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Zoonotic transmission of hepatitis E virus in industrialized countries

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SUMMARY

Hepatitis E is an infectious viral disease with clinical and morphological features of acute hepatitis. The disease represents an important public health problem in developing countries, where it is often related to outbreaks mainly associated with consumption of contaminated water. During recent years, an increasing number of sporadic cases have also been described in industrialized countries. Besides humans, the hepatitis E virus (HEV) has also been identified in animals. In 1997, the virus was first detected in swine, and is now considered ubiquitous. Human and swine HEV strains from the same geographical region present a high level of nucleotide identity, and experimental infections have confirmed the cross-species transmission of swine strains to humans and of human strains to non-human primates. Studies on anti-HEV antibodies detection have demonstrated that people working in contact with swine or wild boar have a higher risk of infection than normal blood donors. In Japan and more recently in France, cases of hepatitis E have been associated with ingestion of uncooked meat from pigs, wild boar, or deer. The disease is currently considered an emerging zoonosis.

KEY WORDS: Emerging diseases, Hepatitis E, Pigs, Public health, Viruses, Zoonoses.

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INTRODUCTION

Hepatitis E is an infectious viral disease with clinical and morphological features of acute hepatitis. The etiological agent is the hepatitis E virus (HEV), first identified in the early 1980s (Emerson and Purcell, 2003). The disease is an important public health concern in developing countries where it is frequently epidemic (Aggarwal, 2011). Industrialized countries were previously thought to be free from HEV, with a limited number of cases reported only in people who had travelled to endemic areas. However, more recent studies have documented a number of sporadic cases in developed areas, including Europe, among patients who had no history of travelling to hepatitis E endemic countries. Furthermore, a high anti-HEV seroprevalence has been detected in a significant proportion of healthy individuals of non-endemic countries (Aggarwal and Jameel, 2011).

Since the early 1990s, serological evidence of HEV infections and virus detection have been reported in many animal species both in developed and developing countries, suggesting the possibility that these species may become infected with HEV-like viruses (Emerson and Purcell, 2003). In 1997, a swine HEV strain was identified for the first time in the USA. The swine HEV strain resulted genetically correlated to two human HEV strains detected in the USA in the same period from patients who had not travelled to endemic areas (Meng *et al.,* 1997). Since then, swine HEV strains have been detected across the globe. Frequently, a strict genetic correlation between human and swine strains from the same geo-

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graphic region has been observed, and the possibility of cross-species transmission of swine strains to humans and of human strains to non-human primates has been demonstrated (Meng, 2011). Furthermore, several seroepidemiological studies have reported high antibody prevalence to HEV in people working in direct contact with swine or wild boar (Carpentier *et al.,* 2012; Withers *et al.,* 2002). The first direct evidence of a possible zoonotic transmission of HEV was provided in Japan in 2003, when cases of hepatitis E were caused by the ingestion of uncooked meat or organs from pigs, wild boar, or deer (Tei *et al.,* 2003; Yazaki *et al.,* 2003). More recently, a study conducted in France confirmed that 13 human cases of hepatitis E were eventually linked to the consumption of raw *figatellu* pig liver sausages (Colson *et al.,* 2010). The disease is now recognized as an emerging zoonosis.

ETIOLOGY

Taxonomy and nomenclature

The HEV is classified as the new genus *Hepevirus* in the family *Hepeviridae* (Emerson *et al.,* 2004; Emerson and Purcell, 2003). HEV strains detected in humans and other mammalian species represent the major genus of *Hepeviridae* (Table 1). Although avian HEV strains share only 50-60% nucleotide identity with mammalian HEV strains (Meng, 2010a), specific antibodies are able to cross-react with the capsid protein of both groups of viruses, demonstrating the presence of common epitopes (Haqshenas *et al.,* 2001). Nonetheless, avian HEV strains have never been associated with cases of infection in human beings (Kamar *et al.,* 2012).

Four genotypes of mammalian HEV are currently recognized (Figure 1), which primarily infect humans, domestic pigs, wild boar, deer, rabbit, and mongoose (Meng, 2011). However, genetically distant HEV strains have more recently been identified in the rat (Johne *et al.,* 2010b), ferrets (Raj *et al.,* 2012), wild boar (Takahashi *et al.*, 2011), bat (Drexler *et al.,* 2012), and cutthroat trout (*Oncorhynchus clarkii*) (Batts *et al.,* 2011), suggesting that the *Hepeviridae* family classification should be reviewed. Based on the sequence comparisons of HEV genomes currently available, strains are classified in genotypes and sub-genotypes. Despite the knowledge that different HEV genotypes occur, the virus seems otherwise to exist as a single serotype (Aggarwal and Naik, 2009). In industrialized countries, autochthonous human cases appear to be related to HEV strains be-

TABLE 1 - *Genotypes and host range of the hepatitis E viruses (adapted from (Meng, 2011).*

HEV strains	*Natural host*	*Geographic distribution*
Mammalian HEV		
Genotype 1	Humans	Burmese-like Asian strains
Genotype 2	Humans	A Mexican strain and some African strains
Genotype 3	Humans, domestic pigs, wild boars, deer, mongoose, rabbits	Worldwide
Genotype 4	Humans, domestic pigs, wild boars	Asia and Europe
Novel unclassified genotype, Rat HEV	Rats	
Novel unclassified genotype, Boar HEV	Wild boars	Japan
Novel unclassified genotype, Bat HEV	Bat	Worldwide
Novel unclassified genotype, Ferret HEV	Ferret	
Avian HEV		
Genotype 1	Chicken	
Genotype 2	Chicken	
Genotype 3	Chicken	
Genotype ?	Chicken	Hungary
Trout HEV		
Genotype ?	Cutthroat trout	USA

longing to genotypes 3 and 4 (Emerson and Purcell, 2003; Scobie and Dalton, 2013), which are still considered the only zoonotic genotypes. Genotype 3 was first identified in autochthonous human cases in the USA, when the 2 human strains US-1 and US-2, showed only 74-75% nucleotide identity with the previously known genotypes 1 and 2, and were accordingly classified separately (Meng *et al.,* 1997). Since then, genotype 3 has been detected worldwide, associated with sporadic cases and small outbreaks in North America, Europe, Japan and New Zealand (Dalton *et al.,* 2007). This genotype is also commonly detected in animals, and a strict genetic correlation has been consistently observed between human and animal strains circulating in the same geographical area. In fact, the first swine HEV strain, identified in the USA in 1997, shared a 92% nucleotide identity in ORF2 with human strains US-1 and US-2. Given the strict genetic correlation, the two viruses were classified in the same genotype 3, and since then pigs have been considered a reservoir of HEV (Meng *et al.,* 1997).

The other zoonotic genotype (genotype 4) is indigenous to Asia, where it has been recovered from both pigs and humans (Colson *et al.,* 2012). Genotype 4 is increasingly described as being endemic in pigs in Asia, and as the cause of sporadic cases of hepatitis E in humans and infection in swine in China, Japan, and recently in Europe (Colson *et al.,* 2012; Garbuglia *et al.,* 2013). The recent and increasingly frequent detection of genotype 4 in Europe is of concern for public health, and raises the question whether genotype 4 was somehow introduced into domestic pigs and may be expected to spread further in farms or whether it was confined to imported pig meat of Asian origin into Europe (Colson *et al.,* 2012).

Structure of the virus and genome organization

HEV is a small (27-34 nm), icosahedral, non-enveloped single-stranded positive-sense RNA virus.

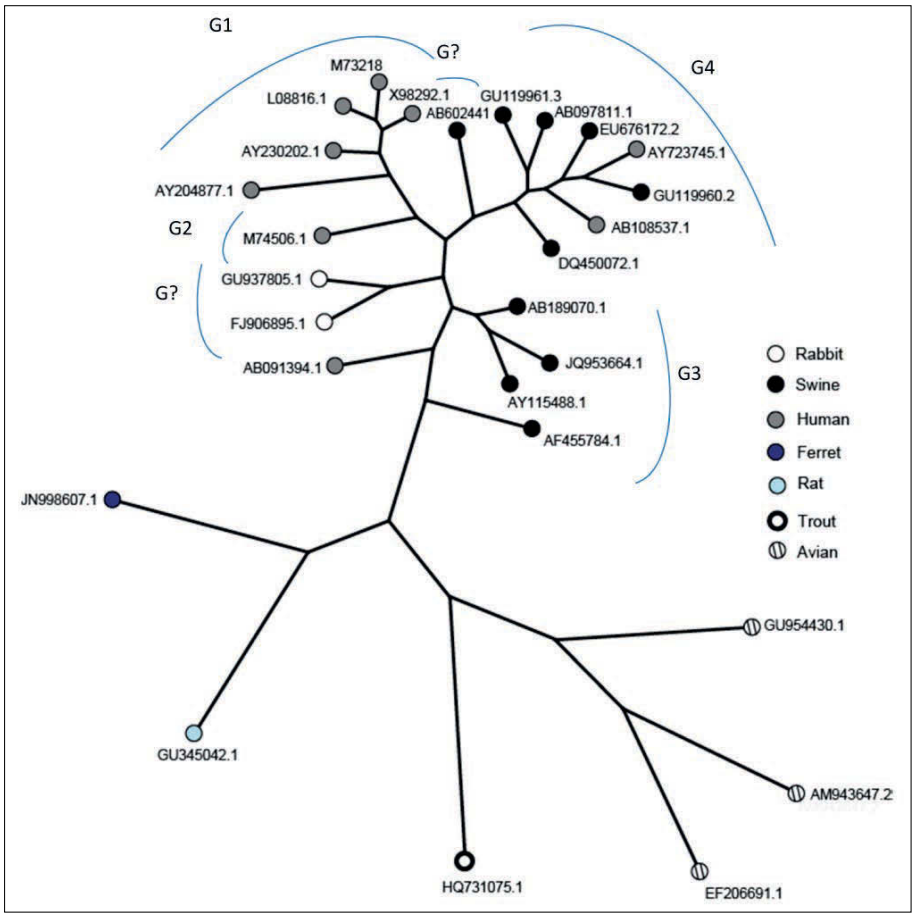

FIGURE 1 - *Phylogenetic tree illustrating different genotypes of hepatitis E. The tree is based on full-length sequences of HEV strains of either human or animal origin. The GenBank accession no. is indicated G1, G2,G3, G4 indicate genotype 1,2,3 and 4 respectively. G? are genotype not jet defined..*

The HEV genome is approximately 7.2 kb in length, and presents a 7-methylguanosine cap followed by three overlapping open reading frames (ORFs) and a second non-coding region of about 65-74 nucleotides with a 3′ poly A tail (Figure 2). The genome length slightly varies between animal strains although the genome organization seemed to be conserved in all these cases. The ORF1 (5073-5124 nt) codes for a non-structural polyprotein of about 1,690 amino acids, which is involved in viral genome replication and viral protein processing. The ORF3 (366-369 nucleotides) follows the ORF1, and overlaps the N-terminal portion of the ORF2 in a different reading frame, encoding a small phosphoprotein (pORF3). Recent studies have shown that pORF3 may be involved in virus release from infected cells (Okamoto, 2011).

The viral capsid protein encoded by ORF2 assembles into the complete virion, binds to host cells, and can elicit neutralizing antibodies. The virus capsid is made of 30 subunits containing homodimers of the pORF2 (Yamada *et al.,* 2009). Among the four major mammalian HEV genotypes, sequence identity between the amino acid residues of the capsid protein is >85%, and most amino acid divergence is found in the N-terminal 111 residues. Expression of a truncated capsid protein lacking the first 111 amino acids and/or the C-terminal 59 amino acids using the baculovirus expression system in insect cells resulted in self-assembly of the capsid protein (Xing *et al.*, 2011). Two types of HEV-like particle (HEV-VLP) were produced with different diameters, corresponding to different proteolytic cleavages. The recombinant HEV capsid protein is currently undergoing clinical trials as a vaccine candidate (Zhao *et al.,* 2013).

Epidemiology of human HEV infection

In developing countries, HEV strains belonging to genotypes 1 and 2 are responsible for most cases of hepatitis E. Infection is usually transmitted among humans by the fecal-oral route, and it has proven able to cause large outbreaks particularly when associated with the consumption of contaminated water.

In industrialized countries, several studies have reported high HEV seroprevalence rates (5-20%) among healthy individuals, suggesting widespread infection, which most likely occurs at a subclinical or asymptomatic level (Emerson and Purcell, 2003). The actual percentage of subjects seropositive to HEV might be even higher, if the more sensitive tests recently developed for anti-HEV antibody detection are used. Accordingly, the seroprevalence among blood donors in Toulose, France, was shown to rise from 16% to 52% using a modern assay with higher sensitivity than previous assays (Kamar *et al.*, 2012). Unlike genotypes 1 and 2, infections caused by genotypes 3 and 4 HEV strains appear to cause clinical hepatitis in middle-aged subjects and the elderly, but this unusual demography remains unexplained. Moreover, the high mortality associated with pregnancy and genotype 1 HEV infections has not been reported with either genotype 3 or 4 strains.

Until a few years ago, the few cases of hepatitis E in industrialized countries were reported only in people who had travelled to endemic areas. However, in the last decade sporadic cases or

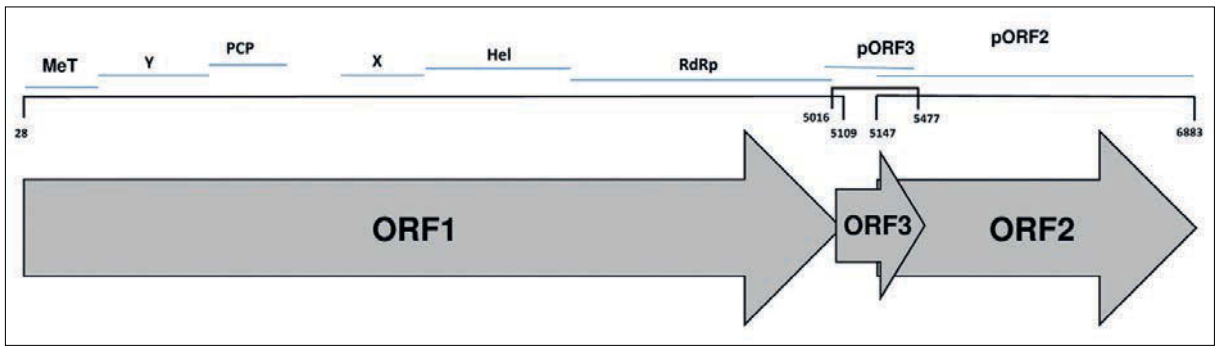

FIGURE 2 - *Genomic organization of HEV, including the 3 ORFs. On the top, functional domains are indicated: MeT methyltransferase, Y domain, PCP protease, X domain, Hel helicase, RdRp RNA dependent RNA polymerase, pORF2 capsid protein, pORF3.*

small clusters of cases have been recorded in subjects without a history of travel abroad, in the USA, Europe (including the United Kingdom, France, the Netherlands, Austria, Spain, Italy, and Greece), and in developed countries of Asia-Pacific (Japan, Taiwan, Hong Kong, Australia), suggesting the presence of autochthonous reservoirs of HEV in these areas (Meng, 2010a). In industrialized countries, infection with HEV normally causes sporadic cases or small outbreaks, and besides imported cases it seems to be at least partially associated with zoonotic transmission (Pavio *et al.*, 2010), and caused by genotype 3 and 4 strains (Figure 3).

As in other developed countries, most cases occurring in Italy are also associated with travel to endemic areas. The first identification of an autochthonous HEV dates back to 1999, when a genotype 3 virus similar to swine strain was identified in a patient who had neither travelled to nor had contact with individuals associated with endemic areas (Schlauder *et al.*, 1999). In Italy,

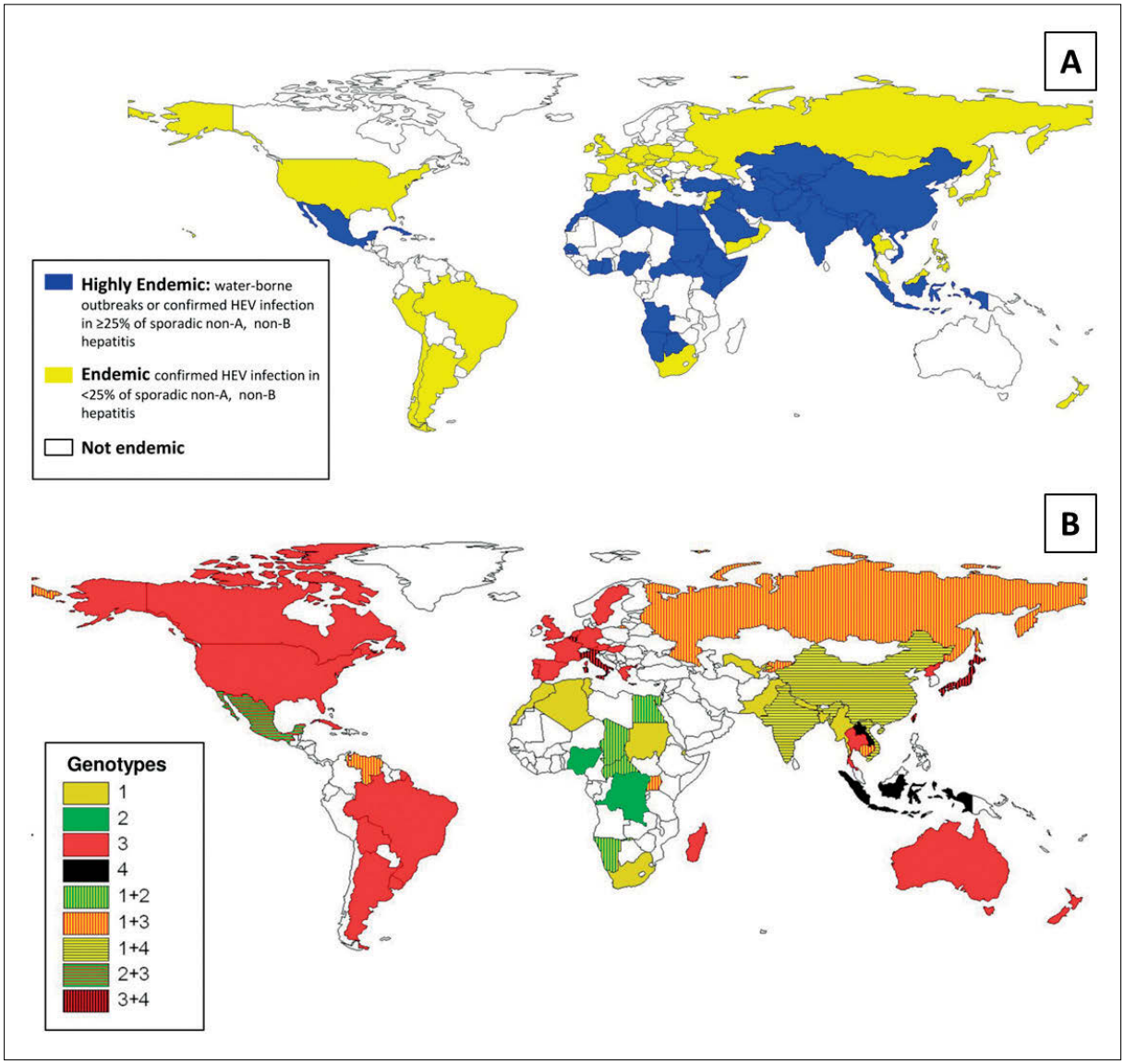

FIGURE 3 - *A: worldwide distribution of HEV infections; B: worldwide distribution of HEV genotypes. The colors used for each country represent the predominant HEV genotypes of human and animal strains (mostly pigs).*

HEV infection is thought to account for approximately 5-10% of reported cases of acute non-A-C viral hepatitis (Zanetti and Dawson, 1994), which would lead to estimates of just a few hundred cases per year. However, the number of reported cases of acute infection is probably underreported, due both to the frequent occurrence of sub-clinical infection and to the lack of specific serological testing in many clinical centers (Zuccaro *et al.,* 2012).
A recent detailed paper has attributed to HEV the etiology of 134 of 651 cases of non A-C hepatitis hospitalized during the period 1994 through 2009 in Northern Italy (Romano *et al.,* 2011). Of these patients, 22 were confirmed as being autochthonous. Although molecular typing of HEV strains was possible for only 5 cases, it is remarkable that these five indigenous strains were all genotype 3 HEV. Similar findings were reported in other studies throughout Europe (Kamar *et al.,* 2012). More recently, rare human cases linked to genotype 4 HEV strains, normally endemic in both man and pigs in Asia, have also been reported in Europe (Garbuglia *et al.,* 2013), but it was not concluded whether any of these cases may have originated via zoonotic or foodborne transmission. Nonetheless, these findings make it obvious to ask whether genotype 4 may spread further in Europe, as is presently the case for genotype 3 HEV.

Epidemiology of HEV infection in animals
Animal strains of HEV were discovered in domestic pigs (Meng *et al.,* 1997), wild boars (Sonoda *et al.,* 2004), chicken (Haqshenas *et al.,* 2001), rabbits (Zhao *et al.,* 2009), rats (Johne *et al.,* 2010b), deer (Tei *et al.,* 2003), mongoose (Nakamura *et al.,* 2006), ferrets (Raj *et al.,* 2012), and possibly also in cattle (Hu and Ma, 2010) and sheep (Wang and Ma, 2010). This together with the existence of other animal species that are seropositive for HEV antibodies (Meng, 2010a; Meng, 2010b) have significantly broadened the host range and diversity of HEV. Once the possibility of zoonotic transmission is accepted, it appears clear that the higher the prevalence is in animals, the greater is the risk of transmission to humans.
Besides the obvious implications of the ubiquitous presence of HEV in animals relevant for the food production chain, its spread in several other animal species raises public health concern for zoonotic infection also through direct contacts with infected animals, environmental contamination, particularly surface waters, via animal HEV shedding in feces, as well as recreational and professional exposure hazards in the countryside (Meng, 2010a; Meng, 2010b; Pavio *et al.,* 2010).

HEV infection in domestic pigs
Since the first identification of swine infection in 1997 in the USA (Meng *et al.,* 1997), several other swine strains have been isolated in North and Central America, Asia, Europe, Africa, New Zealand, and Australia. At least two genotypes of swine HEV, genotypes 3 and 4, have been definitively identified and characterized from pigs worldwide and, as in the case of human strains, swine strains have also been shown to present a high degree of nucleotide and phylogenetic divergence from region to region.
Swine strains, particularly those identified in industrialized countries, have often been related to human cases of disease in which no specific source of infection was identified (Meng *et al.,* 2002). In countries where HEV has been identified and serological studies have been performed, most pigs over the age of three to four months have been shown to possess HEV-antibodies (Clemente-Casares *et al.,* 2003; Meng *et al.,* 1997; van der Poel *et al.,* 2001). Swine younger than two months are usually seronegative or positive at low prevalence, whereas pigs over that age show seropositivity rates that often exceed 80%.
In recent years, virological surveys carried out in many countries (Berto *et al.,* 2012a; Berto *et al.,* 2012b; Di Bartolo *et al.,* 2012; Leblanc *et al.,* 2007) have detected HEV in high sample proportions (often >40%) from apparently healthy animals at slaughterhouses, next to entering the pork production chain and being commercialized.

HEV infection in wild and synanthropic animals
To date, studies aimed at evaluating the presence of HEV RNA or specific antibody in wild boars have been conducted in several European countries (Carpentier *et al.,* 2012; Martelli *et al.,* 2008; Rutjes *et al.,* 2010; Schielke *et al.,* 2009; Widen *et al.,* 2011). As HEV is excreted in the feces of infected animals, it can be speculated that HEV could be transmitted by contact with wild boar

or their feces (Rutjes *et al.,* 2010). These findings, together with the observation that HEV infection may be subclinical and can also be present in animals at an age when they are commonly hunted to be eaten are somewhat worrying because of the possible risk of transmission of HEV to man by either contact with infected boars or ingestion of contaminated undercooked meat or organs.

Antibodies and viruses were also identified in deer and roe deer in Europe and Japan (Matsuura *et al.,* 2007; Rutjes *et al.,* 2010; Tomiyama *et al.,* 2009), and as revealed by sequence analysis on strains from Sika deer in Japan and roe deer in Hungary, both strains belonged to genotype 3 of HEV (Forgach *et al.,* 2010; Takahashi *et al.,* 2004).

Antibodies to HEV and sequences of HEV strains were also identified in rats in Germany (Johne *et al.,* 2010b), in the USA, and in Vietnam (Li *et al.,* 2013). Detection of HEV in rats represent a major concern since rats living close to farms housing pigs might easily transmit the virus to pigs, suggesting a possible role of rats in spreading of the infections (Kanai *et al.,* 2012). However, HEV strains detected in rats can vary substantially. The HEV strains characterized from German rats shared only approximately 60% of sequence identity with human HEV strains (Johne *et al.,* 2010a), and phylogenetic analyses revealed that the rat HEV belonged to a putative novel genotype within the genus *Hepevirus*. Nevertheless, in a recent study conducted in the USA sequences were obtained from HEV-positive liver samples of wild *Rattus* spp., and all isolates belonged to the zoonotic HEV genotype 3, except one close to the recently discovered rat genotype from Germany (Lack *et al.,* 2012). It remains to be determined if this novel rat HEV can effectively cross any species barrier, and infect humans or other animals.

Recently, HEV sequences highly homologous to rat hepatitis E virus were detected in the feces of foxes, in the Netherlands. It is however unclear whether this virus is circulating among foxes or was derived from their prey (e.g., rats) (Bodewes *et al.,* 2013).

Several new HEV strains have also been isolated from farmed rabbits in China (Zhao *et al.,* 2009). The rabbit HEV belongs to the genotype 3, thus it is possible that the rabbit HEV may be zoonotic.

More recently, HEV RNA was detected in asymptomatic ferrets in the Netherlands (Raj *et al.,* 2012) and from 85 different species of bat (Drexler *et al.,* 2012). As revealed by sequence analysis these novel viruses may constitute a distinct genus within the highly diversified family *Hepeviridae*.

Altogether, these findings suggest that hepeviruses may have first appeared in mammalian hosts a long time ago, and have subsequently undergone differentiation into genera according to different host restrictions. Human HEV-related viruses found in farmed and peridomestic animals might thus represent more recent secondary entry of human viruses in these animal species, rather than being animal precursors causally involved in the evolution of human HEV (Drexler *et al.,* 2012).

Other potential animal reservoirs for HEV

Serological evidence of HEV infection has also been reported in a number of other animal species including dogs, cats, goats, rhesus monkeys, cows, cattle, and horses (Arankalle *et al.,* 2001; Geng *et al.,* 2010; Meng, 2010a; Peralta *et al.*, 2009; Tsarev *et al.,* 1994; Zhang *et al.,* 2008). Thus far, the real basis of anti-HEV seropositivity in these animal species is uncertain. In fact, virus or HEV-specific genome sequences were not recovered or were detected only sporadically from these animal species (Hu and Ma, 2010; Wang and Ma, 2010). Thus, it is likely that more and new animal strains of HEV exist, but more studies are needed before the natural history of HEV may be considered fully disclosed.

Non zoonotic transmission

There is no clear demonstration sustaining that direct viral transmission of HEV from person to person is indeed an efficient method for transmitting infection. However, virus spread from infected individuals by fecal shedding in the environment inside confined areas or via contaminated fomites might play a role. Different immigration fluxes from countries where hepatitis E is endemic might explain the difference observed in the anti-HEV seroprevalence between northern and southern regions of Italy (Scotto *et al.,* 2013; Zanetti and Dawson, 1994). However, the habit of eating raw shellfish common in southern Italy could also be considered an additional risk factor, possibly also favored by HEV shedding by infected immigrants into sewage and by consequent coastal seawater pollution.

A marked gradient of anti-HEV seroprevalence was also shown from North to South of France, but in this case the risk factors seemed to be several, including personal water supplies, particular food eating habits, and possession of pet pigs (Renou *et al.,* 2008).
A mix of different transmission modes acting simultaneously may have fed the unique outbreaks involving passengers on a ship returning from a world cruise in 2008, causing overt hepatitis E with jaundice in four patients and IgM seroconversion in 4% of the 789 subjects tested (Said *et al.,* 2009). Overall, 25% of passengers showed anti-HEV IgM and/or IgG, indicating both recent and past infections. The virus detected in patients was a single genotype 3 HEV similar to strains circulating in Europe, suggesting a common source of infection, and seafood consumption was a risk factor. However, from the experience built from norovirus outbreaks aboard cruise ships, it cannot be excluded that virus transmission was favored by other routes, such as water or environmental contamination in common areas.
Unlike other systemic viral infections (such as HIV, HBV, and HCV), diverging data exist on the association between HEV transmission and injection drug use (IDU). Specific seroprevalence ranged from 2% to over 60% in different countries, although significant differences between IDU subjects and healthy blood donors have not been observed in all cases (Gessoni and Manoni, 1996). This might be related to either low viral load in the blood or transient viremic status for HEV, which might explain the higher impact of blood, blood products transfusion or organ transplantation as a risk factor for hepatitis E (Halac *et al.,* 2012). There is no evidence of sexual transmission (Kamar *et al.,* 2012; Scobie and Dalton, 2013).
Finally, vertical transmission from mother to the fetus has been reported frequently, exiting in the death of the fetus (Aggarwal, 2011).

Zoonotic transmission

Early after the discovery of swine HEV in 1997, and a few years later in other animals, the existence of endemic animal reservoirs was questioned with respect to the sporadic cases reported in humans in industrialized countries. The ability of genotype 3 HEV strains to cross species barriers has been widely supported (Meng, 2010a), and the foodborne transmission of animal viruses from pork, wild boar and deer has been confirmed by different evidence.
The ability of HEV to cross the species barrier was confirmed by experimental infections of Specific Pathogen-Free piglets with a human genotype 3 virus, and by similar demonstration that swine genotypes 3 and 4 strains are able to infect non-human primates (Meng, 2011), in support of the observations that genotype 3 strains typically detected in swine can naturally infect humans (Pavio *et al.,* 2010). Since the molecular basis for host-pathogen interaction in HEV is still largely unknown, strains that normally present lower virulence might lead to a more severe course of disease in particular host conditions (Bouquet *et al.,* 2012a). Different levels of restriction in the cross-species infection with animal HEV strains exist, as clearly shown in a recent study conducted on recently reported rat and rabbit HEV strains (Cossaboom *et al.,* 2012).
Finally, important evidence supporting the occurrence of zoonotic transmission of HEV has derived from the phylogenetic analysis of human and swine strains isolated in different regions of the world. Many studies have in fact reported full identity or close nucleotide and amino acid similarities between human and swine strains from the same geographic region (Lu *et al.,* 2006; Meng, 2010b), that were often closer than between strains detected from the same species but in different countries.

Foodborne transmission

There is already some clear evidence linking the onset of hepatitis E to the consumption of contaminated food items, resulting in either sporadic cases or epidemic outbreaks. In Japan, analysis of risk factors and molecular characterization of HEV from 10 patients with fulminant hepatitis E showed that the patients had eaten grilled or undercooked pig liver 2-8 weeks before onset. The HEV RNA sequences found in clinical specimens were identical or similar to HEV detected in packaged pig liver sold in the market or farm swine samples (Yazaki *et al.,* 2003). Further observations confirming the association between pig liver or uncooked meat consumption, wild boar, or deer, and hepatitis E were re-

ported in the following years also in Europe (Bouquet *et al.,* 2012b; Colson *et al.,* 2010; Dalton *et al.,* 2007).

Sequences of HEV strictly related genetically to those recovered from human cases were detected in samples of raw smoked liver sausages (i.e. *figatellu*) during a case-control study carried out in Corsica, France in 2010 (Colson *et al.,* 2010).

In assessing the risk related to the presence of HEV in food, some facts may play a significant role and need to be evaluated, such as the infectious dose, which is still unknown, previous exposures (immunity), pregnancy, and the presence of other diseases.

Pig meat may be a vehicle of infection for consumers. The presence of HEV in pig liver or pork at grocery stores was confirmed in the USA (Feagins *et al.*, 2007), the Netherlands (Bouwknegt *et al.*, 2007), UK (Berto *et al.*, 2012a), Italy, Spain, and the Czech Republic (Di Bartolo *et al.,* 2012), and a recent study confirmed that HEV present in pork liver sausage is infectious, highlighting the actual risk for consumers (Berto *et al.,* 2013).

Cross-contamination can occur during swine slaughtering, and in fact slaughterhouse tools (knives) and surfaces (belt and floor) were found positive for HEV RNA (Di Bartolo *et al.,* 2012). Following hygienic practices and cooking procedures may also play an important role in maintaining a high level of contamination with infectious virus.

To better understand the possible HEV inactivation during industrial processing, pâté-like preparations were produced and different time/temperature combinations were applied, ranging between 62°C and 71°C, and 5 to 20 min (Barnaud *et al.,* 2012). The residual infectivity was tested by experimental inoculation of homogenates intravenously to a group of pigs, testing both the seroconversion and fecal virus shedding. Only treatments of pâté-like preparations at 71°C for 20 min allowed a complete loss of infectivity, which was only partially reduced under different conditions.

In addition to meat from infected animals, the use of HEV-containing pig manure, or water contaminated with animal or human waste for land application and crop field irrigation may lead to contamination of other foodstuffs, such as produce or shellfish, by runoff into rivers and coastal waters, and eventually cause disease among consumers (Halac *et al.,* 2012; Renou *et al.,* 2008).

Infectious swine HEV was demonstrated to be present in pig manure storage facilities in farms in the USA (Kasorndorkbua *et al.,* 2005). Epidemiological evidence strongly suggests that HEV can persist in environmental waters and in soil (Parashar *et al.,* 2011). HEV has been detected in wastewater deriving from gut processing at slaughterhouses and in pig slurry stores (Rutjes *et al.*, 2009). A recent investigation conducted in three European countries demonstrated that HEV was found in 4.8% of lettuce sampled at primary production level and in 3.2% of samples at the point of sale, and in 5% of irrigation water samples (Kokkinos *et al.,* 2012).

HEV was also detected in shellfish, such as mussels and oysters farmed in Scotland (Crossan *et al.,* 2012) and in bivalve shellfish (*Corbicula japonica*) in Japan (Li *et al.,* 2007). An association between shellfish consumption and HEV infection was suspected in outbreaks (Said *et al.,* 2009). Consumer habits play a fundamental role in the risk of HEV transmission. However, it should be noted that compared to norovirus, the presence of HEV in mussels seems to be rare, possibly due to the different binding affinity of HEV to hepatopancreas cells or to a lower release into the environment.

Professional exposure transmission

Human populations with occupational exposure to environmental sources of domestic animal waste and wild animals (professional categories such as veterinary surgeons, farmers, slaughterhouse workers, people assigned to the care of animals may be at risk) have been shown to present higher anti-HEV serum antibody rates than normal blood donors or normal citizens, in several studies. For instance, 26% of veterinarians were found to be seropositive to HEV compared to 18% of regular blood donors in the USA (Meng *et al.,* 2002), and HEV antibody prevalence was reported to be 4.5 times higher in subjects exposed to contact with pigs than in normal people (Withers *et al.,* 2002). In addition, HEV RNA or specific antibodies were detected in slaughterhouse workers or butchers (Dalton *et al.,* 2007), suggesting that HEV transmission from pigs to humans during slaughtering may also occur.

Sewage workers were shown to have a signifi-

cantly higher anti-HEV seroprevalence than normal individuals, that increased with the numbers of years spent in that job, highlighting a specific occupational risk (Vaidya *et al.,* 2003).

CONCLUSIONS

Epidemiological and virological studies conducted in the last few years have clearly demonstrated that hepatitis E should be considered an emerging zoonosis. Swine appears to represent the major animal reservoir for the virus, and the large global relevance of the pork food chain creates public health concerns for the future. HEV infection can be transmitted through food by the ingestion of infected meat products (Tei *et al.,* 2003). However, the possibility of cross-contamination between raw meat products and the risk of virus spread in the environment through manure from pig farms, with the consequent possible contamination of vegetables and drinking or bathing water, should also be taken into consideration. Contamination of water could also lead to the contamination of filtering shellfish, thereby further compounding public health risks.

Another possible route of transmission of hepatitis E virus to humans is direct contact with infected animals. In this case, people such as farmers, workers attending the animals, and veterinarians who work in contact with pigs during the viremic period or when the virus is excreted with the feces may be at greater risk of infection (Withers *et al.,* 2002; Yazaki *et al.,* 2003). Furthermore, for these population categories the possibility of infection by indirect contact with instruments and tools contaminated with infected feces cannot be ruled out. Knowledge of these risks should thus encourage the implementation of hygiene and biosecurity procedures that may help avoid or minimize the chances of infection.

The enzootic nature of swine HEV infection in pigs in many countries, together with the virus ability to cross the species barrier, raise concerns with regard to the possibility of zoonotic transmission, and food and environmental safety. Nevertheless, many veterinary aspects of infection are not yet known. Knowledge is still limited with respect to the genetic correlation between different animal and human strains of HEV. The natural history of infection in pigs also requires further study, as does the economic impact of the disease on pig production. The host range of the infection is not fully known, and the cases associated with the ingestion of uncooked meat from wild boars and deer in Japan indicate that the role of wild animals, as well as swine and ruminants, should be further considered in the epidemiology of the disease.

Despite the obvious health implications of this emerging zoonosis, information on the significance of HEV circulation in swine herds, and in other animals in developed countries including Europe is still insufficient. In countries like Spain, Italy, the Netherlands, and the UK, where larger epidemiological studies have been conducted, it has been demonstrated that the virus is circulating actively in the pig herds. Considering the possibility of zoonotic transmission of the infection as actual, a higher prevalence in animals would certainly imply a greater risk of transmission to humans, requiring more attention to the veterinary public health and food safety aspects of pork production.

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