

## Hepatitis E: a curious zoonosis

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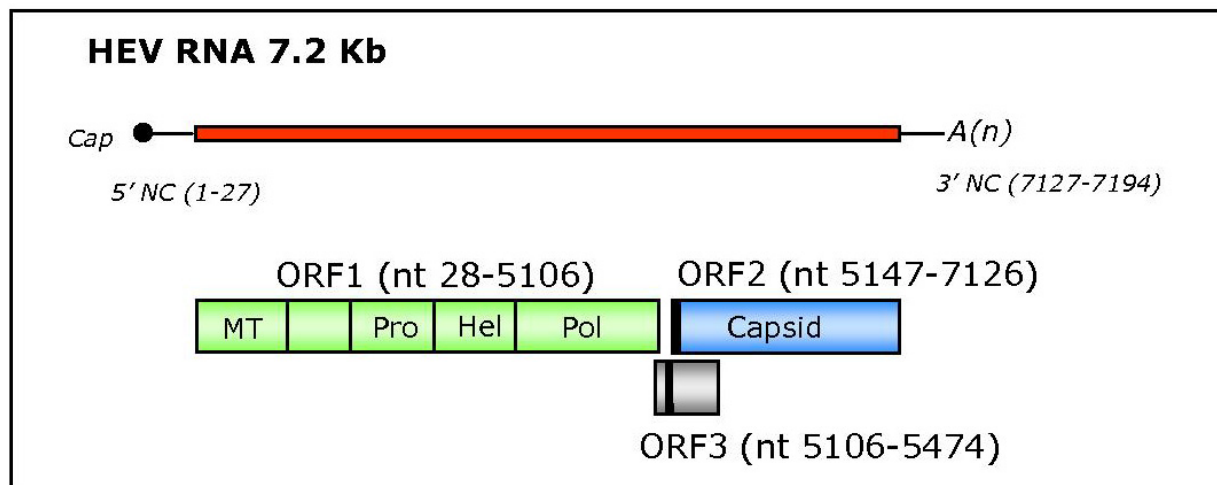
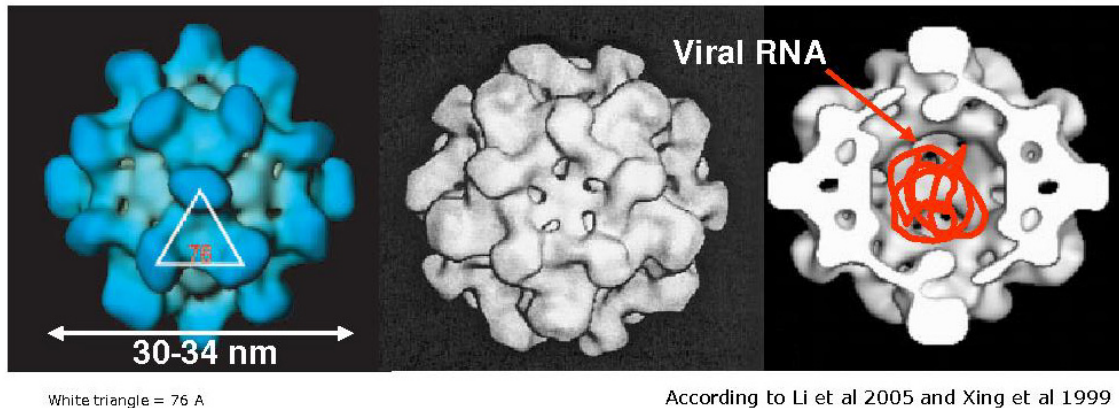
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## 1. ABSTRACT

Hepatitis E virus (HEV) is responsible for large waterborne epidemics of acute hepatitis in endemic regions and for sporadic autochthonous cases in non endemic regions. Although the water vector has been thoroughly documented in endemic regions, very little is known about the modes of contamination occurring in non endemic regions. Unlike the other hepatitis viruses, HEV has an animal reservoir. Several lines of evidence, such as the results of phylogenic analysis and studies on direct contamination via infected food products, have suggested that some cases of animal to human transmission occur. However, all the possible sources of human contamination in non endemic areas have not yet been defined, and this point needs to be investigated. The high genetic variability of HEV, which might be an important factor, involved in zoonotic contamination processes, also needs a surveillance plan.

## 2. INTRODUCTION

Until recently, viral Hepatitis E was still considered as an imported disease associated with travel in endemic regions. This acute hepatitis, very similar to hepatitis A or drug induced hepatitis, was very under diagnosed, since most of the cases were thought being imported. Nowadays, markers of hepatitis E infection are investigated more frequently and increasing number of reports described autochthonous cases. These autochthonous cases are related to viral genotype different from endemic areas confirming that they are acquired locally. In contrast to other hepatitis virus, Hepatitis E virus (HEV) infects animals and more particularly pigs. Swine represent a large reservoir of HEV and share the same genotypes with sporadic cases. Autochthonous acquired Hepatitis E has lead to investigate possible transmission pathways in industrialized countries where the water vector is not dominant. This review aims at



MT: methyl transferase; Pro: Cystein protease ; Hel: helicase; Pol: RNA polymerase

**Figure 1.** HEV schematic structure and genome organization.

presenting HEV viral biology, clinic and reservoir to characterize the possible zoonotic risk of infection.

### 3. HEPATITIS E VIRUS BIOLOGY

#### 3.1. HEV characteristics and virus life cycle

HEV was characterized in the 80's in India as a viral agent responsible for large waterborne epidemics but different from Hepatitis A Virus (1). HEV was identified in 1983 by electron microscopy (2) and cloned in 1990. HEV is classified in the Hepeviridae family, gender Hepevirus (3) ([http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs\\_hepev.html](http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/fs_hepev.html)). Its genomic organization has similarities with members of the Caliciviridae family and also a certain number of homologies with Rubella virus of the Togaviridae family or plants furovirus.

HEV is a small, non enveloped virus of approximately 30 nm in diameter. The viral capsid has an icosahedral symmetry and is composed of 60 copies of a single protein: core or ORF-2 (Figure 1). This capsid

contains a single stranded molecule of RNA of positive polarity, capped and polyadenylated, of 7.2 Kb. It encodes for 3 open reading frames (ORF). The first corresponds to the non structural proteins and carries consensus motif of various enzymatic functions such as methyl transferase, papain-like protease, helicase and RNA dependent RNA polymerase (RdRp). The second encodes for a capsid protein of 660 amino-acids (approximately 88 Kd) with 3 putative sites of N-glycosylation. The third one encodes for a small phosphoproteine of 123 amino-acids associated with cytoskeleton, which function is not known. ORF-3 is necessary for viral infection in macaque cynomolgus *in vivo* but not *in vitro* (4, 5). The capsid protein has an endoplasmic reticulum signal peptide and when it is expressed *in vitro*, it is found glycosylated in the cytoplasm and on the cell surface (6). It interacts with the 5' end of the viral RNA which probably plays a role in RNA encapsidation. Using also *in vitro* expression system, it has been shown that the ORF-2 and ORF-3 proteins interact together suggesting that ORF-3 may play a role in viral assembly of de novo particles (7). The capsid protein

carries major epitopes of neutralization (8). Truncated form of the capsid protein with a N-terminal deletion of the first 111 amino-acids can assemble into virus-like particle (VLP) (9). These VLPs are smaller than the native particles (23-24 nm in diameter) but have identical antigenic properties (10).

Since there is no *in vitro* model with robust viral production, the virus life cycle remains hypothetical. For viral entry, the cellular receptor of HEV is still unknown. The HSC70 protein might be a receptor or co-receptor since it interacts *in vitro* with the C-terminal region of the capsid protein, but it remains to be demonstrated *in vivo* (11). After being released in the cytoplasm, HEV RNA is putatively translated into non structural proteins followed by the synthesis of genomic and sub-genomic RNAs. Replication complexes have been observed, *in vitro*, at the endoplasmic reticulum membrane. Then the translation of the capsid and the ORF-3 would occur, leading to the assembly of viral particles and release of new virions. There is no evidence of a direct cytopathic effect of HEV, more likely hepatocytes cytolysis is mediated by the cellular immune response.

### 3.2. HEV genetic variability

HEV is divided into four main genotypes and 24 subtypes. These 4 genotypes present 72 to 77 % of homology in nucleotides, between them, and each subtype shares approximately 85 to 90 % of homology. There are 5 subtypes of genotype 1 (1a, 1b, 1c, 1d, 1th), 2 subtypes of genotype 2 (2a, 2b), 10 subtypes of genotype 3 (3a, 3b, 3c, 3d, 3rd, 3f, 3g, 3 hours, 3i, 3j) and 7 subtypes of genotype 4 (4a, 4b, 4c, 4d, 4th, 4f, 4g). The current classification was elaborated by combining five different methods of phylogeny (5' ends of ORF1 and -2, 3' ends of ORF1 and -2 and full length genomes), it may vary at the level of subtypes, according to the region of the genome compared (ORF1, -2 or -3), bringing a certain degree of complexity to this classification (12) (Figure 2). There is only a limited number of full size genomes (70) which do not represent all subtypes. Genotypes 1 and 2 were isolated in humans, during large epidemics in developing countries where they are endemic. These two genotypes have distinct geographical distribution. Genotype 1 is present in Asia (India, Pakistan, Nepal, China, Bangladesh, Uzbekistan, Kyrgyzstan) and Africa (Central African Republic, Morocco, Algeria, Namibia, Sudan, Egypt, Chad). Genotype 2 is present in Mexico and occasionally in Africa (Nigeria, Namibia, Egypt, Central African Republic, Chad) (Figure 3). For the genotype 3, the situation is more complex, first, the geographical distribution is different from that of genotypes 1 & 2, second it is only associated with sporadic cases and no epidemic was described with this genotype and third, it is found in animals (pig, wild boar, deer). Genotype 3 is present in Europe (France, Germany, Austria, Greece, Italy, Spain, Great Britain, Netherlands, Sweden) and also in the United States, Canada, Argentina, Brazil, Chile, Mexico, Australia, New Zealand, Russia, Kurdistan, Korea, Kampuchea, Thailand, Taiwan and South Africa. Initially, genotype 3 was first isolated in a pig in the USA (13), then it was identified in two American human cases with acute

hepatitis (14) and later in other countries. Molecular epidemiology and comparison of genotypes 3 strains isolated from human or pigs suggested that HEV could be a zoonotic agent. Furthermore, phylogenetic analyses have shown that some genotype 3 strains are specific to certain countries and others may have been imported by the trade of animals. Thus, in Japan, there are "autochthonous" Japanese strains and others imported from Great Britain following the trade of domestic pigs in the years 1900' (15).

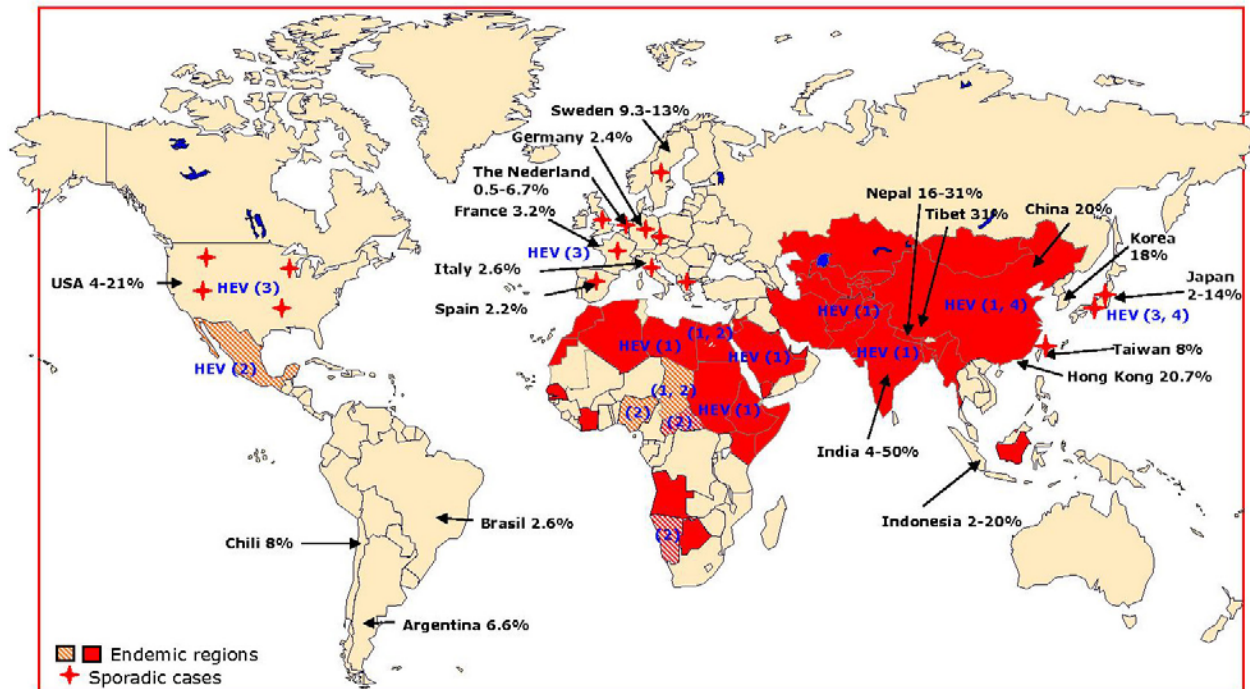
For genotype 4, the geographical distribution is more limited: Japan, China, Indonesia, South Africa, Taiwan, Vietnam and India. Nevertheless, genotype 4 was isolated in human as well as in pigs. The genetic proximity of human and animal strains also suggests that barrier species crossing occur (12).

In endemic areas like India and China where genotypes 3 and 4 circulate in the animal reservoir, the genotype 1 remains responsible for the large epidemics in human (16).

Like other RNA viruses, the genetic variability of HEV is dependent on the absence of proofreading activity of its RdRp and the miss incorporation of nucleotides, which accumulate at every replication cycle. HEV's RdRp error rate is estimated to  $0.8 \times 10^{-3}$  substitution per site per year (15). As hepatitis viruses A and C, HEV can be present as quasi-species in individual (17). In France, a study carried out by the National Reference Centre (NCR) of enterotransmissible hepatitis has focused on intra-individual variation of HEV during an epidemic in Tanefdour, Algeria. This study, based on RFLP (Restriction Fragment Length Polymorphism) has shown that several viral variants coexisted in the same patient (18), thus confirming that HEV exists as quasi-species. Moreover, it was shown that inter genotypic co-infection can occur in one patient (genotype 3 and 4) (19). In addition, genetic analysis of certain sequences reveals the possibility of genetic recombination. A study evokes the possibility of an event of recombination between a human genotype 3 strain and a swine genotype 3 strain (20). This phenomenon, well-known for other RNA viruses such as enterovirus, might be increased in the case of HEV since other non human reservoir exists. Combining HEV quasi-species variability with possible co-infection and recombination events, this suggests that viral variant with increased pathogenicity for human might be selected. Surveillance of HEV variability in the different reservoirs must be recommended.

More recently, an avian strain was identified in the United States in poultry. In opposite to the genotype 3 and 4, that cause only subclinical infections in animals, this avian strain is associated with hepatitis splenomegaly (21). The avian strain has 50 to 60% of homology in nucleotides with the 4 other genotypes of HEV (Figure 2). It has never been isolated in human but the current diagnostic tools (molecular and serological) do not guarantee its detection.





**Figure 3.** HEV geographic distribution (According to CDC data).

#### 4. PATHOGENESIS AND SYMPTOMS

Hepatitis E involves a feco-oral transmission pathway, usually as the result of digestive contamination when soiled food or water are ingested by humans. Contrary to what occurs with the hepatitis A virus and other enteric viruses, inter-human transmission of HEV is a rare occurrence, which explains the unimodal pattern of the epidemic curves (22). The hepatotropic HEV is replicated in the cytoplasm of hepatocytes before being transported by the bile and eliminated via the fecal pathway. However, several aspects of the HEV viral cycle still remain to be elucidated, such as the possible existence of extra-hepatic replication sites and that of intestinal viral multiplication processes. Viremia occurs only briefly, mainly during the prodromic phase, and disappears at the onset of the symptoms. Fecal excretion of the virus begins a few days (5 days on average) prior to jaundice, and regresses at the onset of jaundice before ending within 2 to 3 weeks (23). HEV infection does not progress to chronicity. Specific IgM antibodies develop two weeks on average before the clinical signs: their maximum levels coincide with the peak in the serum ALAT levels, which in turn is concomitant with the jaundice, and disappear within 3 months on average. The anti-HEV IgG antibodies which develop soon after the IgM antibodies persist for several years, although probably do not persist as long as the anti-HAV antibodies. The time lag between the onset of the viral replication process and the biochemical signs suggests that the virus may be only slightly if at all cytopathic (24). Contaminated subjects acquire secondary immunity by synthesizing antibodies, some of which are neutralising antibodies (25). However, the exact duration of the immune response has not yet been established.

The HEV incubation time is 40 days (2-9 weeks) on average. The prodromic phase can be either inexistent or quite short, but it can sometimes continue for up to 2 weeks. In 1994, HEV was responsible for less than 1% of all cases of acute hepatitis occurring in France (26). Like hepatitis A, hepatitis E is an acute condition which never becomes chronic. However, since HEV sometimes continues to be excreted for several months (up to 24 months) by immunocompromised patients, this route could constitute another potential source of contamination (27).

The clinical symptoms, which are similar to that of hepatitis A and the other forms of acute hepatitis, often includes asthenia, hepatomegaly and jaundice, but the bilirubin levels have been reported to be higher than in the case of acute hepatitis A and B (28, 29). Digestive clinical signs have sometimes also been reported, such as vomiting and diffuse abdominal pain, associated or not with generally fairly moderate hyperthermia. Elevated transaminase levels are commonly observed, as with the other forms of acute viral hepatitis. The histological features of the liver, which tend to be diffuse, are characterized by the presence of biliary and hepatocytic cholestasis and that of an inflammatory infiltrate, which is usually rated as moderate. In endemic countries of the world, the main targets of HEV are young adults, whereas the population contaminated in non endemic regions consists mostly of elderly males (30). HEV seropositive subjects have been reported to account for 3.2% of the French population (31). The overall prevalence of anti-HEV antibodies among blood donors in Europe and United States ranges from 2 to 8%, reflecting a generally asymptomatic contact with HEV: acute HEV infection gives rise to clinical symptoms in only 10% of cases (32). One explanation for this situation may be that the viral



strain (genotype 3) existing in weakly endemic geographical regions may be somewhat attenuated (33, 34). Since HEV causes a self-limiting hepatitis similar to hepatitis A, hepatitis E generally runs its course and disappears spontaneously leaving no sequelae within a period of 2 to 4 weeks. However, prolonged cholestatic forms involving the persistence of jaundice for more than one month and relapsing forms have been observed, but less frequently than with acute hepatitis A. The severity of the disease is said to be greater than that of hepatitis A, since the overall mortality rate ranges between 0.4 and 4%, including 1 to 2% of fulminant hepatitis (24, 35). There exists no specific treatment for the fulminant cases: liver grafts are the only means of preventing the death of the patient in this case. A particularly high rate of incidence of fulminant forms (20%) has generally been reported to occur among pregnant women during the third term of pregnancy, which is associated with a high risk of morbidity (miscarriages, premature childbirth) and perinatal mortality (35-38). Mother-to-infant transmission has been observed in 50 to 100% of the cases recorded (36, 39). Several possible physiopathological explanations have been put forward for the severity of the infection in the late stages of pregnancy, including depression of the Th1 cell-mediated immune response and a concomitant increase in the Th2-mediated immune response in pregnant women in comparison with non pregnant women with acute hepatitis E (40), but none of these hypotheses have been actually confirmed so far. In addition, it is worth noting that the rates of mortality published have been based on epidemiological data collected in endemic regions, which therefore do not correspond to the rates of occurrence of fulminant hepatitis E encountered among pregnant women in countries where HEV is not endemic. Autochthonous fulminant hepatitis E in non endemic countries has been only very rarely reported to occur in pregnant women following exposure to a region at risk (41) or without any such exposure (42, 43). To our knowledge, only two cases of fulminant hepatitis E involving pregnant women inhabiting non endemic regions have been described, but in both cases, the women had recently returned from a stay in India (37). Lastly, the prior existence of a chronic liver disease might account for the more severe and fulminant hepatic feature of acute hepatitis E (42, 44, 45). More recently chronic HEV infections have been described in patients after transplantation and with immunosuppressive treatment. Viral shedding in feces was observed up to 12 months after infection and liver fibrosis was observed in few cases.

In view of the recent increase in the number of cases of acute autochthonous hepatitis E in countries such as France where the disease is not endemic, in addition to the potential severity of this viral infection, it is recommended to search for the presence of anti-HEV antibodies and RNA in patients' blood and stools when performing the initial etiological tests for acute hepatitis.

## 5. EXPERIMENTAL MODELS

### 5.1. *In vivo* models

Since HEV doesn't grow efficiently *in vitro*, animal models have been developed to characterize HEV pathogenesis.

The first one involves nonhuman primate: macaque cynomolgus (*Macaca fascicularis*) or less frequently chimpanzee and was mainly used to study genotype 1 and 2. The second one involves pig in which genotype 3 and 4 grow efficiently. Both models were used to demonstrate cross species infections. More recently, a third model was developed in chicken (free from specific pathogen) to study the avian strains of HEV (46).

Although these models represent limited clinical aspects of the viral pathogenesis, since the animals remain asymptomatic, viremia with virus shedding in feces and seroconversion are observed. A moderate increase in hepatic enzymes activities and minor hepatic lesions are occasionally observed.

Initially, the macaque model was developed with genotype 1 & 2 strains isolated in human but it is sensitive to all 4 genotypes. This model was used to demonstrate that cross species infections occur by inoculation of a swine genotype 3 strain and the observation of a productive infection (viremia, virus shedding and seroconversion). Comparison of lesion profiles after infection with human or swine genotype 3 HEV did not show major differences. Similar enzymes elevation, viral excretion and seroconversion were observed suggesting that genotype 3 strains have no specific host restriction (47). Attempt of cross infection of macaque with avian strain failed, suggesting that this strain represent a limited risk for human contamination (48) (Table 1).

Swine reservoir is naturally infected by genotype 3 or 4 and pig model was used to study viral dissemination, transmission, and cross species infection. Using this model extra hepatic sites of HEV replication were identified. High virus titres are found in the liver and the bile but HEV is also found in the small intestine, colon, lymph nodes and tonsils. The significance of these other replication sites remains to be defined in HEV pathogenesis and transmission pathways (49). The experimental infection of pregnant gilts did not lead to any fulminate hepatitis, abortion or vertical transmission (50), which shows the limit of this model in studying human pathogenesis. A contact between infected animal and naive animals is sufficient for HEV transmission with genotype 3 (51). In contrast, pig is not sensitive to infection with genotype 1 strain SAR-55 (Sarghoda, Pakistan) nor to infection with the Mexico strain of genotype 2 (47, 52, 53). The presence of a genotype 1 strain was exceptionally found a pig in Cambodia, but it remains a unique single case (54). The molecular mechanism of this host restriction remains unknown. Genotype 3 and 4 isolated in human, are infectious when inoculated into pigs. The comparison of pathogenesises between swine and human strains of genotype 3 does not show major difference (55). The natural infection of domestic pig is asymptomatic and does

**Table 1.** Barrier species crossing of hepatitis E viruses

Genotype	Natural Host	Exp Models	Infection
1 & 2	Human	Macaque	+
		swine	-
		rat	+
3	Human	Macaque	+
		Swine	+
	Swine	Macaque	+
		swine	+
		Chicken	nd
		rat	nd
		Agneau	+
Avian	Chicken	Macaque	-
		swine	-
		chicken	+
		rat	nd
		turkey	+

nd: not determined

not lead to growth delay nor weight lost and it is observed in young animals between 12 and 15 weeks of age (56).

In contrast to the macaque model, the avian strain is infectious when inoculated into pigs (Meng XJ 2005, Iowa State University Report 2005, A.S. leaflet R1982) (Table 1). Viremia, viral shedding in feces and seroconversion are observed with very mild hepatic lesions. Since pigs and chickens are commonly present together in farms, it raises the possibility that natural infection may occur and that the avian strain may cross species barrier. Particular attention must be taken on possible genetic recombination between avian and swine strains which could then represent a potential risk of infection to human. It currently remains unknown if swine HEV is transmissible to chickens.

Studies in SPF chicken model have shown an oro-nasal transmission pathway associated with clinical symptoms and hepato-splenomegaly (46). After inoculation, chickens become viremic and shed virus in feces. Avian HEV RNA is found in bile and liver samples. No study has address yet if vertical transmission occurs and if the virus is present in eggs of infected poultries. Cross species infection was demonstrated in turkey. Even if the avian strains do not infect macaque, transmission to pig and turkey suggest that it is a zoonotic virus and may infect human.

The development of a rat model was investigated since anti-HEV antibodies were identified in field rat and mice. However, attempt to experimentally infect rats with either human genotype 1 or 3, swine genotype 3 or avian HEV did not lead to productive infection. Infection with genotype 1 induced rare seroconversion; hepatic lesions are very mild and viral shedding in feces is short (57). Serial passages were not observed due to the low and short viral production (personal communication of Pr Myint, Bangkok, Thailand). Rats inoculated with genotype 3 or avian HEV did not become infected as evidenced by the lack of viremia, virus shedding in feces or seroconversion. These data suggest that rat or mice caught in farms are infected by a HEV-like virus, but additional work is needed to determine the origin of the rat viruses as well as the potential role of rodents in HEV transmission

## 5.2. *In vitro* models

Efficient cell culture systems to support strong HEV replication are not available yet but several attempts, using various cell lines or primary cultures, are described in the literature. Other alternative models to characterize HEV biology, in stable or transiently transfected cell lines with full length HEV genome or single gene, have been used but won't be detailed in this review.

The development of an *in vitro* model would be also very useful to correlate the presence of HEV RNA with infectious virus. This model would contribute to identify contaminated products and thus contamination pathways.

*Ex vivo* model were first attempted; Macaque hepatocytes isolated after *in vivo* infection or infected *in vitro* after culture can support HEV replication with production of infectious viral particles in the supernatant (58, 59). Both positive- and negative-strand viral RNA are detected and viral neutralization using antibodies are efficient, thus confirming viral replication. However, these cells are difficult to obtain and freezing procedure are not optimized, thus feasibility is limited.

A model of HEV infection was developed in the hepatoma cell lines HepG/C3A using genotype 1 SAR55 or Akluj strains. This model was used to study HEV thermal stability or neutralizing antibodies (60, 61). However high viral titres are required for initial infection and genotype 3 or 4 infections have not been described. Another cell culture system was established using the hepatocarcinoma cell line PLC/PRF/5 infected with faecal suspension with high titre of HEV ( $2.10^7$  copies of genome equivalent/ml of genotype 3). Viral production is observed after 12 to 60 days post inoculation but it is dependant on the initial titre. Lower titres do not allow efficient infection which is a limiting factor (62). More recently a system was developed in a subclone of hepatoma cell line HuH7, HuH7 10.3, which produce intracellular infectious particle after transfection with a HEV full length RNA of genotype 1 (5). These cells can be re infected by the intra cellular virion neo synthesised. The viral production is not robust enough to permit structural or biochemical analysis, but can provide answers to many relevant questions. Virions produced have similar properties (thermal stability, neutralization, sedimentation) to virions produced *in vivo*. Further investigations are needed to validate this model with other genotypes.

## 6. HEV RESERVOIRS AND ZOONOTIC TRANSMISSIONS

### 6.1. Animal reservoirs

The high genetic similarity of human and swine strains of genotypes 3 and 4 (up to 97-100% of homology in nucleotides and 98-100% in amino-acids) indicate that HEV may have several hosts. Nonetheless, it is not clear yet if there is a natural host, which would be the pig and an occasional host: human. Among the various sub-types of genotypes 3 and 4, there is no distinct separation between animal and human strains and so far no specific "species" strain have been described. In industrialized countries, such as the United States, Japan or Great Britain, a low HEV

seroprevalence is observed in human population (2 to 8%). In contrast, high seroprevalence, up to 80%, is observed in swine herds (63). Thus, it suggests that swine are the main reservoir and that human are occasionally exposed. Contamination of both species from the same source cannot be excluded and with this hypothesis, swine would be more exposed than human. In order to determine the transmission direction, animal to human or human to animal, a chronological approach was used to date different genotype 4 strains in China. Phylogenetic analysis of human and swine strains isolated at various periods of time in the same geographical regions have shown that certain subgroups of HEV circulated initially in the swine reservoir, between 2002 and 2004, and then they were isolated later on in human in 2004 and 2005. Although, nucleotide sequences isolated in human and swine were not strictly identical it indicated that swine reservoir was probably contaminated first and contributed to human secondary contamination (64).

Anti-HEV antibodies have been detected in other animals' species such as wild boar, deer, rat, dog, cat, mongoose, cow, sheep, goat or horse suggesting that they are exposed and sensitive to HEV infection or to a closely related agent (65, 66). While viruses of genotype 3 and/or 4 were isolated in wild boar and deer, no virus was associated with the seropositivity of the other animals. Pig, wild boars and deer have thus potential of being authentic reservoirs of HEV. Finally, the avian strain which is genetically far enough from the other HEV strains, does not seem to represent a significant risk for human contamination. There is no cross species infection from the avian strain to non human primates in experimental infection (48).

## 6.2. Zoonotic transmissions

Several cases of HEV transmission, directly from animals to humans, have been described: symptoms of acute hepatitis were observed 15 to 60 days after consumption of food products raw or under cooked: deer meat sushi (67), wild boar barbecue (30, 67) or roasted or uncooked pork liver (68). The demonstration that these cases had an animal origin relies on several evidence: identity of the sequences (100% of similarity at the nucleotide level) between the human cases and the strain isolated from food (67), IgM and/or IgG antibodies in the majority of people having consumed the same food product that the index case (30, 69), cluster of human cases among people having consumed the same food product. These data were reinforced by experimental data, which showed that strains of genotype 3 are transmissible from humans to pigs, and from pigs to monkeys.

The contamination of human by direct contact with animals is equally supported by an higher anti-HEV antibody prevalence within slaughterhouse staff, veterinarians and pig breeders, than in the general population, suggesting that a direct or indirect contamination is possible (70-73). A survey in a cohort of 295 veterinarians from 8 American states showed an antibody prevalence of 27% versus 16% in the general population (70). In Sweden, a study showed also a high prevalence within the pig breeders population (13%) (73). At last, a recent case described an acute Hepatitis E in a French subject that had been given a pet

pig 2 months before the onset of the symptoms. Our team showed that the pet pig was infected by a genotype 3 strain, very close to the one of the patient (98% of equivalence in amino acids) (74, 75).

## 6.3. Possible source of contamination in non endemic regions

In endemic countries, the routes of direct contamination by HEV, are ingestion of contaminated water or of soiled food products. Contamination by direct contacts with infected animals are not frequent and may only concern people with professional occupations at risk (63) (70, 71). In non endemic regions, contamination by the water vector is not formally established. Viral sequences are rarely amplified from environment samples. HEV sequences were isolated in waste water in urban areas of several European countries (Spain, France, Greece and Sweden) and in the US. This observation confirms the viral circulation in human population, but does not prove that the water vector is responsible for HEV contamination (76). Infectious virus is found in manure storage samples from pig farms (77). Although the viral load seems to decrease after long term storage, spreading of this manure as a fertilizer may represent a source of contamination for the environment (vegetables, ground water).

In contrast to HAV, which is transmitted through sea shells (mussels, oysters) there is no evidence of HEV transmission through such food product. HEV RNA was found in a sort of clam (*corbicula Japonica*) in Japan but no human case was associated. Little is known about HEV resistance in highly salted environment (78). Consumption of food products derived from pig are at risk, especially raw or undercooked meat as shown in Japan. HEV contaminations have been associated with consumption of sushi or sashimi containing pig liver, wild boar or deer meat. These food habits are unique to Japan, but more generally consumption of pork constitutes a risk factor associated with HEV contamination. Indeed, a study carried out in Bali (Indonesia) on HEV prevalence in pregnant women has shown a seroprevalence of 20% among Hindus (20%) versus 2% among Muslim women who do not consume pork meat ( $P < 0.001$ ) (79). Usually pork meat is consumed well cooked but the thermal stability of the HEV is not well characterized. One hour heating at 60 °C, completely inactivates a viral suspension of genotype 1 (SAR-55) and partially, a suspension of genotype 2 (Mexico) (61). Unfortunately there is no data on genotypes 3 and 4 which are presumably more at risk of zoonotic transmission. It is thus possible that meat or prepared dish with pork meat undercooked may still contain infectious virus. In France there is a strong tradition of local preparation derived from pork meat "charcuterie": mid dry liver sausage, dry sausages etc... Thus, it is possible that HEV may resist to these processes since it resists under unfavourable conditions (manure). It also should be noted that HEV is still infectious after more than ten years of freezing thus its must be stable (61).

Lastly, it is also necessary to consider swine as a possible source of biological products used in human health (pancreatic extract, xenograft) (80). This type of vector was not tested yet but a survey should be considered.



## 7. DIAGNOSIS, PREVENTION, VACCINATION

In clinical practice, HEV diagnosis is based on serological data while the search for viral RNA or the genotyping are not systematically performed. In many countries hepatitis E is not a disease with mandatory statement, thus it is under diagnosed. For serology detection, the current kits used are based on the recognition of peptides or fraction of the capsid protein of genotypes 1 and 2. The existence of a single serotype was shown after inoculation of genotype 1 capsid protein in monkeys and observation of a cross protection after challenge with genotype 2 or 3. The single serotype was also confirmed by comparison of seroprevalence using two recombinant capsids of genotypes 1 (SAR-55) or 3 (porcine the USA) and several serums from patients or pigs from various origin (USA, Canada, China, Korea and Thailand in an ELISA test. These two proteins have 95% of homology in amino-acids. Overall, the comparative analysis has shown similar prevalence values for each group, but some serums were positive only with one of the two proteins (81). It is thus possible that some minor epitopes are not recognized by heterotypic antibodies. This observation may explain why there is proven cases of hepatitis E diagnosed on the basis of clinical symptoms compatible with acute hepatitis associated with the presence of viral RNA in the serum or stools, but where there was no even late seroconversion (82). There is such a high genetic variability in genotype 3 associated with autochthonous cases in Europe, that further characterization of immunodominant epitopes is needed.

The prevention of the disease is limited; in endemic areas the improvement of sanitation decreases water contamination by sewage. In areas where there are sporadic cases the first goal is to identify all possible vectors of contamination and then to prevent any risk of transmission.

A vaccine based on genotype 1 VLPs is under development. This vaccine confers, to the macaque *Cynomolgus*, a protection against a virulent challenge with a homotypic strain of genotype 1 and also a cross protection to genotypes 2 and 3 (83). The first clinical trial of phase I was carried out successfully in the Nepal, where 90% of acute hepatitis are related to HEV (84). A phase 2, randomized, double-blind, placebo-controlled trial was performed recently. The estimated efficacy of three doses of vaccine is 95.5% (85). This vaccine could be used in endemic areas for the general population or for travellers to these areas. Moreover, it could be recommended to pregnant women and all personnel with professional occupation at risk of over-exposure to HEV.

## 8. CONCLUSION

The zoonotic potential of HEV is now well established but all possible contamination pathways remain to be identified especially in non endemic countries where the number of autochthonous cases is in regular increase. An important issue is the evaluation of risk factors associated to transmission and the clinical consequences related to the virus exposure. Qualitative and quantitative

risk assessment must be determined to define recommendations on HEV surveillance. Impact of HEV animal reservoir on human contamination must be measured to take proper control on zoonotic transmissions. Particular attention on HEV genetic variability and recombination in animals must be paid to prevent the emergence of highly pathogenic strains of HEV.

## 9. ACKNOWLEDGMENTS

NP, AB and ME are grateful to MedVetNet WP31 ZOOVIRNET for the financial support of this publication.

## 10. REFERENCES

1. Arankalle, V. A., M. S. Chadha, S. A. Tsarev, S. U. Emerson, A. R. Risbud, K. Banerjee & R. H. Purcell: Seroepidemiology of water-borne hepatitis in India and evidence for a third enterically-transmitted hepatitis agent. *Proc Natl Acad Sci U S A*, 91, 3428-32 (1994)
2. Balayan, M. S., A. G. Andjaparidze, S. S. Savinskaya, E. S. Ketiladze, D. M. Braginsky, A. P. Savinov & V. F. Poleschuk: Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology*, 20, 23-31 (1983)
3. Emerson, S. U., D. Anderson, A. A., M. X.-J., P. M., S. G. G. & T. S. A.: Hepevirus. In: Virus taxonomy. Eighth report of the International Committee on Taxonomy of Viruses. Ed: M. A. M. C. M. Fauquet, J. Maniloff, U. Desselberger, and L. A. Ball (ed.). *Academic Press*, London, United Kingdom., (2004)
4. Graff, J., H. Nguyen, C. Yu, W. R. Elkins, M. St Claire, R. H. Purcell & S. U. Emerson: 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, 6680-9 (2005)
5. Emerson, S. U., H. Nguyen, U. Torian & R. H. Purcell: ORF3 protein of hepatitis E virus is not required for replication, virion assembly, or infection of hepatoma cells in vitro. *J Virol*, 80, 10457-64 (2006)
6. Zafrullah, M., M. H. Ozdener, R. Kumar, S. K. Panda & S. Jameel: Mutational analysis of glycosylation, membrane translocation, and cell surface expression of the hepatitis E virus ORF2 protein. *J Virol*, 73, 4074-82 (1999)
7. Zafrullah, M., M. H. Ozdener, S. K. Panda & S. Jameel: The ORF3 protein of hepatitis E virus is a phosphoprotein that associates with the cytoskeleton. *J Virol*, 71, 9045-53 (1997)
8. Zhang, Y., P. McAtee, P. O. Yarbough, A. W. Tam & T. Fuerst: Expression, characterization, and immunoreactivities of a soluble hepatitis E virus putative capsid protein species expressed in insect cells. *Clin Diagn Lab Immunol*, 4, 423-8 (1997)
9. Li, T. C., N. Takeda, T. Miyamura, Y. Matsuura, J. C. Wang, H. Engvall, L. Hammar, L. Xing & R. H. Cheng: Essential elements of the capsid protein for self-assembly into empty virus-like particles of hepatitis E virus. *J Virol*, 79, 12999-3006 (2005)
10. Xing, L., K. Kato, T. Li, N. Takeda, T. Miyamura, L. Hammar & R. H. Cheng: Recombinant hepatitis E capsid protein self-assembles into a dual-domain T = 1 particle

- presenting native virus epitopes. *Virology*, 265, 35-45 (1999)
11. Zhou, Y. & S. U. Emerson: P.302 Heat shock cognate protein 70 may mediate the entry of hepatitis E virus into host cells. *J Clin Virol*, 36, S155 (2006)
  12. Lu, L., C. Li & C. H. Hagedorn: Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Rev Med Virol*, 16, 5-36 (2006)
  13. Meng, X. J., R. H. Purcell, P. G. Halbur, J. R. Lehman, D. M. Webb, T. S. Tsareva, J. S. Haynes, B. J. Thacker & S. U. Emerson: A novel virus in swine is closely related to the human hepatitis E virus. *Proc Natl Acad Sci U S A*, 94, 9860-5 (1997)
  14. Schlauder, G. G., G. J. Dawson, J. C. Erker, P. Y. Kwo, M. F. Knigge, D. L. Smalley, J. E. Rosenblatt, S. M. Desai & I. K. Mushahwar: The sequence and phylogenetic analysis of a novel hepatitis E virus isolated from a patient with acute hepatitis reported in the United States. *J Gen Virol*, 79 ( Pt 3), 447-56 (1998)
  15. Tanaka, Y., K. Takahashi, E. Orito, Y. Karino, J. H. Kang, K. Suzuki, A. Matsui, A. Hori, H. Matsuda, H. Sakugawa, Y. Asahina, T. Kitamura, M. Mizokami & S. Mishiro: Molecular tracing of Japan-indigenous hepatitis E viruses. *J Gen Virol*, 87, 949-54 (2006)
  16. Arankalle, V. A., L. P. Chobe, M. V. Joshi, M. S. Chadha, B. Kundu & A. M. Walimbe: Human and swine hepatitis E viruses from Western India belong to different genotypes. *J Hepatol*, 36, 417-25 (2002)
  17. Pawlotsky, J. M.: Hepatitis C virus population dynamics during infection. *Curr Top Microbiol Immunol*, 299, 261-84 (2006)
  18. Grandadam, M., S. Tebbal, M. Caron, M. Siriwardana, B. Larouze, J. L. Koeck, Y. Buisson, V. Enouf & E. Nicand: Evidence for hepatitis E virus quasispecies. *J Gen Virol*, 85, 3189-94 (2004)
  19. Takahashi, M., T. Nishizawa, A. Yoshikawa, S. Sato, N. Isoda, K. Ido, K. Sugano & H. Okamoto: Identification of two distinct genotypes of hepatitis E virus in a Japanese patient with acute hepatitis who had not travelled abroad. *J Gen Virol*, 83, 1931-40 (2002)
  20. van Cuyck, H., J. Fan, D. L. Robertson & P. Roques: Evidence of recombination between divergent hepatitis E viruses. *J Virol*, 79, 9306-14 (2005)
  21. Haqshenas, G., H. L. Shivaprasad, P. R. Woolcock, D. H. Read & X. J. Meng: 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, 2449-62 (2001)
  22. Aggarwal, R. & S. R. Naik: Hepatitis E: intrafamilial transmission versus waterborne spread. *J Hepatol*, 21, 718-23 (1994)
  23. Aggarwal, R., D. Kini, S. Sofat, S. R. Naik & K. Krawczynski: Duration of viraemia and faecal viral excretion in acute hepatitis E. *Lancet*, 356, 1081-2 (2000)
  24. Castera, L. & J. M. Pawlotsky: [Hepatitis E virus infection: epidemiology and prevention]. *Gastroenterol Clin Biol*, 25, 521-37 (2001)
  25. Tsarev, S. A., T. S. Tsareva, S. U. Emerson, S. Govindarajan, M. Shapiro, J. L. Gerin & R. H. Purcell: Successful passive and active immunization of cynomolgus monkeys against hepatitis E. *Proc Natl Acad Sci U S A*, 91, 10198-202 (1994)
  26. Pham, B. N., V. Guignonis, F. Durand, J. Bernuau, L. Udin & A. Bezeaud: Anti-HEV antibodies in acute hepatitis in France. *J Hepatol*, 20, 679 (1994)
  27. Banas, B., U. Tausch, F. Hofstadter, M. Woenckhaus, M. C. Pietrzyk, G. A. J. Riegger, S. Boehm, B. K. Kramer & W. Jilg: P.328 Infection with hepatitis E virus: first report of a chronic case and molecular characterization of the virus. *J Clin Virol*, 36, S162 (2006)
  28. Khuroo, M. S.: 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, 818-24 (1980)
  29. Wu, J. C., I. J. Sheen, T. Y. Chiang, W. Y. Sheng, Y. J. Wang, C. Y. Chan & S. D. Lee: The impact of traveling to endemic areas on the spread of hepatitis E virus infection: epidemiological and molecular analyses. *Hepatology*, 27, 1415-20 (1998)
  30. Tamada, Y., K. Yano, H. Yatsushashi, O. Inoue, F. Mawatari & H. Ishibashi: Consumption of wild boar linked to cases of hepatitis E. *J Hepatol*, 40, 869-70 (2004)
  31. Boutrouille, A., L. Bakkali-Kassimi, C. Cruciere & N. Pavio: Prevalence of anti-Hepatitis E Virus Antibodies in French Blood Donors. *J Clin Microbiol*, 45, 2009-1 (2007)
  32. Mast, E. E. & K. Krawczynski: Hepatitis E: an overview. *Annu Rev Med*, 47, 257-66 (1996)
  33. Mushahwar, I. K., G. J. Dawson & G. R. Reyes: Hepatitis E virus: molecular biology and diagnosis. *Eur J Gastroenterol Hepatol*, 8, 312-8 (1996)
  34. Emerson, S. U. & R. H. Purcell: Hepatitis E virus. *Rev Med Virol*, 13, 145-54 (2003)
  35. Krawczynski, K., R. Aggarwal & S. Kamili: Hepatitis E. *Infect Dis Clin North Am*, 14, 669-87 (2000)
  36. Kumar, R. M., S. Uduman, S. Rana, J. K. Kochiyil, A. Usmani & L. Thomas: Sero-prevalence and mother-to-infant transmission of hepatitis E virus among pregnant women in the United Arab Emirates. *Eur J Obstet Gynecol Reprod Biol*, 100, 9-15 (2001)
  37. Hussaini, S. H., S. J. Skidmore, P. Richardson, L. M. Sherratt, B. T. Cooper & J. G. O'Grady: Severe hepatitis E infection during pregnancy. *J Viral Hepat*, 4, 51-4 (1997)
  38. Khuroo, M. S., S. Kamili & S. Jameel: Vertical transmission of hepatitis E virus. *Lancet*, 345, 1025-6 (1995)
  39. Singh, S., A. Mohanty, Y. K. Joshi, D. Deka, S. Mohanty & S. K. Panda: Mother-to-child transmission of hepatitis E virus infection. *Indian J Pediatr*, 70, 37-9 (2003)
  40. Pal, R., R. Aggarwal, S. R. Naik, V. Das, S. Das & S. Naik: Immunological alterations in pregnant women with acute hepatitis E. *J Gastroenterol Hepatol*, 20, 1094-101 (2005)
  41. Zijlstra, J. G., E. B. Haagsma, J. E. Tulleken & T. S. van der Werf: Hepatitis-E-associated fulminant hepatic failure in non-pregnant young women. *Lancet*, 350, 289-90 (1997)
  42. Mennecier, D., E. Nicand, M. Grandadam, J. A. Bronstein, C. Thiolet, O. Farret, Y. Buisson & C. Molinie: [Subfulminant hepatitis E in France]. *Gastroenterol Clin Biol*, 24, 467-9 (2000)

43. Suzuki, K., T. Aikawa & H. Okamoto: Fulminant hepatitis E in Japan. *N Engl J Med*, 347, 1456 (2002)
44. Hamid, S. S., M. Atiq, F. Shehzad, A. Yasmeen, T. Nissa, A. Salam, A. Siddiqui & W. Jafri: Hepatitis E virus superinfection in patients with chronic liver disease. *Hepatology*, 36, 474-8 (2002)
45. Peron, J. M., J. M. Mansuy, H. Poirson, C. Bureau, E. Dupuis, L. Alric, J. Izopet & J. P. Vinel: Hepatitis E is an autochthonous disease in industrialized countries. Analysis of 23 patients in South-West France over a 13-month period and comparison with hepatitis A. *Gastroenterol Clin Biol*, 30, 757-62 (2006)
46. Billam, P., F. F. Huang, Z. F. Sun, F. W. Pierson, R. B. Duncan, F. Elvinger, D. K. Guenette, T. E. Toth & X. J. Meng: Systematic pathogenesis and replication of avian hepatitis E virus in specific-pathogen-free adult chickens. *J Virol*, 79, 3429-37 (2005)
47. Meng, X. J., P. G. Halbur, J. S. Haynes, T. S. Tsareva, J. D. Bruna, R. L. Royer, R. H. Purcell & S. U. Emerson: Experimental infection of pigs with the newly identified swine hepatitis E virus (swine HEV), but not with human strains of HEV. *Arch Virol*, 143, 1405-15 (1998)
48. Huang, F. F., Z. F. Sun, S. U. Emerson, R. H. Purcell, H. L. Shivaprasad, F. W. Pierson, T. E. Toth & X. J. Meng: Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. *J Gen Virol*, 85, 1609-18 (2004)
49. Williams, T. P., C. Kasorndorkbua, P. G. Halbur, G. Haqshenas, D. K. Guenette, T. E. Toth & X. J. Meng: Evidence of extrahepatic sites of replication of the hepatitis E virus in a swine model. *J Clin Microbiol*, 39, 3040-6 (2001)
50. Kasorndorkbua, C., B. J. Thacker, P. G. Halbur, D. K. Guenette, R. M. Buitenwerf, R. L. Royer & X. J. Meng: Experimental infection of pregnant gilts with swine hepatitis E virus. *Can J Vet Res*, 67, 303-6 (2003)
51. Kasorndorkbua, C., D. K. Guenette, F. F. Huang, P. J. Thomas, X. J. Meng & P. G. Halbur: Routes of transmission of swine hepatitis E virus in pigs. *J Clin Microbiol*, 42, 5047-52 (2004)
52. Meng, X. J., P. G. Halbur, M. S. Shapiro, S. Govindarajan, J. D. Bruna, I. K. Mushahwar, R. H. Purcell & S. U. Emerson: Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *J Virol*, 72, 9714-21 (1998)
53. Cooper, K., F. F. Huang, L. Batista, C. D. Rayo, J. C. Bezanilla, T. E. Toth & X. J. Meng: Identification of genotype 3 hepatitis E virus (HEV) in serum and fecal samples from pigs in Thailand and Mexico, where genotype 1 and 2 HEV strains are prevalent in the respective human populations. *J Clin Microbiol*, 43, 1684-8 (2005)
54. Caron, M., V. Enouf, S. C. Than, L. Dellamonica, Y. Buisson & E. Nicand: Identification of genotype 1 hepatitis E virus in samples from swine in Cambodia. *J Clin Microbiol*, 44, 3440-2 (2006)
55. Halbur, P. G., C. Kasorndorkbua, C. Gilbert, D. Guenette, M. B. Potters, R. H. Purcell, S. U. Emerson, T. E. Toth & X. J. Meng: Comparative pathogenesis of infection of pigs with hepatitis E viruses recovered from a pig and a human. *J Clin Microbiol*, 39, 918-23 (2001)
56. Wu, J. C., C. M. Chen, T. Y. Chiang, W. H. Tsai, W. J. Jeng, I. J. Sheen, C. C. Lin & X. J. Meng: Spread of hepatitis E virus among different-aged pigs: two-year survey in Taiwan. *J Med Virol*, 66, 488-92 (2002)
57. Maneerat, Y., E. T. Clayson, K. S. Myint, G. D. Young & B. L. Innis: Experimental infection of the laboratory rat with the hepatitis E virus. *J Med Virol*, 48, 121-8 (1996)
58. Tam, A. W., R. White, E. Reed, M. Short, Y. Zhang, T. R. Fuerst & R. E. Lanford: In vitro propagation and production of hepatitis E virus from in vivo-infected primary macaque hepatocytes. *Virology*, 215, 1-9 (1996)
59. Tam, A. W., R. White, P. O. Yarbough, B. J. Murphy, C. P. McAtee, R. E. Lanford & T. R. Fuerst: In vitro infection and replication of hepatitis E virus in primary cynomolgus macaque hepatocytes. *Virology*, 238, 94-102 (1997)
60. Emerson, S. U., P. Clemente-Casares, N. Moiduddin, V. A. Arankalle, U. Torian & R. H. Purcell: Putative neutralization epitopes and broad cross-genotype neutralization of Hepatitis E virus confirmed by a quantitative cell-culture assay. *J Gen Virol*, 87, 697-704 (2006)
61. Emerson, S. U., V. A. Arankalle & R. H. Purcell: Thermal stability of hepatitis E virus. *J Infect Dis*, 192, 930-3 (2005)
62. Tanaka, T., M. Takahashi, E. Kusano & H. Okamoto: Development and evaluation of an efficient cell-culture system for Hepatitis E virus. *J Gen Virol*, 88, 903-11 (2007)
63. Meng, X. J., S. Dea, R. E. Engle, R. Friendship, Y. S. Lyoo, T. Sirinarumit, K. Urairong, D. Wang, D. Wong, D. Yoo, Y. Zhang, R. H. Purcell & S. U. Emerson: Prevalence of antibodies to the hepatitis E virus in pigs from countries where hepatitis E is common or is rare in the human population. *J Med Virol*, 59, 297-302 (1999)
64. Zheng, Y., S. Ge, J. Zhang, Q. Guo, M. H. Ng, F. Wang, N. Xia & Q. Jiang: Swine as a principal reservoir of hepatitis E virus that infects humans in eastern China. *J Infect Dis*, 193, 1643-9 (2006)
65. Arankalle, V. A., M. V. Joshi, A. M. Kulkarni, S. S. Gandhe, L. P. Chobe, S. S. Rautmare, A. C. Mishra & V. S. Padbidri: Prevalence of anti-hepatitis E virus antibodies in different Indian animal species. *J Viral Hepat*, 8, 223-7 (2001)
66. Nakamura, M., K. Takahashi, K. Taira, M. Taira, A. Ohno, H. Sakugawa, M. Arai & S. Mishiho: Hepatitis E virus infection in wild mongooses of Okinawa, Japan: Demonstration of anti-HEV antibodies and a full-genome nucleotide sequence. *Hepatol Res*, 34, 137-40 (2006)
67. Tei, S., N. Kitajima, K. Takahashi & S. Mishiho: Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet*, 362, 371-3 (2003)
68. Yazaki, Y., H. Mizuo, M. Takahashi, T. Nishizawa, N. Sasaki, Y. Gotanda & H. Okamoto: Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *J Gen Virol*, 84, 2351-7 (2003)
69. Masuda, J., K. Yano, Y. Tamada, Y. Takii, M. Ito, K. Omagari & S. Kohno: Acute hepatitis E of a man who consumed wild boar meat prior to the onset of illness in Nagasaki, Japan. *Hepatol Res*, 31, 178-83 (2005)

70. Meng, X. J., B. Wiseman, F. Elvinger, D. K. Guenette, T. E. Toth, R. E. Engle, S. U. Emerson & R. H. Purcell: Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. *J Clin Microbiol*, 40, 117-22 (2002)
71. Hsieh, S. Y., X. J. Meng, Y. H. Wu, S. T. Liu, A. W. Tam, D. Y. Lin & Y. F. Liaw: Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. *J Clin Microbiol*, 37, 3828-34 (1999)
72. Drobeniuc, J., M. O. Favorov, C. N. Shapiro, B. P. Bell, E. E. Mast, A. Dadu, D. Culver, P. Iarovoi, B. H. Robertson & H. S. Margolis: Hepatitis E virus antibody prevalence among persons who work with swine. *J Infect Dis*, 184, 1594-7 (2001)
73. Olsen, B., D. Axelsson-Olsson, A. Thelin & O. Weiland: Unexpected high prevalence of IgG-antibodies to hepatitis E virus in Swedish pig farmers and controls. *Scand J Infect Dis*, 38, 55-8 (2006)
74. Renou, C., J. F. Cadranet, M. Bourlière, P. Halfon, D. Ouzan, H. Rifflet, P. Carencu, A. Harafa, J. J. Bertrand, A. Boutrouille, P. Muller, J. P. Igual, A. Decoppet, M. Eloit & N. Pavio: Possible zoonotic transmission of hepatitis E from a pet pig to its owner. *Emerg Infect Dis*, 13, 1094-1096 (2007)
75. Pavio, N., A. Boutrouille, C. Fablet, N. Rose, C. Renou, J. Bertrand, J. Naudy, A. Decoppet & M. Eloit: P.329 Presence of hepatitis E virus (HEV) in pigs enhances their possible role as reservoir for animal-to-human transmission in France. *J Clin Virol*, 36, S162-S163 (2006)
76. Pina, S., J. Jofre, S. U. Emerson, R. H. Purcell & R. Girones: Characterization of a strain of infectious hepatitis E virus isolated from sewage in an area where hepatitis E is not endemic. *Appl Environ Microbiol*, 64, 4485-8 (1998)
77. Kasorndorkbua, C., T. Opriessnig, F. F. Huang, D. K. Guenette, P. J. Thomas, X. J. Meng & P. G. Halbur: Infectious swine hepatitis E virus is present in pig manure storage facilities on United States farms, but evidence of water contamination is lacking. *Appl Environ Microbiol*, 71, 7831-7 (2005)
78. Teo, C. G.: Hepatitis E indigenous to economically developed countries: to what extent a zoonosis? *Curr Opin Infect Dis*, 19, 460-6 (2006)
79. Surya, I. G., K. Kornia, T. G. Suwardewa, Mulyanto, F. Tsuda & S. Mishiro: Serological markers of hepatitis B, C, and E viruses and human immunodeficiency virus type-1 infections in pregnant women in Bali, Indonesia. *J Med Virol*, 75, 499-503 (2005)
80. Meng, X. J.: Swine hepatitis E virus: cross-species infection and risk in xenotransplantation. *Curr Top Microbiol Immunol*, 278, 185-216 (2003)
81. Engle, R. E., C. Yu, S. U. Emerson, X. J. Meng & R. H. Purcell: Hepatitis E virus (HEV) capsid antigens derived from viruses of human and swine origin are equally efficient for detecting anti-HEV by enzyme immunoassay. *J Clin Microbiol*, 40, 4576-80 (2002)
82. Mansuy, J. M., J. M. Peron, F. Abravanel, H. Poirson, M. Dubois, M. Miedouge, F. Vischi, L. Alric, J. P. Vinel & J. Izopet: Hepatitis E in the south west of France in individuals who have never visited an endemic area. *J Med Virol*, 74, 419-24 (2004)
83. Emerson, S. U. & R. H. Purcell: Recombinant vaccines for hepatitis E. *Trends Mol Med*, 7, 462-6 (2001)
84. Purcell, R. H., H. Nguyen, M. Shapiro, R. E. Engle, S. Govindarajan, W. C. Blackwelder, D. C. Wong, J. P. Prieels & S. U. Emerson: Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. *Vaccine*, 21, 2607-15 (2003)
85. Shrestha, M. P., R. M. Scott, D. M. Joshi, M. P. Mammen, Jr., G. B. Thapa, N. Thapa, K. S. Myint, M. Fourneau, R. A. Kuschner, S. K. Shrestha, M. P. David, J. Seriwatana, D. W. Vaughn, A. Safary, T. P. Endy & B. L. Innis: Safety and efficacy of a recombinant hepatitis E vaccine. *N Engl J Med*, 356, 895-903 (2007)

**Key Words** :Hepatitis E virus, zoonosis, animal reservoir, cross-species infection.

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