MOLECULAR EPIDEMIOLOGY OF HEPATITIS E VIRUS (HEV) IN SOUTH AMERICA: CURRENT STATUS

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ABSTRACT

Hepatitis E virus (HEV) is a common causative agent of acute hepatitis in many developing countries where standards of sanitation and hygiene are poor. HEV is transmitted primarily by the fecal–oral route. However, zoonotic transmission from animal reservoirs to human by consumption of raw or undercooked meat has also been suggested. In endemic areas, HEV infection occurs as large waterborne epidemics and/or small outbreaks. Sporadic cases of HEV infection have also been reported in developed countries, where its occurrence is not always associated with travel to highly endemic areas. HEV mammalian isolates are classified into four genotypes 1-4, which appear to have a specific host range and geographical distribution. The epidemiology of HEV in South America seems to be complex and seroprevalence in human and domestic pigs differs both among populations within the same countries and between countries. Additionally, HEV strains have been detected and molecularly characterized and genotype 3 is the most frequently genotype found in the region. Moreover, except for two strains isolated from autochthonous cases of acute hepatitis that occurred in Venezuela and belonged to genotype 1, all sequences detected in South America were classified as within genotype 3. Our understanding of HEV epidemiology has undergone major changes in recent years and despite the fact that several attempts to shed light over the epidemiology of this infection have been carried out in South America, data about the molecular characterization of HEV isolates is still lacking and further investigation is needed. This review summarizes the current status of HEV molecular epidemiology from a regional point of view.

Keywords: Hepatitis E, molecular epidemiology, South America.

INTRODUCTION

Hepatitis E virus (HEV), formerly known as enterically transmitted non-A, non-B hepatitis virus, is the aetiological agent of acute hepatitis E. It is considered to be endemic in many developing countries in Asia, Africa, including the Mediterranean region and Mexico where standards of sanitation and hygiene are poor (Aggarwal and Krawczynski, 2000; Purcell and Emerson, 2001). HEV infection is moderately severe and generally self-limiting, with death rates of ≤4% in the general population, and as high as 20% in pregnant women (Khuroo, 1980). Symptomatic disease is observed most frequently in young people and adults (14-40 years old) (Purcell and Emerson, 2008). The earliest well-documented report of the disease was a large epidemic of water-borne hepatitis occurring in New Delhi, India in 1955 (Vishwanathan, 1957). Even tough it was initially believed to have been related to hepatitis A virus, subsequent testing of stored sera from this epidemic and another outbreak during 1978 to 1979 in Kashmir, India failed to demonstrate serological markers for hepatitis A and B viruses (Khuroo, 1980). HEV is transmitted primarily by the fecal–oral route, and has been reported to occur as large waterborne epidemics and small outbreaks. Sporadic cases of HEV infection have also been reported in non-endemic, developed countries, where its occurrence is usually associated with travel to endemic areas (Hino et al. 1991; Dawson et al. 1992; Herrera, 1993; Ishikawa et al. 1995; Donati et al. 1997). Recently, accumulating lines of evidence indicate that HEV-associated hepatitis also occurs among individuals in industrialized countries who have no history of travel to endemic areas (Kwo et al. 1997; Harrison, 1999; Purcell and Emerson, 2001; Smith, 2001; Okamoto et al. 2003; Mansuy et al. 2004; Ijaz et al. 2005; Mizuo et al. 2005; Amon et al. 2006). Over the last decade, an increasing number of autochthonous locally acquired cases have been reported in several developed and developing countries and evidence for a zoonotic reservoir has been uncovered (Meng et al. 1997, 1998; Erker et al. 1999; Harrison, 1999; Meng, 2000, 2003; Okamoto et al. 2001; Smith, 2001; Nishizawa et al. 2003;
RNA polymerase. ORF3 encodes a small phosphoprotein (Papain-like protease; ORF1 encodes a non-structural polyprotein comprising approximately 660 amino acids (aa), is the only structural protein of the virus which assemble into a highly structured multimer (60 copies) (Reyes et al. 1993; Mushahwar et al. 1992; Huang et al. 1995). Notably, the size differences among different HEV strains are confined mainly to this region (Tsarev et al. 2001). Moreover, genotypes 1 and 2 appear to be anthroponotic, since they have been isolated exclusively from human cases (Lu et al. 2006; Teo, 2010). By contrast, genotypes 3 and 4 are extremely diverse (Okamoto, 2007), being divided into ten (3a–j) and seven (4a–g) subtypes, respectively (Lu et al. 2006). HEV strains of these genotypes have been found in outbreaks of HEV, which are usually due to consumption of contaminated drinking water (Purcell and Emerson, 2001). Moreover, genotypes 1 and 2 appear to be anthroponotic, since they have been isolated exclusively from human cases (Lu et al. 2006; Teo, 2010).

HEV genome organization

Viral genome is approximately 7200 nucleotides (nt) in size and is capped and polyadenylated (Reyes et al. 1993; Kabrane-Lazizi et al. 1999b). It consists of a short 5′ untranslated region (UTR) (27 to 35 nt), tree discontinuous and partially overlapping open reading frames (ORFs); ORF1, ORF2 and ORF3, and a short 3′ UTR (65 to 74 nt) that is terminated by a poly (A) tract (Tam et al. 1991) (Fig. 1). The 5′ and 3′ ends of the viral genome are non-coding regions and cis-acting elements, probably involved in the regulation of the viral replication and translation (Tam et al. 1991).

ORF1 encompassing approximately two thirds of the viral genome, is located at the 5′ end and is approximately 5000 nt in size. This region of the genome contains several conserved domains and encodes viral non-structural proteins that are involved in RNA synthesis including putative methyl transferase, guananyl transferase, papain-like cysteine protease, RNA helicase and RNA-dependent RNA polymerase (Koonin et al. 1992; Agrawal et al. 2001; Magden et al. 2001). The hypervariable region (HVR), a non-coding region within ORF1, displays substantial genetic diversity (Tsarev et al. 1992; Huang et al. 1995). Notably, the size differences among different HEV strains are confined mainly to this region (Huang et al. 2004). Recently, it was proposed that HVR modulates the efficiency of HEV replication and translation (Pudupakam et al. 2011).

ORF2, a coding region located at the 3′ end of the genome, is approximately 2000 nt in size and encodes the capsid protein. Probably, this protein of about 660 amino acids (aa), is the only structural protein of the virus which assemble into a highly structured multimer (60 copies) (Reyes et al. 1993; Mushahwar et al. 1996; Pavio et al. 2010). ORF2 is putatively translated from a subgenomic RNA (Purcell and Emerson, 2001) and contains 3 putative N-glycosylation sites and an endoplasmic reticulum signal peptide (Graff et al. 2008).

ORF3 consists of 372 nt and overlaps ORF1 by one nucleotide at 5′ end and extensively overlaps ORF2 by 331 nt at the 3′ end (Okamoto, 2007; Mushahwar, 2008). The third ORF encodes for a small phosphoprotein of 123 aa associated with cytoskeleton, and is involved in virus replication and virion morphogenesis. Recently, its role in virus release from infected cells has been demonstrated (Yamada et al. 2009; Nagashima et al. 2011). In addition, ORF3 is necessary for viral infection in Rhesus macaques in vivo, but not in vitro (Graff et al. 2005).

Genetic variability

Although a single serotype has been proposed (Purcell, 1994), extensive genomic diversity has been observed among HEV isolates (Okamoto et al. 2007). Mammalian HEV sequences have been classified into four major genotypes (1– 4) (Schlauder and Mushahwar, 2001), according to analysis of the complete genome sequence (Lu et al. 2006) and/or variable partial HEV genomic regions including a 371-nt region in ORF1 and a 147-nt region in ORF2 (Schlauder et al. 1998; Zanetti et al. 1999; Schlauder and Mushahwar, 2001). Recently, the existence of a new HEV genotype infecting rabbits was proposed (Zhao et al. 2009).

HEV genotypes were further subdivided into subtypes which are defined on the basis of five different phylogenetic reconstructions; 5′ ORF1, 3′ ORF1, 5′ ORF2, 3′ ORF2, and complete genome (Lu et al. 2006).

HEV genotype 1 is classified into five subtypes, 1a–e, and genotype 2 into two subtypes, 2a and 2b (Lu et al. 2006). These genotypes are relatively conserved and are mostly associated with large epidemics and outbreaks of HEV, which are usually due to consumption of contaminated drinking water (Purcell and Emerson, 2001). Moreover, genotypes 1 and 2 appear to be anthropoctic, since they have been isolated exclusively from human cases (Lu et al. 2006; Teo, 2010).

By contrast, genotypes 3 and 4 are extremely diverse (Okamoto, 2007), being divided into ten (3a–j) and seven (4a–g) subtypes, respectively (Lu et al. 2006). HEV strains of these genotypes have been found in

**Figure 1.** Organization of the HEV genome. Scheme showing the organization of the three viral open reading frames (ORF’s); ORF1 encodes a non-structural polyprotein comprising Met, Methyl transferase; Y, Y-domain (non-function assigned); Pr, Papain-like protease; H, Hypervariable region (HVR, in text); X, X-domain (non-function assigned); Hel, RNA helicase; RdRp, RNA polymerase. ORF3 encodes a small phosphoprotein (Ph). ORF 2 encodes the capsid protein (Cap).
humans and animal reservoirs including swine, wild boar and deer (Pavio et al. 2010). It is proposed that the high diversity of genotypes 3 and 4 appears to relate to their zoonotic origin (Lu et al. 2006).

A report regarding pairwise comparison of the entire genome of 75 HEV isolates revealed an inter-genotype difference of 23.6–27.7% (Okamoto, 2007). Furthermore, genotype 1 has up to 11.8% of intra-genotypic diversity, while genotypes 3 and 4 show a wide range of intra-genotype variability (0–19.3% and 0.1–17.0%, respectively) (Okamoto, 2007). However, in view of the fact that the sequences available of genotype 2 are scant, the information of their intra-genotypic diversity is more limited. In this case, the intra-genotype differences was observed on the basis of the only complete nucleotide sequence, the single Mexican strain (MEX–14) (Huang et al. 1992) and partial nucleotide sequences of a 3’ ORF2 region of 16 African strains. The pairwise comparison of these sequences indicated that the 16 African isolates differ from each other by up to 10.3% and from Mexican isolate by 12.3–16.8% (Okamoto, 2007).

**Geographical distribution**

HEV is endemic worldwide and the different genotypes appear to have a specific geographical distribution (Fig. 2).

Genotype 1 consists of epidemic strains and has been reported in various countries in Asia (Bangladesh, China, India, Vietnam, Pakistan, Nepal) and Africa (Chad, Egypt, Sudan, South Africa), Cuba and, recently, Argentina (an imported case from Asia) and Venezuela (Okamoto, 2007; Montalvo et al. 2008; Munné et al. 2011; García et al. 2012). Genotype 2 sequences were first reported from an epidemic in Mexico (Huang et al. 1992) and subsequently identified from endemic cases in several African countries (Buisson et al. 2000; Maila et al. 2004; Nicand et al. 2005). Genotype 3 is widely distributed and has been isolated from sporadic cases of acute human HEV infection and/or domestic pigs in several countries throughout the world (Okamoto, 2007). In addition, strains of HEV genotype 3 have been identified in many other animal reservoirs including wild boar (Sonoda et al. 2004; De Deus et al. 2008) and deer (Tei et al. 2003; Reuter et al. 2009). Moreover, genotype 3, the most prevalent HEV genotype worldwide, possesses a varied distribution of its 10 subtypes. Subtypes 3a and 3j strains have been mainly identified in North America; 3b, 3d, and 3g strains in Asia; and 3c, 3e and 3f in Europe. Subtypes 3h and 3i are rare but have been found in Italy, Austria and New Zealand (Lu et al. 2006). Genotype 4 comprises strains from human and domestic pigs and it is found in Asian countries (Meng et al. 1998; Schlauder and Mushahwar, 2001; Lu et al. 2006; Lorenzo et al 2007; Okamoto, 2007). Recently, a strain isolated from swine in central Europe was also reported (Hakze-van der Honing et al. 2011). Furthermore, it has been reported that in addition to pigs, wild boars are also reservoirs for genotype 4 HEV in humans (Sato et al. 2011).

**HEV infection as a zoonosis**

HEV is unique among the known hepatitis viruses in that it has an animal reservoir (Pavio et al. 2010). In fact, after the successful experimental transmission of human HEV to domestic pigs, accumulating lines of evidence have suggested that HEV is a zoonosis. The isolation of a novel swine HEV closely related to human HEV...
achieved by Meng et al. (1997), provided direct evidence of the involvement of domestic pigs in the spread of this infection. Since then, many swine HEV isolates have been identified in many countries worldwide (Okamoto, 2007).

In addition to pigs, other animals also have been shown to be susceptible to infection with HEV, and as such serve as reservoir of HEV in nature. These animals are boars, deer, cows, sheep, goats, camels, horses, dogs, cats, rats, mongoose and probably rabbits (Kabrane-Lazizi et al. 1999a; Favorov et al. 2000; Arankalle et al. 2001; Matsuda et al. 2003; Tei et al. 2003; Takahashi et al. 2004; Nakamura et al. 2006; Saad et al. 2007; Geng et al. 2011). Even though several animal species have been identified as potential HEV reservoirs, domestic pigs and wild boars are the main animal reservoir for the genotypes 3 and 4 strains worldwide (Meng, 2009).

HEV infection by eating contaminated food was supported by several pieces of evidence. Consumption of raw or undercooked meat from wild boar and deer has been identified as a source of HEV infection in humans (Matsuda et al. 2003; Sonoda et al. 2004; Takahashi et al. 2004; Tamada et al. 2004; Tei et al. 2004; Li et al. 2005). Yazaki et al. (2003) investigated a series of 29 cases of sporadic hepatitis E occurring in Japan and identified nine patients with a history of having recently ingested grilled or undercooked pig liver 2–8 weeks before the onset of the disease. Additionally, HEV RNA has been detected in 6.5 and 11% of commercial pig liver in Netherlands and United States, respectively (Bouwknecht et al. 2007; Feagins et al. 2007). All strains detected were classified as HEV genotype 3.

These reports suggest that zoonotic foodborne transmission of HEV plays an important role in the occurrence of hepatitis E and also raise a public health concern.

**HEV Status in South America**

In South America, where endemic areas exist, data regarding HEV prevalence and molecular epidemiology in humans and animal reservoirs is still scant. However, in the last few years several reports on this matter have arisen and HEV has become a main focus of interest for researchers.

**Seroprevalence in humans and pigs**

Although HEV was discovered in the early 80’s (Balayan et al. 1983), the first serological surveys regarding the circulation of HEV in humans in South America arose more than 10 years later.

In Venezuela, Puyol et al. (1994) reported seroprevalence rates ranging from 1.6 to 5.4% in three different populations (pregnant women of urban area, rural people and Amerindians).

Meanwhile, in Chile the presence of anti-HEV antibodies was studied in 1.773 samples. Anti-HEV was detected in 8.0%, 12.5%, 7.5% and 17% in blood donors, health care workers, inmates and Araucanian Indians, respectively. Prevalence of anti-HEV was not related to age and/or sex (Ibarra et al. 1997). In addition, anti-HEV specific antibodies were detected in 1.2% of low-social economic status children (Ibarra et al. 2006).

On the other hand, studies conducted with select Uruguayan and Argentine blood donor populations revealed a specific prevalence of anti-HEV antibodies of 1.2 and 1.8%, respectively (Cruells et al. 1997; Rey et al. 1997).

A study carried out by Bartoloni et al. (1999) with rural populations in Bolivia showed a seroprevalence ranging from 4.4 to 10.4 % with no significant differences between genders. In Peru, although data regarding seroprevalence in general population do not exist, anti-HEV IgG was detected in 10.5% of the subjects comprising a risk group (Vildosola et al. 2000).

In several select population in Brazil, the prevalence of anti-HEV IgG has been reported as 1.0 and 4.3% in pregnant women and intravenous drug users, respectively (Trinta et al. 2001). In blood donors the seroprevalence was 2.3 and 11.8% in two different studies (Trinta et al. 2001; Bortolierio et al. 2006). Da Silva et al. (2012) have recently reported a seroprevalence of 8.4% in individuals exposed to swine in Matto Grosso, which did not characterise this type of exposure as a risk factor for HEV infection in this region.

Once the potential role of pig as reservoir HEV infection was suggested, several studies have been carried out in order to determine the prevalence of anti-HEV antibodies in swine herds in South America.

The average seroprevalence among the different surveys varies greatly from study to study: 23% in Argentina (Munné et al. 2006), 81% in Brazil (Guimaraes et al. 2005) and 5% in Chile (Reinhardt et al. 2003). The observed high variability of seroprevalence rate results from important differences within each herd (4 to 58% for the Argentine study, 15 to 100% for the Brazilian study). Similarly, in Chile the HEV seroprevalence in pigs varies significantly among geographical regions with rates ranging 0.6 to 9.5% (Reinhardt et al. 2003).

In Uruguay, as well as in most of the countries in South America, the HEV serological status of swine herds remains unknown and no data has been reported.

**Molecular epidemiology**

The molecular epidemiology of HEV in South America is complex and the modes of transmission and sources of infections of HEV are not well understood. Despite the fact that several attempts to shed light over the epidemiological status of this infection have been carried out in South America, data about the molecular characterization of HEV isolates is still lacking and further investigation is needed.

HEV RNA in South American countries was
first isolated from two Argentinean patients with acute hepatitis who reported no history of travel to regions in which HEV was considered endemic at the time (Schlauder et al. 2000). In this study, strains showed no relatedness with other isolates previously described of HEV and were preliminarily classified as a new viral genotype. Later, Munné et al. (2006) reported for the first time in the region, the detection and molecular characterization of the first swine strains of HEV isolated from faecal samples in a herd located in Buenos Aires. Based on the analysis of a 287-bp fragment within ORF1, these sequences grouped into genotype 3 and exhibited a close relationship to the HEV variants previously identified in Argentina from sporadic cases of acute human hepatitis E infection. According to the phylogenetic reconstruction reported in Munné’s work, the variants described by Schlauder et al. (2000) were reassigned within the genotype 3.

Recently, in a study conducted to better understand the molecular epidemiology of HEV infection in Argentina, the co-circulation of polyphyletic variants was clearly demonstrated by phylogenetic analysis of the 287-bp region within ORF1 and a small sequence of the ORF2 (capsid gene) (Munné et al. 2011). In fourteen out of fifteen diagnosed cases of acute HEV infection included in this study, HEV genotype 3 isolates were detected and strains of subtypes 3a, 3b and 3i were identified. Despite the fact that genotype 3 was the most prevalent virus, and probably responsible for most of the autochthonous cases of HEV infection in Argentina, a strain belonging to genotype 1 was also detected. This single case was demonstrated to be imported from India and the first to be characterized in South America. This, together with the widespread circulation of polyphyletic variants of genotype 3 suggests multiple sources of infection of HEV in Argentina.

In Brazil, the evolutionary history of HEV is different from that of Argentina. In fact, the first HEV strain described, classified as genotype 3 by full-length genome molecular analysis, was isolated from a pig in 2007, when no cases of HEV infection in humans had been reported in this country (Paiva et al. 2007).

Two years later, Lopes dos Santos et al. (2009) detected and molecular characterized, by analysing the 287-bp region within ORF1, four new porcine HEV strains which were also classified into genotype 3. Even though the sequences were not compared to the strain described by Paiva et al. (2007), these isolates showed to be closely related to each other but only distantly related to Argentine HEV isolates, suggesting that they probably had different origins.

Later, in a retrospective study, the same group reported the first case of a human autochthonous HEV infection in Brazil (Lopes dos Santos et al. 2010). The patient infected was a 30-year-old male who presented clinical and paraclinical sings compatible with acute hepatitis in January of 2006. The strain isolated from the patient serum sample clustered within genotype 3 sequences (subtype 3b) and was closely related to two porcine strains previously characterized in Brazil (Lopes dos Santos et al. 2009). The data reported in this work clearly suggested a zoonotic origin for the infection. Furthermore, the newly detected swine HEV strains (Lopes dos Santos et al. 2011) were classified within subtype 3b and also showed to be closely related to the sample obtained from the first reported autochthonous human case and the porcine HEV strains that were previously reported in Brazil.

Recently, Dell’Amico et al. (2011) reported the molecular characterization of the first strains of both human and porcine HEV detected in Bolivia. In a preliminary analysis, based on the phylogenetic reconstruction using a 348-bp fragment corresponding to the ORF2, the sequences obtained from pigs and human showed to be very closely related and were classified within genotype 3. Further analysis allowed the authors to show that swine and human sequences belonged to subtype 3i and 3e, respectively (Purdy et al. 2012). Nevertheless, their phylogenetic relationship with other HEV strains isolated in South America still remains to be elucidated.

In Venezuela, newly detected HEV infection was reported by García et al. (2012). In this work two strains were classified as genotype 1 and one as genotype 3, which were closely related to Yam 67 (north of India) and US1 reference isolates from the USA, respectively. Despite the fact that the Venezuelan genotype 1 isolate displayed 100% nucleotide identity with the strain Yam 67 within the RNA dependent RNA polymerase coding region of the ORF1, the infection was considered autochthonous since patients did not report traveling outside the country. Additionally, the findings of this work imply that HEV is an important cause of acute hepatitis in Venezuela, with high morbidity in children and young adults, suggesting that this infection is endemic in this country.

In November 2009 the first sporadic case of HEV infection was detected in Montevideo, Uruguay, and during the period December 2009- June 2010 eight new autochthonous cases were identified (Mirazo et al. 2011). All patients were adults (39-65 years old) with no history of travel outside the country in the previous 40 days. HEV RNA was isolated from all patients and a preliminary molecular analysis of the viral strains was conducted on the basis of a 137-pb sequence within the highly conserved overlapping region of ORF2-3. Sequence analysis and comparison of the isolates with published HEV strains revealed that all samples belonged to genotype 3. Since the strains were very closely related to sequences of swine HEV isolates from Germany and although patients reported that they did not have contact with animal reservoirs, this was evidence of possible zoonotic transmission of the disease. In order to analyze
the genetic variability of HEV strains detected in this work and contribute to shedding light on the epidemiological status of this emergent infection in South America, HEV isolates were further characterized (Mirazo et al. unpublished data). Molecular analysis of the HVR within the ORF 1 revealed that, though the isolates showed very high nucleotide sequence identity (96-98%), four distinct sub-clusters were observed, thus suggesting that several sources of infection have likely existed. On the other hand, the phylogenetic reconstruction based on the 287-pb fragment of the methyl transferase gene within ORF 1 allowed to determining the evolutionary relationships between these isolates with the Argentinean and Brazilian strains. In fact, Uruguayan strains exhibited a nucleotide identity higher than 90% with the viral sequence isolated from an autochthonous case of HEV infection in Brazil (Lopes dos Santos et al. 2010) but in contrast were distantly related to Argentinean isolates. Finally, by analyzing this ORF 1 region, the subtyping studies showed that all Uruguayan strains belonged to subtype 3h (Mirazo et al. unpublished data), a rare subtype previously reported in Italy and New Zealand (Lu et al. 2006).

**Concluding remarks**

Recent advancements in molecular epidemiology of HEV infection in South America have revealed an epidemiological picture more complex that the initially assumed. The occurrence of sporadic cases in developing areas mainly associated to genotype 3 strains suggests that epidemiology of HEV may be undergoing changes. A more extensive sampling of HEV variants in different epidemiological settings and improvement in accurate identification of genetic relatedness among HEV strains using longer or even whole-genome sequences are required.

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