Human isolates (references)	Swine isolates (references)
I	Algeria	van Cuyck-Gandre et al., 1997	nd
	Burma	Tam et al., 1991	nd
	Chad	van Cuyck-Gandre et al., 1997; Nicand et al., 2005	nd
	China	Yin et al., 1993	nd
	India	Arankalle et al., 1999	nd
	Kyrgyzstan	Lu et al., 2004	nd
	Morocco	Chatterjee et al., 1997	nd
	Nepal	Shrestha et al., 2003	nd
	Pakistan	Tsarev et al., 1992	nd
	Spain[1]	Buti et al., 2004	nd
	Sudan	Nicand et al., 2005	nd
	Thailand	Cooper et al., 2005	nd
	Tunisia	Chatterjee et al., 1997	nd
	Uzbekistan	Chatterjee et al., 1997	nd
II	Chad	Nicand et al., 2005	nd
	Mexico	Huang et al., 1992	nd
	Namibia	Maila et al., 2004	nd
	Nigeria	Buisson et al., 2000	nd
III[2]	Argentina	Schlauder et al., 2000	Munne et al., 2006
	Austria	Worm et al., 2000	nd
	France	Mansuy et al., 2004	nd
	Germany	Preiss et al., 2006	nd
	Greece	Schlauder et al., 1999	nd
	Great Britain	Banks et al., 2004b	Banks et al., 2004a
	Hungary	Reuter et al., 2006	nd
	Italy	Zanetti et al., 1999	nd
	Japan	Yazaki et al., 2003	Okamoto et al., 2001; Nishizawa et al., 2003; Yazaki et al., 2003
	Korea	Ahn et al., 2005	Choi et al., 2003
	Kyrgyzstan	Lu et al., 2004	Lu et al., 2004
	Mexico	nd	Cooper et al., 2005
	New Zealand	nd	Garkavenko et al., 2001
	Spain	Buti et al., 2004	Pina et al., 2000
	Thailand	nd	Cooper et al., 2005
	The Netherlands	Widdowson et al., 2003	van der Poel et al., 2001
	USA	Schlauder et al., 1998	Meng et al., 1997; Huang et al., 2002b
IV	China	Wang et al., 1999; Li et al., 2006a	Wang et al., 2002
	India	nd	Arankalle et al., 2002; Chobe et al., 2006
	Indonesia	nd	Wibawa et al., 2004
	Japan	Yazaki et al., 2003	Yazaki et al., 2003
	Taiwan	Hsieh et al., 1998	Wu et al., 2002

[1]a patient with a travel history to Ethiopia; [2]comprises hepatitis E virus strains originating from countries with occasional occurrence of hepatitis E; nd = never detected

cial breeds of hens in Australia. Comparison of sequenced genome segments of avian HEV and BLSV revealed a similarity of about 80% (Payne et al., 1999; Haqshenas et al., 2001).

Sequence analysis of the genome of avian HEV revealed 50% to 60% similarity in nucleotide sequence with human and swine HEV strains. Organisation of the genome of avian HEV is similar to the genome arrangement found in other HEV strains. Dissimilarity in the localisation of the ORF3 was also observed: ORF3 does not overlap with ORF1, similarly to HEV-T1. It is not clear whether avian HEV has a novel genotype (V) of HEV or whether it is another member of the *Hepevirus* genus (Wang et al., 2000; Haqshenas et al., 2001).

5. Replication and expression of HEV

The exact mode of replication and expression of HEV has not been recognised yet. The assumed course of events has been mostly based on analogy with other viruses (where the genome is formed by a positive-sense RNA) and on the knowledge of conservative segments of non-structural HEV domains (Worm et al., 2002).

5.1. Hepatocytes – primary host target cells for HEV

The primary target cells for HEV are hepatocytes. The positive-sense chain of viral RNA in their cytoplasm is translated into a non-structural protein necessary for viral replication. Negative-sense RNA serves as a template. It is not clear whether a non-structural polyprotein is split; if it was, the viral or host protease would be responsible for this process. The product of a non-structural gene comprises of RNA-dependent RNA polymerase, which evidently participates in the formation of both template sense and positive sense chains. This polymerase is only detectable at the beginning of replication (Panda et al., 2000). Translation is likely initiated by capping (Zhang et al., 2001). Structural protein ORF2 is posttranslationally glycosylated, which is usual for surface proteins of encapsulated viruses (Zafrullah et al., 1999). The ORF3 phosphorylated protein function, encapsulation and excretion of virions from hepatocytes remains obscure (Worm et al., 2002; Emerson and Purcell, 2003).

5.2. The other HEV infected host tissues

Using *in situ* hybridisation, HEV RNA was also detected on the luminal surface of epithelial cells in the biliary duct; which indicates a transient high HEV RNA concentration in bile. Other studies demonstrated HEV transfer from hepatocytes into epithelial cells and to biliary duct cells in an acute stage of the disease (Kawai et al., 1999; Williams et al., 2001). The template RNA was detected in the small intestine, spleen, lymph nodes, tonsils (Williams et al., 2001) and the HEV RNA was detected in bone marrow, salivary glands, mesenteric and inguinal lymphatic nodes, kidneys and urine (Banks et al., 2004a; de Deus et al., 2006). Possible replication of swine HEV outside the liver increased the fear of pathogen transmission with xenotransplantations (Williams et al., 2001).

6. HEV transmission

HEV is classified as one of the foodborne and waterborne viruses and it could be regarded as both an emerging anthroponosis and zoonosis. Developing countries of Asia, Africa, South and Central America (countries with endemic occurrence of HE) are considered as risk areas (Hubalek, 2003; Ashbolt, 2004; Koopmans and Duizer, 2004; Vasickova et al., 2005). Sporadic occurrence of HE has been described in industrialised countries where affected people have been associated with a travel history to countries with an increased risk of infection (Skidmore et al., 1991; Dawson et al., 1992). Later studies reported HEV infections in people without a travel history to these countries (Schlauder et al., 1998; Smith, 2001). Further studies of HEV suggested other routes of transmission and the zoonotic potential of HEV has been discussed.

6.1. The most common HEV transmission route

HEV is usually transmitted via the faecal-oral route. Insufficient drinking water treatment and low standards of sanitation have been implicated in major outbreaks in developing countries. Contamination of drinking water with animal or human faeces is common (Balayan et al., 1983; Balayan, 1997; Tsega et al., 1991; Ashbolt, 2004).

Close genetic similarity of human and swine HEV viruses indicates that semi-liquid manure from pigs may also be a source of water contamination (Smith, 2001).

The first HE outbreak was recorded in Delhi, India, after the faecal contamination of a drinking water pump chamber. The outbreak, during which 29,300 people fell ill, arose in December, 1955: it culminated in two weeks and subsided within two months (Vishwanathan, 1957, as quoted by Worm et al., 2002).

6.2. Person to person contact and transplacental transmission

Person-to-person transmission of HE between family members has been documented in only 1% to 2% of cases, in contrast to 15% person-to-person transmission of hepatitis A (Khuroo, 1980). Transplacental transmission of HEV in the third trimester of pregnancy has been described; it is associated with a high perinatal mortality of the affected newborns (Khuroo et al., 1995; Bednar et al., 1999). Preterm delivery was recorded in two thirds of women infected with HE viremia included in the study. This group was reported to have a fatality rate approaching 26.9%; vertical transmission of HEV was described in 33.3% of cases (Kumar et al., 2004).

6.3. HEV transmission via foodstuffs

6.3.1. Pork and pig offal

Yazaki et al. (2003) and Tamada et al. (2004) referred to an infection caused by the consumption of undercooked liver or meat from domestic pigs and wild boars in Japan. In nine out of ten cases, patients had consumed grilled or raw pig liver, two months to 19 days prior to the onset of the first symptoms. Subsequently, packages with raw liver from grocery stores in the area where the infected people lived (Hokkaido) were tested. Of a total of 363 samples HEV RNA (ORF2) was detected in seven packages (1.9%). After ORF2 gene sequencing, the strains were classified as genotype groups III and IV. By comparison with human HEV genotypes detected in Japanese patients, the sample (swJL145) from genotype group IV showed 97.7 to 100% similarity of nucleotide sequences with HEV from ten HE patients. Two isolates (swJV234 and swJV235) belonging to genotype group III had 96.6 to 100% homology with HEV from five people. Finally, 3 people from the investigated group admitted to the consumption of undercooked pig intestine.

Due to the fact that viral particles are secreted in the intestine together with bile and that replication of both swine and human HEV in the intestine has been demonstrated (Williams et al., 2001, as quoted by Yazaki et al., 2003), this foodstuff might also be a potential source of infection (Yazaki et al., 2003).

A case of HE was recorded in the UK, where the causative agent had 98% homology with a local swine HEV strain. The patient admitted to the consumption of raw pork in the past. However, it was not demonstrated whether this foodstuff was the source of the viral infection (Banks et al., 2004a). In Bali, IgG class antibodies to HEV were detected in 20% (54/276) of the tested population, in remarkable contrast with 4% (17/446) in Lombok and 0.5% (2/393) in Surabaya (Indonesia). Although the majority of the population in Indonesia is Muslim, Balinese people are Hindu and can consume pork. Therefore, serum samples were obtained from the 99 farm pigs in Bali and tested for anti-HEV antibodies. The sera from 71 pigs (72%) were positive for anti-HEV antibodies and a 2-month-old pigs had detectable HEV RNA (Wibawa et al., 2004). This accumulating evidence suggests that eating undercooked liver or meat from domestic pigs and wild boars is associated with a high risk of acquiring HEV infection.

6.3.2. Undercooked foodstuffs made from venison

Foodstuffs made from raw or undercooked venison are viewed as a risk factor for HEV transmission. For a group of 45 people with a history of raw venison consumption, anti-HEV IgG antibodies were found in 8 (17.7%). In contrast, in a control group of 45 people (from the same area who did not consume raw venison), specific antibodies were detected in only one (2.2%) person. These results indicate that this route of transmission of the infection should not be ignored (Tei et al., 2004). Takahashi et al. (2004) examined wild boars living in the forests of Hyogo prefecture, Japan, and found HEV RNA in three of seven boars. A full-genome HEV isolate from them was revealed to be 99.7%

identical to a previous isolate from wild Sika deer (*C. n. nippon*) hunted in the same forest and to those isolates from four patients who contracted HE after eating raw meat form the deer. These findings suggest an interspecies HEV transmission between boar and deer in the wild and that both animal species might serve as a source of infection for humans (Takahashi et al., 2004).

6.3.3. Shellfish

The primary cause of HE outbreaks in developing countries is poor sanitation. Particularly during heavy rainfall, contamination of both drinking water supplies and coastal waters with human and animal faeces occurs (Balayan et al., 1983, 1997; Tsega et al., 1991). The propensity for waterborne transmission suggests that shellfish could also become contaminated and thus acts as a vector for transmission of HEV. Although direct evidence is not currently available, the association of shellfish with transmission of HEV is highly suggestive (Lees, 2000). Cacopardo et al. (1997) consider a stay in a tropical zone and consumption of raw or undercooked shellfish as risk factor for HEV transmission.

6.4. Zoonotic potential of HEV

The hypothesis of the zoonotic potential of HEV came first from a study by Balayan et al. (1990) who reported an experimental infection of domestic swine with human strains of HEV. Meng et al. (1998a) documented that swine HEV has the ability to cross the species barrier and to infect rhesus monkeys, chimpanzees and *vice versa*. Human HEV (US-2) has also been shown to infect SPF pigs. Viraemia and HEV antibodies immediately appeared in the infected pigs, showing that human HEV replication occurred in the pig organism. Identification of nucleotide sequences of HEV from pigs and rodents and their close relationship to human HEV from the same geographical regions (Meng et al., 1997; Tsarev et al., 1998; Yazaki et al., 2003) supports the hypothesis that a HEV reservoir may exist in animals.

The number of studies indicating that HE can be a zoonotic disease is now overwhelming (Favorov et al., 1998; van der Poel et al., 2001; Wang et al., 2002; Withers et al., 2002; Meng, 2003; Nishizawa et al., 2003; Tei et al., 2003, 2004; Banks et al., 2004b; Takahashi et al., 2004; Tamada et al., 2004; Li et al., 2005; Masuda et al., 2005; Mizuo et al., 2005; Lu et al., 2006).

7. Risk groups in the population and HEV prevention

The WHO has specified groups of people exposed to HEV infection risk (http://www.who.int/disease/hepatitis/HepatitisE_whocdscsredc2001_12.pdf):

(i) people residing in areas where community outbreaks occur
(ii) people travelling to areas with an endemic occurrence of HE
(iii) people in overcrowded refugee camps, such as in Sudan, Somalia, Kenya and Ethiopia
(iv) people with chronic liver disease
(v) people handling non-human primates, pigs, cows, sheep and goats

Due to the fact that almost all HEV infections are spread by the faecal-oral route, good personal hygiene, high quality of standards for public water supplies and proper disposal of sanitary waste in developing countries have been recommended.

For travellers to highly endemic areas, the usual elementary local food hygiene precautions are recommended. These include avoiding drinking water and/or ice of unknown purity as well as eating uncooked shellfish and uncooked fruits or vegetables that are not peeled or prepared by the travellers themselves.

8. Diagnostic methods

8.1. Molecular detection of HEV

Nucleic acid-based techniques, especially nested RT-PCR and real-time RT-PCR, have emerged rapidly as the method of first choice for sensitive and specific detection of RNA viruses. This method is very useful in research for the characterisation of divergent HEV strains whose serological responses have not been detected by some assays, especially in countries where infection is not endemic (Hsieh et al., 1998; Schlauder et al., 1999; Wang et al., 1999; Worm et al., 2000, 2002).

RT-PCR is a conventional method to detect viral RNA, not only in sera and faeces during the acute stage of the disease in humans (Erker et al., 1999; Inoue et al., 2006), clinical samples of domestic pig (Table 4) and other animal species, such as,

Table 4. Detection of hepatitis E virus RNA in domestic pigs using RT-PCR

Clinical sample	References
Bone marrow	Banks et al., 2004a
Plasma	Banks et al., 2004a
Serum	Meng et al., 1997; Okamoto et al., 2001; Huang et al., 2002b; Wu et al., 2002; Banks et al., 2004a; Cooper et al., 2005; de Deus et al., 2006
Salivary glands	Banks et al., 2004a
Mesenteric lymph nodes	Banks et al., 2004a; de Deus et al., 2006
Inguinal lymph nodes	Banks et al., 2004a
Kidneys	Banks et al., 2004a
Urine	Banks et al., 2004a
Faeces	Garkavenko et al., 2001; van der Poel et al., 2001; Huang et al., 2002b; Wu et al., 2002; Banks et al., 2004a; Lu et al., 2004; Cooper et al., 2005; de Deus et al., 2006
Liver	Yazaki et al., 2003; de Deus et al., 2006
Bile	de Deus et al., 2006

wild boar (*S. scrofa leucomystax*; Yazaki et al., 2003; Tamada et al., 2004), Sika deer (*C. n. nippon*; Tei at al., 2003; Takahashi et al., 2004; Li et al., 2005), mongoose (*Herpestes* sp.; Nakamura et al., 2006) and chickens (Wang et al., 2000; Haqshenas et al., 2001), but also in contaminated water (Pina et al., 2000; Grimm and Fout, 2002).

This molecular method consists of two or three steps (nested RT-PCR). The first step of RT-PCR, reverse transcription, uses specific primers, random hexamers or Oligo dT to rewrite viral RNA into cDNA. In the second or third step, PCR or nested PCR uses specific primers to amplify specific segments of viral RNA. Primer binding sites can be spread over the whole genome. However, nested RT-PCR is prone to contamination and virus quantification cannot be undertaken. To overcome these difficulties, rapid and sensitive real-time RT-PCR assays have been developed for the detection of HEV RNA in clinical samples (Ahn et al., 2006; Enouf et al., 2006; Jothikumar et al., 2006).

It should be noted that the choosing a suitable method for RNA extraction to ensure an adequate recovery of intact viral RNA and the elimination of inhibitory substances, is very important for the successful detection of viral genomes.

8.2. Immunologic diagnosis

8.2.1. Enzyme immunoassay (EIA)

EIA is a practical, highly sensitive and inexpensive diagnostic method for detection of anti-HEV antibodies. Antigenic domains have been found in all ORFs proteins of HEV (Khudyakov et al., 1999):

(i) 12 antigenic domains in ORF1 (particularly in the domain of the putative RNA-dependent RNA polymerase)
(ii) six antigenic domains in the ORF2 protein
(iii) three antigenic domains within the ORF3 protein

Recombinant proteins, originating from the ORF2 and ORF3 C-end domain or from a larger ORF2 segment and complete ORF3, are used for the detection of IgG and IgM anti-HEV. A wider range of antigens expressed from a larger part of ORF2 or "capsid-like" particles are more effective in the detection of antibodies in the convalescent stage of the disease rather than rare antigens from the ORF2 and 3 C-ends or the whole of ORF3 (Ghabrah et al., 1998). Synthetic peptides may also be used as antigens; however, antibodies cannot be reliably detected in the convalescence stage due to a low sensitivity. These peptide antigens are usually used for the confirmation of the EIA result with recombinant proteins and for the exclusion of non-specific reactions. Their use might increase the reaction specificity and determine the genotype group of HEV in the acute stage of hepatitis (Worm et al., 2002).

It should be noted that pair-wise comparison of 12 different EIA tests showed a concordance in blood donor sera ranging from 41% to 94% (mean, 68%), also a concordance among reactive sera from 0% to 89%: mean, 32% (Mast et al., 1998).

At present, three commercial EIA tests for the detection of anti-HEV antibodies are available. The

first two are Genelabs®-EIA; using four short recombinant proteins derived from the 3´end of ORF2 (42 amino acids) and ORF3 (33 amino acids) of the Burmese (genotype I) and Mexican (genotype II) strains for the detection of IgG or IgM antibodies.

The third commercially available EIA test (Abbott®-EIA) is based on the detection of anti-HEV IgG antibodies using two recombinant proteins obtained from the complete ORF3 (123 amino acids) of the Burmese (genotype I) strain. All tests may be used for routine diagnosis or epidemiological studies; however, because sensitivity and specificity have not been precisely established, their usefulness for seroepidemiological studies is limited (Worm et al., 2000, 2002). Evaluation of the sensitivity, specificity, predictive positive and predictive negative values of the serology is difficult as it is not clear what the true positive is. Waar et al. (2005) defined the sera that were positive in the HEV PCR as true positive and ruled out the serum that was not available for PCR. The values for a combination of all three commercially available EIA's were: sensitivity, 100%; specificity, 99.5%; positive predictive value, 75%; negative predictive value, 100%.

8.2.2. Immune fluorescence microscopy (IFE)

A few specialised laboratories use this technique for the detection of antibodies. IFE detects antibodies that react against the HEV antigen semiquantitatively. Anti-HEV antibodies block the binding of fluorescein-conjugated anti-HEV IgG to HEV antigen in frozen liver tissue. The concentration of anti-HEV antibodies is estimated semiquantitatively (Krawczynski and Bradley, 1989). This method is laborious and expensive and thus not useful for routine diagnosis.

8.3. Virus isolation

Establishment of a practical cell culture system to allow the propagation of HEV *in vitro* is vital for virological characterisation as well as for diagnosis and prevention of HEV infection. Several *in vitro* culture systems, such as human lung, kidney or liver (2BS, A549, Hep-G2) and macaques hepatocytes for HEV replication have been reported. Most of these, however, cannot provide authentic HEV particles or a high titre of VLPs and have poor reproducibility (Tam et al., 1997; Wei et al., 2000; Worm et al., 2002). Currently there is no reliable cell culture system for HEV.

8.4. Immune electron microscopy (IEM)

IEM detects VLPs in clinical specimens (Balayan et al., 1983). HEV particles are precipitated with the native antibody to HEV derived from acute- or convalescent-phase sera. Anti-HEV antibodies concentrations can be determined semiquantitatively by rating the antibody coating. Although IEM is a superior technique for specificity, the sensitivity of the assay is insufficient for routine analysis. IEM is difficult to perform and most clinical specimens do not contain sufficient VLPs to be detected (Yarbough, 1999). Other antigen detection methods have not been reported (Anderson and Shrestha, 2002).

9. Conclusions

HEV was first observed and described in 1983 (Balayan et al., 1983). The virus has been extensively investigated since that time. After a long-term effort to assign this virus to a taxonomic class, the virus has been classified in the *Hepevirus* genus, Hepeviridae family in 2004 (Emerson et al., 2004; http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs_hepev.htm). Most of the information concerning HEV is based on molecular biology.

More thorough studies may be expected in this field, which would better clarify the virus-encoded protein types, their structures, functions and consequential biological characteristics presented inside the host organism and its cells.

The ecology of the virus has been insufficiently studied so far. The point which should be addressed is the presence and circulation of HEV in populations of wild mammals, largely artiodactyls (wild boar, Sika deer), rodents (*Rattus* spp.) and carnivores. There is also a very urgent need for epizootiological studies contributing to a better understanding of the relationship between rats and domestic pigs in keeping the HEV circulation in the rural foci.

The studies performed so far on HEV transmission show that classification of this agent among waterborne and foodborne viruses was justified. However, more information in this field of re-

search is desirable. Above all direct confirmation of HEV transmission via foodstuffs is still needed. Viral RNA has been detected in the above mentioned foodstuffs; however, it does not mean that the virus present is infectious. HE patients with a history of undercooked meat consumption have been noted. During subsequent inspection of sources of these foodstuffs, HEV RNA was detected. Unfortunately, it could not be confirmed that the foodstuffs consumed by these people contained the infectious agent although there are significant epidemiological links. The fact that viral RNA detected in some patients showed a relatively high homology with HEV RNA found in meat intended for consumption supported the hypothesis that undercooked meat was the source of infection.

Anti-HEV antibodies (or viral RNA) have not only been found in people, but also detected in various animal species (Arankalle et al., 1994, 2001; Favorov et al., 1998). HEV capability to cross the species barrier was confirmed experimentally (Meng et al., 1998a). Knowledge of inter-species transmission supports the hypothesis about the zoonotic potential of HEV. However, further research of HEV incidence into other animal species and the possibility of species barrier crossing should be performed. More detailed information obtained by this investigation might extend the knowledge of potential risk factors of the HEV transmission and facilitate the adoption of preventive measures.

In general, the research concerning HEV is far from finished and various aspects of this disease remain obscure. Accordingly, it is necessary to obtain more information in this field.

10. Acknowledgments

Special thanks are expressed to Dr. Nigel Cook (Central Science Laboratory, Sand Hutton, York, UK) for critical reading of the manuscript. The authors are indebted to Anna Maslanova and Zdenka Gregorova (Veterinary Research Institute, Brno) for the help with cited references, Ing. Ludmila Faldikova, CSc. (Veterinary Research Institute, Brno) for English translation, and Mgr. Maria Vass (Swinburne University of Technology, Victoria, Australia) and Catherine Murdoch (Aberdeen University in Scotland, UK) for grammatical corrections.

11. REFERENCES

Acha P.N., Szyfres B. (2003): Zoonoses and Communicable Diseases Common to Man and Animals. 3rd ed. Pan American Health Organization, Washington.

Aggarwal R., Kini D., Sofat S., Naik S.R., Krawczynski K. (2000): Duration of viremia and faecal viral excretion in acute hepatitis. Lancet, 356, 1081–1082.

Ahn J.M., Kang S.G., Lee D.Y., Shin S.J., Yoo H.S. (2005): Identification of novel human hepatitis E virus (HEV) isolates and determination of the seroprevalence of HEV in Korea. Journal of Clinical Microbiology, 43, 3042–3048.

Ahn J.M., Rayamajhi N., Kang S.G., Yoo H.S. (2006): Comparison of real-time reverse transcriptase-polymerase chain reaction and nested or commercial reverse transcriptase-polymerase chain reaction for the detection of hepatitis E virus particle in human serum. Diagnostic Microbiology and Infectious Disease, 56, 269–274.

Anderson D.A., Shrestha I.L. (2002): Clinical Virology, Hepatitis E virus. 2nd ed. ASM Press, American Society for Microbiology, Washington, DC, USA.

Arankalle V.A., Goverdhan M.K., Banerjee K. (1994): Antibodies against hepatitis E virus in Old World monkeys. Journal of Viral Hepatitis, 1, 125–129.

Arankalle V.A., Paranjape S., Emerson S.U., Purcell R.H., Walimbe A.M. (1999): Phylogenetic analysis of hepatitis E virus isolates from India (1976–1993). Journal of General Virology, 80, 1691–1700.

Arankalle V.A., Joshi M.V., Kulkarni A.M., Gandhe S.S., Chobe L.P., Rautmare S.S., Mishra A.C., Padbidri V.S. (2001): Prevalence of anti-HEV antibodies in different Indian animal species. Journal of Viral Hepatitis, 8, 223–227.

Arankalle V.A., Chobe L.P., Joshi M.V., Chadha M.S., Kundu B., Walimbe A.M. (2002): Human and swine hepatitis E viruses from Western India belong to different genotypes. Journal of Hepatology, 36, 417–425.

Ashbolt N.J. (2004): Microbial contamination of drinking water and disease outcomes in developing regions. Toxicology, 198, 229–238.

Balayan M.S. (1997): Epidemiology of hepatitis E virus infection. Journal of Viral Hepatitis, 4, 155–165.

Balayan M.S., Andzhaparidze A.G., Savinskaya S.S., Ketiladze E.S., Braginsky D.M., Savinov A.P., Poleschuk V.F. (1983): Evidence for virus in non-A/non-B hepatitis transmitted via the faecal-oral route. Intervirology, 20, 23–31.

Balayan M.S., Usmanov R., Zamyatina N. (1990): Brief report: experimental hepatitis E infection in domestic pigs. Journal of Medical Virology, 32, 58–59.

Banks M., Heath G.S., Grierson S.S., King D.P., Gresham A., Girones R., Widen F., Harrison T.J. (2004a): Evidence for the presence of hepatitis E virus in pigs in the United Kingdom. Veterinary Record, 154, 223–227.

Banks M., Bendall R., Grierson S., Heath G., Mitchell J., Dalton H. (2004b): Human and porcine hepatitis E virus strains, United Kingdom. Emerging Infectious Diseases, 10, 953–955.

Bednar M., Frankova V., Schindler J., Soucek A., Vavra J. (1999): Medical Microbiology (in Czech). 1st ed. (Reissue), Nakladatelstvi Marvil, Prague. 558 pp.

Berke T., Matson D.O. (2000): Reclassification of the Caliciviridae into distinct genera and exclusion of hepatitis E virus from the family on the basis of comparative phylogenetic analysis. Archives of Virology, 150, 1421–1436.

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. Journal of Virology, 79, 3429–3437.

Buisson Y., Grandadam M., Nicand E., Cheval P., van Cuyck-Gandre H., Innis B., Rehel P., Coursaget P., Teyssou R., Tsarev S. (2000): Identification of novel hepatitis E virus in Nigeria. Journal of General Virology, 81, 903–909.

Buti M., Clemente-Casares P., Jardi R., Formiga-Cruz M., Schaper M., Valdes A., Rodriguez-Frias F., Esteban R., Girones R. (2004): Sporadic cases of acute autochthonous hepatitis E in Spain. Journal of Hepatology, 41, 126–131.

Cacopardo B., Russo R., Preiser W., Benanti F., Brancati G., Nunnari A. (1997): Acute hepatitis E in Catania (eastern Sicily) 1980–1994. The role of hepatitis E virus Infection, 25, 313–316.

Chatterjee R., Tsarev S., Pillot J., Coursaget P., Emerson S.U., Purcell R.H. (1997): African strains of hepatitis E virus that are distinct from Asian strains. Journal of Medical Virology, 53, 139–144.

Chauhan A., Jameel S., Dilawari J.B., Chawla Y.K., Kaur U., Ganguly N.K. (1993): Hepatitis E virus transmission to a volunteer. Lancet, 341, 149–150.

Chobe L.P., Lole K.S., Arankalle V.A. (2006): Full genome sequence and analysis of Indian swine hepatitis E virus isolates of genotype 4. Veterinary Microbiology, 114, 240–251.

Choi I.S., Kwon H.J., Shin N.R., Yoo H.S. (2003): Identification of swine hepatitis E virus (HEV) and prevalence of anti-HEV antibodies in swine and human populations in Korea. Journal of Clinical Microbiology, 41, 3602–3608.

Clayson E.T., Myint K.S., Snitbhan R., Vaughn D.W., Innis B.L., Chan L., Cheung P., Shrestha M.P. (1995): Viremia, faecal shedding, and IgM and IgG responses in patients with hepatitis E. Journal of Infectious Diseases, 172, 927–933.

Cooper K., Huang F.F., Batista L., Rayo C.D., Bezanilla J.C., Toth T.E., Meng X.J. (2005): Identification of genotype 3 hepatitis E virus (HEV) in serum and faecal samples from pigs in Thailand and Mexico, where genotype 1 and 2 HEV strains are prevalent in the respective human populations. Journal of Clinical Microbiology, 43, 1684–1688.

Dawson G.J., Mushahwar I.K., Chau K.H. (1992): Detection of long-lasting antibody to hepatitis E virus in U. S. traveller to Pakistan. Lancet, 340, 426–427.

de Deus N., Seminati C., Pina S., Mateu E., Martin M., Segales J. (2006): Detection of hepatitis E virus in liver, mesenteric lymph node, serum, bile and faeces of naturally infected pigs affected by different pathological conditions. Veterinary Microbiology, 119, 105–114.

Emerson S.U., Purcell R.H. (2003): Hepatitis E virus. Reviews in Medical Virology, 13, 145–154.

Emerson S.U., Anderson D., Arankalle A.V., Meng X.J., Purdy M., Schlauder G.G., Tsarev S.A. (2004): Hepevirus. In: Fauquet C.M., Mayo M.A., Maniloff J., Desselberger U., Ball L.A. (eds.): Virus Taxonomy. The Eighth Report of the International Committee on Taxonomy of Viruses. Elsevier/Academic Press, London. 851–855.

Enouf V., Dos Reis G., Guthmann J.P., Guerin P.J., Caron M., Marechal V., Nicand E. (2006): Validation of single real-time TaqMan® PCR assay for detection and quantisation of four major genotypes of hepatitis E virus in clinical specimens. Journal of Medical Virology, 78, 1076–1082.

Erker J.C., Desai S.M., Mushahwar I.K. (1999): Rapid detection of hepatitis E virus RNA by reverse transcription-polymerase chain reaction using universal oligonucleotide primers. Journal of Virological Methods, 81, 109–113.

Favorov M.O., Nazarova O., Margolis H.S. (1998): Is hepatitis E an emerging zoonotic disease? American Journal of Tropical Medicine and Hygiene, 59, 242.

Favorov M.O., Kosoy M.Y., Tsarev S.A., Childs J.E., Margolis H.S. (2000): Prevalence of antibody to hepatitis E virus among rodents in the United States. Journal of Viral Hepatitis, 8, 223–227.

Garkavenko O., Obriadina A., Meng J., Anderson D.A., Bernard H.J., Schroeder B.A., Khudyakov Y.E., Fields H.A., Croxson M.C. (2001): Detection and characterisation of swine hepatitis E virus in New Zealand. Journal of Medical Virology, 65, 525–529.

Ghabrah T.M., Tsarev S.A., Yarbough P.O., Emerson S.U., Strickland G.T., Purcell R.H. (1998): Comparison of tests for antibody to hepatitis E virus. Journal of Medical Virology, 55, 134–137.

Grimm A.C., Fout G.S. (2002): Development of molecular method to identify hepatitis E virus in water. Journal of Virological Methods, 101, 175–188.

Halbur P.G., Kasorndorkbua 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. Journal of Clinical Microbiology, 39, 918–923.

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. Journal of General Virology, 82, 2449–2462.

Hirano M., Ding X., Li T.C., Takeda N., Kawabata H., Koizumi N., Kadosaka T., Goto I., Masuzawa T., Nakamura M., Taira K., Kuroki T., Takinawa T., Watanabe H., Abe K. (2003): Evidence for widespread infection of hepatitis E virus among wild rats in Japan. Hepatology Research, 27, 1–5.

Hsieh S.Y., Yang P.Y., Ho Y.P., Chu C.M., Liaw Y.F. (1998): Identification of a novel strain of hepatitis E virus responsible for sporadic acute hepatitis in Taiwan. Journal of Medical Virology, 55, 300–304.

Hsieh S.Y., Meng X.J., Wu Y.H., Liu S.T., Tam A.W., Lin D.Y., Liaw Y.F. (1999): Identity of novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. Journal of Clinical Microbiology, 37, 3828–3834.

Huang C.C., Nguyen D., Fernandez J., Yun K.Y., Fry K. E., Bradley D.W., Tam A.W., Reyes G.R. (1992): Molecular cloning and sequencing of the Mexico isolate of hepatitis E virus (HEV). Virology, 191, 550–558.

Huang F.F., Haqshenas G., Shivaprasad H.L., Guenette D.K., Woolcock P.R., Larsen C.T., Pierson F.W., Elvinger F., Toth T.E., Meng X.J. (2002a): Heterogeneity and seroprevalence of a newly identified avian hepatitis E virus from chicken in the United States. Journal of Clinical Microbiology, 40, 4197–4202.

Huang F.F., Haqshenas G., Guenette D.K., Halbur P.G., Schommer S.K., Pierson F.W., Toth T.E., Meng X.J. (2002b): Detection by reverse transcription-PCR and genetic characterization of field isolates of swine hepatitis E virus from pigs in different geographic regions of the United States. Journal of Clinical Microbiology, 40, 1326–1332.

Hubalek Z. (2003): Emerging human infectious diseases: anthroponoses, zoonoses, and sapronoses. Emerging Infectious Diseases, 9, 403–404. http://www.cdc.gov/ncidod/eid/vol9no3/02-0208.htm

Hussaini S.H., Skidmore S.J., Richardson P., Sherratt L. M., Cooper B.T., O'Grady J.G. (1997): Severe hepatitis E infection during pregnancy. Journal of Viral Hepatitis, 4, 51–54.

Inoue J., Takahashi M., Yazaki Y., Tsuda F., Okamoto H. (2006): Development and validation of an improved RT-PCR assay with nested universal primers for detection of hepatitis E virus strains with significant sequence divergence. Journal of Virological Methods, 137, 325–333.

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. Journal of Virological Method, 131, 65–71.

Kabrane-Lazizi Y., Fine J.B., Elm J., Glass G.E., Higa H., Diwan A., Gibbs C.J., Meng X.J., Emerson S.U., Purcell R.H. (1999a): Evidence for widespread infection of wild rats with hepatitis E virus in the United States. American Journal of Tropical Medicine and Hygiene, 61, 331–335.

Kabrane-Lazizi Y., Meng X.J., Purcell R.H., Emerson S.U. (1999b): Evidence that the genomic RNA of hepatitis E virus is capped. Journal of Virology, 73, 8848–8850.

Kasorndorkbua C., Halbur P.G., Thomas P.J., Guenette D.K., Toth T.E., Meng X.J. (2002): Use of a swine bioassay and a RT-PCR assay to assess the risk of transmission of swine hepatitis E virus in pigs. Journal of Virological Methods, 101, 71–78.

Kawai H.F., Koji T., Iida F., Kaneko S., Kobayashi K., Nakane P.K. (1999): Shift of hepatitis E virus RNA from hepatocytes to biliary epithelial cells during acute infection of rhesus monkey. Journal of Viral Hepatitis, 6, 287–297.

Khudyakov Y.E., Lopareva E.N., Jue D.L., Crews T.K., Thyagarajan S.P., Fields H.A. (1999): Antigenic domains of the open reading frame 2-encoded protein of hepatitis E virus. Journal of Clinical Microbiology, 37, 2863–2871.

Khuroo M.S. (1980): Study of an epidemic of non-A, non-B hepatitis: possibility of another human hepatitis virus distinct from posttransfusion non-A, non-B type. American Journal of Medicine, 68, 818–824.

Khuroo M.S., Kamili S., Jameel S. (1995): Vertical transmission of hepatitis E virus. Lancet, 345, 1025–1026.

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 virus. Proceedings of the National Academy of Sciences, USA, 89, 8259–8263.

Koopmans M., Duizer E. (2004): Foodborne viruses: an emerging problem. International Journal of Food Microbiology, 90, 23–41.

Krawczynski K., Bradley D.W. (1989): Enterically-transmitted non-A, non-B hepatitis: identification of virus-associated antigen in experimentally infected cynomolgus macaques. Journal of Infectious Diseases, 159, 1042–1049.

Kumar A., Beniwal M., Kar P., Sharma J.B., Murthy N.S (2004): Hepatitis E in pregnancy. International Journal of Gynecology and Obstetrics, 85, 240–241.

Lees D. (2000): Viruses and bivalve shellfish. International Journal of Food Microbiology, 59, 81–116.

Li F., Zhuang H., Kolivas S., Locarnini S., Anderson D.A. (1994): Persistent and transient antibody responses to hepatitis E virus detected by Western immunoblot using open reading frame 2 and 3 and glutathione S-transferase fusion protein. Journal of Clinical Microbiology, 32, 2060–2066.

Li T.C., Yakamawa Y., Suzuki K., Tatsumi M., Razak M.A., Uchida T., Takeda N., Miyamura T. (1997): Expression and self-assembly of empty virus-like particles of hepatitis E virus. Journal of Virology, 71, 7207–7213.

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. Emerging Infectious Diseases, 11, 1958–1960.

Li R.C., Ge S.X., Li Y.P., Zheng Y.J., Nong Y., Guo Q.S., Zhang J., Ng M.H., Xia N.S. (2006a): Seroprevalence of hepatitis E virus infection, rural southern People's Republic of China. Emerging Infectious Diseases, 12, 1682–1688.

Li T.C., Saito M., Ogura G., Ishibashi I., Miyamura T., Takeda N. (2006b): Serologic evidence for hepatitis E virus infection in mongoose. American Journal of Tropical Medicine and Hygiene, 74, 932–936.

Lu L., Drobeniuc J., Kobylnikov N., Usmanov R.K., Robertson B.H., Favorov M.O., Margolis H.S. (2004): Complete sequence of Kyrgyzstan swine hepatitis E virus (HEV) isolated from piglet thought to be experimentally infected with human HEV. Journal of Medical Virology, 74, 556–562.

Lu L., Li C., Hagedorn C.H. (2006): Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. Review of Medical Virology, 16, 5–36.

Magden J., Takeda N., Li T., Auvinen P., Ahola T., Miyamura T., Merits A., Kaariainen L. (2001): Virus-specific mRNA capping enzyme encoded by hepatitis E virus. Journal of Virology, 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. Journal of General Virology, 85, 89–95.

Mansuy J.M., Peron J.M., Abravanel F., Poirson H., Dubois M., Miedouge M., Vischi F., Alric L., Vinel J.P., Izopet J. (2004): Hepatitis E in the south west of France in individuals who have never visited an endemic area. Journal of Medical Virology, 74, 419–424.

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, 27, 857–861.

Masuda J., Yano K., Tamada Y., Takii Y., Ito M., Omagari K., Kohno S. (2005). Acute hepatitis E of a man who consumed wild boar meat prior to the onset of illness in Nagasaki, Japan – Case report. Hepatology Research, 31, 178–183.

Meng X.J. (2003): Swine hepatitis E virus: Cross-species infection and risk in xenotransplantation. Current Topics in Microbiology and Immunology, 278, 185–216.

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. Proceedings of the National Academy of Sciences, USA, 94, 9860–9865.

Meng X.J., Halbur P.G., Sharipo M.S., Govindarajan S., Bruna J.D., Mushahwar I.K., Purcell R.H., Emerson S.U. (1998a): Genetic and experimental evidence for cross-species infection by the swine hepatitis E virus. Journal of Virology, 72, 9714–9721.

Meng X.J., Halbur P.G., Tsareva T.S., Bruna J.D., Royer R.L., Purcell R.H., Emerson S.U. (1998b): Experimental infection of pigs with the newly identified swine hepatitis E virus (swine HEV), but not with human strain of HEV. Archives of Virology, 143, 1405–1415.

Meng X.J., Dea S., Engle R.E., Friendship R., Lyoo Y.S., Sirinarumitr T., Urairong K., Wang D., Wong D., Yoo D., Zhang Y., Purcell R.H., Emerson S.U. (1999): Prevalence of antibodies to the hepatitis E virus in pig from countries where hepatitis E is common or is rare in the human population. Journal of Medical Virology, 59, 297–302.

Mizuo H., Yazaki Y., Sugawara K., Tsuda F., Takahashi M., Nishizawa T., Okamoto H. (2005): Possible risk factors for the transmission of hepatitis E virus and for the severe form of hepatitis E acquired locally in Hokkaido, Japan. Journal of Medical Virology, 76, 341–349.

Munne M.S., Vladimirsky S., Otegui L., Castro R., Brajterman L., Soto S., Guarnera E., Molina V., Monfellano M., Schlauder G.G., Gonzalez J.E. (2006): Identification of the first strain of swine hepatitis E virus in South America and prevalence of anti-HEV antibodies in swine in Argentina. Journal of Medical Virology, 78, 1579–1583.

Nakamura M., Takahashi K., Taira K., Taira M., Ohno A., Sakugawa H., Arai M., Mishiro S. (2006): Hepatitis E virus infection in wild mongooses of Okinawa, Japan: demonstration of anti-HEV antibodies and a full-genome nucleotide sequence. Hepatology Research, 34, 137–140.

Nicand E., Armstrong G.L., Enouf V., Guthmann J.P., Guerin J.P., Caron M., Nizou J.Y., Andraghetti R. (2005): Genetic heterogeneity of hepatitis E virus in Darfur, Sudan, and neighbouring Chad. Journal of Medical Virology, 77, 519–521.

Nishizawa T., Takahashi M., Mizuo H., Miyajima H., Gotanda Y., Okamoto H. (2003): Characterization of Japanese swine and human hepatitis E virus isolates of genotype IV with 99% identity over the entire genome. Journal of General Virology, 84, 1245–1251.

Okamoto H., Takahashi M., Nishizawa T., Fukai K., Muramatsu U., Yoshikawa A. (2001): Analysis of complete genome of indigenous swine hepatitis E virus isolated in Japan. Biochemical and Biophysical Communications, 289, 929–936.

Okamoto H., Takahashi M., Nishizawa T., Usui R., Kobayashi E. (2004): Presence of antibodies to hepatitis E virus in Japanese pet cats. Infection, 32, 57–58.

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. Journal of Virology, 74, 2430–2437.

Payne C.J., Ellis T.M., Plant S.L., Gregory A.R., Wilcox G.E. (1999): Sequence data suggests big liver and spleen disease virus (BLSV) is genetically related to hepatitis E virus. Veterinary Microbiology, 68, 119–125.

Pina S., Buti M., Cotrina M., Piella J., Girones R. (2000): HEV identified in serum from humans with acute hepatitis and sewage of animal origin in Spain. Journal of Hepatology, 33, 826–833.

Preiss J.C., Plentz A., Engelmann E., Schneider T., Jilg W., Zeitz M., Duchmann R. (2006): Autochthonous hepatitis E virus infection in Germany with sequence similarities to other European isolates. Infection, 34, 173–175.

Reuter G., Fodor D., Katai A., Szucs G. (2006): Identification of a novel variant of human hepatitis E virus in Hungary. Journal of Clinical Virology, 36, 100–102.

Schlauder G.G., Mushahwar I.K. (2001): The genetic heterogeneity of hepatitis E virus. Journal of Medical Virology, 65, 282–292.

Schlauder G.G., Dawson G.J., Erker J.C., Kwo P.Y., Knigge M.F., Smalley D.L., Rosenblatt J.E., Desai S.M., Mushahwar I.K. (1998): The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. Journal of General Virology, 79, 447–456.

Schlauder G.G., Desai S.M., Zanetti A.R., Tassopoulos N.C., Mushahwar I.K. (1999): Novel hepatitis E virus (HEV) isolates from Europe: Evidence for additional genotypes of HEV. Journal of Medical Virology, 57, 243–251.

Schlauder G.G., Frider B., Sookoian S., Castano G.C., Mushahwar I.K. (2000): Identification of 2 novel isolates of hepatitis E virus in Argentina. Journal of Infectious Diseases, 182, 294–297.

Shrestha S.M., Shrestha S., Tsuda F., Nishizawa T., Gotanda Y., Takeda N., Okamoto H. (2003): Molecular investigation of hepatitis E virus infection in patients with acute hepatitis in Kathmandu, Nepal. Journal of Medical Virology, 69, 207–214.

Skidmore S.J., Yarbough P.O., Gabor K.A., Tam A.W., Reyes G.R., Flower A.J. (1991): Imported hepatitis E in UK. Lancet, 337, 1541.

Smith J.L. (2001): A review of hepatitis E virus. Journal of Food Protection, 64, 572–586.

Sonoda H., Abe M., Sugimoto T., Sato Y., Bando M., Fukui E., Mizuo H., Takahashi M., Nishizawa T., Okamoto H. (2004): Prevalence of hepatitis E virus (HEV) infection in wild boars and deer and genetic identification of a genotype 3 HEV from a boar in Japan. Journal of Clinical Microbiology, 42, 5371–5374.

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.

Tam A.W., Smith M.M., Guerra M.E., Huang C.C., Bradley D.W., Fry K.E., Reyes G.R. (1991): Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. Virology, 185, 120–131.

Tam A.W., White R., Yarbough P.O., Murphy B.J., McAtee C.P., Lanford R.E., Fuerst T.R. (1997): *In vitro* infection and replication of hepatitis E virus in primary cynomolgus macaque hepatocytes. Virology, 238, 94–102.

Tamada Y., Yano K., Yatsuhashi H., Inoue O., Mawatari F., Ishibashi H. (2004): Consumption of wild boar linked to cases of hepatitis E. Journal of Hepatology, 40, 869–870.

Tei S., Kitajima N., Takahashi K., Mishiro S. (2003): Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet, 362, 371–373.

Tei S., Kitajima N., Ohara S., Inoue Y., Miki M., Yamatani T., Yamabe H., Mishiro S., Kinoshita Y. (2004): Consumption of uncooked deer meat as a risk factor for hepatitis E virus infection: an age- and sex-matched case-control study. Medical Virology, 74, 67–70.

Tien N.T., Clayson H.T., Khiem H.B., Sac P.K., Corwin A.L., Myint K.S., Vaughn D.W. (1997): Detection of immunoglobulin G to the hepatitis E virus among several animal species in Vietnam. American Journal of Tropical Medicine and Hygiene, 57, 211.

Tsarev S.A., Emerson S.U., Reyes G.R., Tsareva T.S., Legters L.J., Malik I.A., Iqbal M., Purcell R.H. (1992): Characterization of a prototype strain of hepatitis E virus. Proceeding of the National Academy of Science, USA, 89, 559–563.

Tsarev S.A., Tsareva T.S., Emerson S.U., Yarbough P.O., Legters J.L., Moskal T., Purcell R.H. (1994): Infectivity titration of prototype strain of hepatitis E virus in cynomolgus monkeys. Journal of Medical Virology, 43, 135–142.

Tsarev S.A., Shrestha M.P., He J., Scott R.M., Vaung D. W., Clayson E.T., Gigliotti S., Longer C.F., Innis B.L. (1998): Naturally acquired hepatitis E virus (HEV) infection in Nepalese rodents. American Journal of Tropical Medicine and Hygiene, 59, 242.

Tsega E., Krawczynski K., Hansson B.G., Nordenfelt E., Negusse Y., Alemu W., Bahru Y. (1991): Outbreak of acute hepatitis E virus infection among military personnel in northern Ethiopia. Journal of Medical Virology, 34, 232–236.

van Cuyck-Gandre H., Zhang H.Y., Tsarev S.A., Clements N.J., Cohen S.J., Caudill J.D., Buisson Y., Coursaget P., Warren R.L., Longer C.F. (1997): Characterization of hepatitis E virus (HEV) from Algeria and Chad by partial genome sequence. Journal of Medical Virology, 53, 340–347.

van der Poel W.H.M., Verschoor F., van der Heide R., Herrera M.I., Vivo A., Kooreman M., de Roda Husman A.M. (2001): Hepatitis E virus sequences in swine related to sequences in human, the Netherlands. Emerging Infectious Diseases, 7, 970–976.

Vasickova P., Dvorska L., Lorencova A., Pavlik I. (2005): Viruses as a cause of foodborne diseases: a review of the literature. Veterinarni Medicina 50, 89–104. http://www.vri.cz/docs/vetmed/50-3-89.pdf

Vishwanathan R. (1957): Infectious hepatitis in Delhi (1955–1956): a critical study. Indian Journal of Medical Research, 45, 1–30.

Vitral C.L., Pito M.A., Lewis-Ximenez L.L., Khudyakov Y.E., dos Santos D.R., Gaspar A.M.C. (2005): Serological evidence of hepatitis E virus infection in different animal species from the Southeast of Brazil. Memorias do Instituto Oswaldo Cruz, 100.

Waar K., Herremans M.M.P.T., Vennema H., Koopmans M.P.G., Benne C.A. (2005): Hepatitis E is cause of unexplained hepatitis in The Netherlands. Journal of Clinical Virology, 33, 145–149.

Wang Y.C., Ling R., Erker J.C., Zhang H., Li H., Desai S., Mushahwar I.K., Harrison T.J. (1999): A divergent genotype of hepatitis E virus in Chinese patients with acute hepatitis. Journal of General Virology, 80, 169–177.

Wang Y.C., Zhang H., Ling R., Li H., Harrison T.J. (2000): The complete sequence of hepatitis E virus genotype 4 reveals an alternative strategy for translation of open reading frames 2 and 3. Journal of General Virology, 81, 1675–1686.

Wang Y.C., Zhang H.Y., Xia N.S, Peng G., Lan H.Y., Zhuang H., Zhu Y.H., Li S.W., Tian K.G., Gu W.J., Lin J.X., Wu X., Li H.M., Harrison T.J. (2002): Prevalence, isolation, and partial sequence analysis of hepatitis E virus from domestic animals in China. Journal of Medical Virology, 67, 516–521.

Wei S., Walsh P., Huang R., To S.S.T. (2000): 93G, Novel sporadic strain of hepatitis E virus in South China isolated by cell culture. Journal of Medical Virology, 61, 311–318.

Wibawa I.D., Muljono D.H., Mulyanto, Suryadarma I. G., Tsuda F., Takahashi M., Nishizawa T., Okamoto H. (2004): Prevalence of antibodies to hepatitis E virus among apparently healthy humans and pigs in Bali, Indonesia: Identification of a pig infected with genotype 4 hepatitis E virus. Journal of Medical Virology, 73, 38–44.

Widdowson M.A., Jaspers W.J.M., van der Poel W.H.M., Verschoor F., de Roda Husman A.M., Winter H.L.J., Zaaijer H.L., Koopmans M. (2003): Cluster of cases of acute hepatitis associated with hepatitis E virus infection acquired in the Netherlands. Clinical Infectious Disease, 36 1, 29–33.

Williams T.P.E., Kasorndorkbua C., Halbur P.G., Haqshenas G., Guenette D.K., Toth T.E., Meng X.J. (2001): Evidence of extrahepatic sites of replication of the hepatitis E virus in a swine model. Journal of Clinical Microbiology, 39, 3040–3046.

Withers M.R., Corea M.T., Morrow M., Stebbins M.E., Seriwatana J., Webster W.D., Boak M.B., Vaughn D.W. (2002): Antibody levels to hepatitis E virus in North Carolina swine workers, non-swine workers, swine, and murids. American Journal of Tropical Medicine and Hygiene, 66 (4), 384–388.

Worm H.C., Schlauder G.G., Wurzer H., Mushahwar I.K. (2000): Identification of a novel variant of hepatitis E virus in Austria: sequence, phylogenetic and serological analysis. Journal of General Virology, 81, 2885–2890.

Worm H.C., van der Poel W.H.M., Brandstatter G. (2002): Hepatitis E: an overview. Microbes and Infection, 4, 657–666.

Wu J.C., Chen C.M., Chiang T.Y., Tsai W.H., Jeng W.J., Sheen I.J., Lin C.C., Meng X.J. (2002): Spread of hepatitis E virus among different-aged pigs: two-year survey in Taiwan. Journal of Medical Virology, 66, 488–492.

Xing L., Kato K., Li T., Takeda N., Miyamura T., Hammar L., Cheng R.H. (1999): Recombinant hepatitis E capsid protein self-assembles into dual-domain T=1 particle presenting native virus. Virology, 265, 35–45.

Yarbough P.O. (1999): Hepatitis E virus. Intervirology, 42, 179–184.

Yazaki Y., Mizuo H., Takahashi M., Nishizawa T., Sasaki N., Gotanda Y., Okamoto H. (2003): Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be foodborne, as suggested by the presence of hepatitis E virus in pig liver as food. Journal of General Virology, 84, 2351–2357.

Yin S., Tsarev S.A., Purcell R.H., Emerson S.U. (1993): Partial sequence comparison of eight new Chinese strains of hepatitis E virus suggests the genome sequence is relatively stable. Journal of Medical Virology, 41, 230–241.

Yoo D., Willson P., Pei Y., Hayes M.A., Deckert A., Dewey C.E., Friendship R.M., Yoon Y., Gottschalk M., Yason C., Giulivi A. (2001): Prevalence of hepatitis E virus antibodies in Canadian swine herds and identification of novel variant of swine hepatitis E virus. Clinical and Diagnostic Laboratory Immunology, 8, 1213–1219.

Zafrullah M., Ozdener M.H., Panda S.K., Jameel S. (1997): The ORF3 protein of hepatitis E is phosphoprotein that associates with the cytoskeleton. Journal of Virology, 71, 9045–9053.

Zafrullah M., Ozdener M.H., Kumar R., Panda S.K., Jameel S. (1999): Mutational analysis of glycosylation, membrane translocation, and cell surface expression of the hepatitis E virus ORF2 protein. Journal of Virology, 73, 4074–4082.

Zanetti A.R., Schlauder G.G., Romano L., Tanzi E., Fabris P., Dawson G.J., Mushahwar I.K. (1999): Identification of a novel variant of hepatitis E virus in Italy. Journal of Medical Virology, 57, 356–360.

Zhang M., Purcell R.H., Emerson S.U. (2001): Identification of the terminal sequence of the SAR-55 and MEX-14 strains of hepatitis E virus and confirmation that the genome is capped. Journal of Medical Virology, 65, 293–295.

Received: 2007–01–05
Accepted after corrections: 2007–07–13

Corresponding Author:

Prof. MVDr. Ivo Pavlik, CSc., Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic
Tel.: +420 533 331 601, fax: +420 541 211 229, e-mail: pavlik@vri.cz