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Classification and Genomic Diversity of Enterically Transmitted Hepatitis Viruses

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Hepatitis A virus (HAV) and hepatitis E virus (HEV) are significant human pathogens and are responsible for a substantial proportion of cases of severe acute hepatitis worldwide. Genetically, both viruses are heterogeneous and are classified into several genotypes that differ in their geographical distribution and risk group association. There is, however, little evidence that variants of HAV or HEV differ antigenically or in their propensity to cause severe disease. Genetically more divergent but primarily hepatotropic variants of both HAV and HEV have been found in several mammalian species, those of HAV being classified into eight species within the genus *Hepatovirus* in the virus family *Picornaviridae*. HEV is classified as a member of the species *Orthohepevirus A* in the virus family *Hepeviridae,* a species that additionally contains viruses infecting pigs, rabbits, and a variety of other mammalian species. Other species (*Orthohepevirus B–D*) infect a wide range of other mammalian species including rodents and bats.

Hepatitis A virus (HAV) and hepatitis E virus (HEV) show moderate genetic diversity, both being classified into several genotypes infecting humans, and several additional species infecting a wide range of other mammalian hosts. The genetic diversity of both viruses has been extensively used as a tool to investigate their molecular epidemiology and transmission. Further studies have sought to determine the existence of differences between genotypes in their clinical presentations and pathogenicity and will be reviewed.

CLASSIFICATION

HAV and HEV are positive-stranded RNA viruses that share a propensity to cause liver disease in humans. However, the two viruses are quite distinct in terms of their replication strategy, virion structure, and taxonomy (see Kenney and Meng 2018; McKnight and Lemon 2018). HAV is classified as a member of the *Hepatovirus* genus in the large family *Picornaviridae* (Zell et al. 2017). Although HAV is spread by the fecal–oral route, it is not known whether HAV replicates within tissues of the gastrointestinal tract, and its disease manifestations arise from the spread of virus infection to the liver.

HEV is classified as a member of the *Orthohepevirus* genus in the family *Hepeviridae* (Emerson and Purcell 2003; Purdy et al. 2015). Virions of hepeviruses are structurally distinct from picornaviruses (see Kenney and Meng 2018) and their structural proteins are translated

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from a separate subgenomic RNA expressed during virus replication rather than as part of a single polyprotein as in the picornaviruses. Although there is detectable homology between the RNA-dependent RNA polymerase and helicase genes of picornaviruses and hepeviruses, they have been assigned to different RNA virus supergroups (I and III, respectively) (Koonin 1991), indicating an extremely distant evolutionary relationship between them.

DIVERSITY AND TAXONOMY OF HAV AND RELATED VIRUSES

Hepatovirus Species

Until very recently, the genus *Hepatovirus* included a single species, HAV, whose members comprise a group of viruses known to infect humans and various nonhuman primate species. The species was renamed *Hepatovirus A* in 2014 for consistency with nomenclature conventions adopted elsewhere in the picornavirus family. Since 2015, however, a wide range of further, genetically divergent hepatoviruses have been described in seals (Anthony et al. 2015), shrews, and hedgehogs (order Eulipotyphla), several different rodent (Rodentia) and bat (Chiroptera) host species (Drexler et al. 2015), and, most recently, woodchucks (Yu et al. 2016; and see Sander et al. 2018). Collectively, these viruses are substantially more divergent from each other and from HAV than are existing human and nonhuman primate HAV strains, consistent with them being assigned to several additional species. Using translated sequences from the structural gene region, VP2, variants showing >7% divergence from all other variants might be assigned to as many as 13 new species (Drexler et al. 2015). In 2017, the ICTV accepted the proposal to create new species to classify those hepatovirus variants for which (near-) complete genome sequences were available and that clustered separately on phylogenetic analysis. Accordingly, the genus *Hepatovirus* now includes nine species (*Hepatovirus A–I*) based on phylogenetic groupings (Fig. 1). Although distance thresholds have not been specified in the current species definition, nucleotide sequence divergence ranges from 3.8% among members of *Hepatovirus A* to 29% among members of *Hepatovirus G* (mean intraspecies divergence of 16%), compared with 30%–42% among members of different species (mean, 38%). The respective amino acid sequence divergence ranges are 0.8%–26% (mean, 11.6%) compared with 31%–48% (mean, 41%).

Members of these new hepatovirus species share a number of genome characteristics with HAV, such as low G+C content (37.0%–37.8% within HAV genotypes) compared with 33.7%–39.3% in members of the new species. They also share the massive suppression of CpG dinucleotide frequencies (10%–34% of frequencies expected from their G+C content). Although there is some variability in genome length and positions of cleavage sites for the different structural and nonstructural proteins, members of all hepatovirus species generally show comparable genome organization and features. The latter includes their universal possession of internal ribosomal entry sites (IRES) in the 5′-untranslated region (5′UTR), of which most resemble the type III IRES of HAV in sequence and predicted structure (Anthony et al. 2015; Drexler et al. 2015; Yu et al. 2016). However, viruses belonging to *Hepatovirus C* and *Hepatovirus E* (infecting a bat and rodent species, respectively) possessed a type IV IRES (Drexler et al. 2015), structurally resembling those of certain other picornavirus genera (e.g., *Tremovirus*, *Duck hepatitis virus*, and *Sapelovirus*). Hepatovirus species are hepatotropic (Drexler et al. 2015), infect a wide range of hosts, but show limited evidence for host specificity. Although members of the species *Hepatovirus A* are restricted to humans and primates (macaques and African green monkey), related hosts such as hedgehogs and shrews (order Eulipotyphla) are infected with members of other hepatovirus species (*Hepatovirus H* and *Hepatovirus I*), as are rodents and bats. Conversely, members of a single species (*Hepatovirus H*) can infect three quite different hosts—hedgehogs (*Erinaceus europaeus)*, tupias (*Tupaia belangeri chinensis*), and bats (*Eidolon helvum*). Although the distribution of hepatovirus species is consistent with frequent jumps

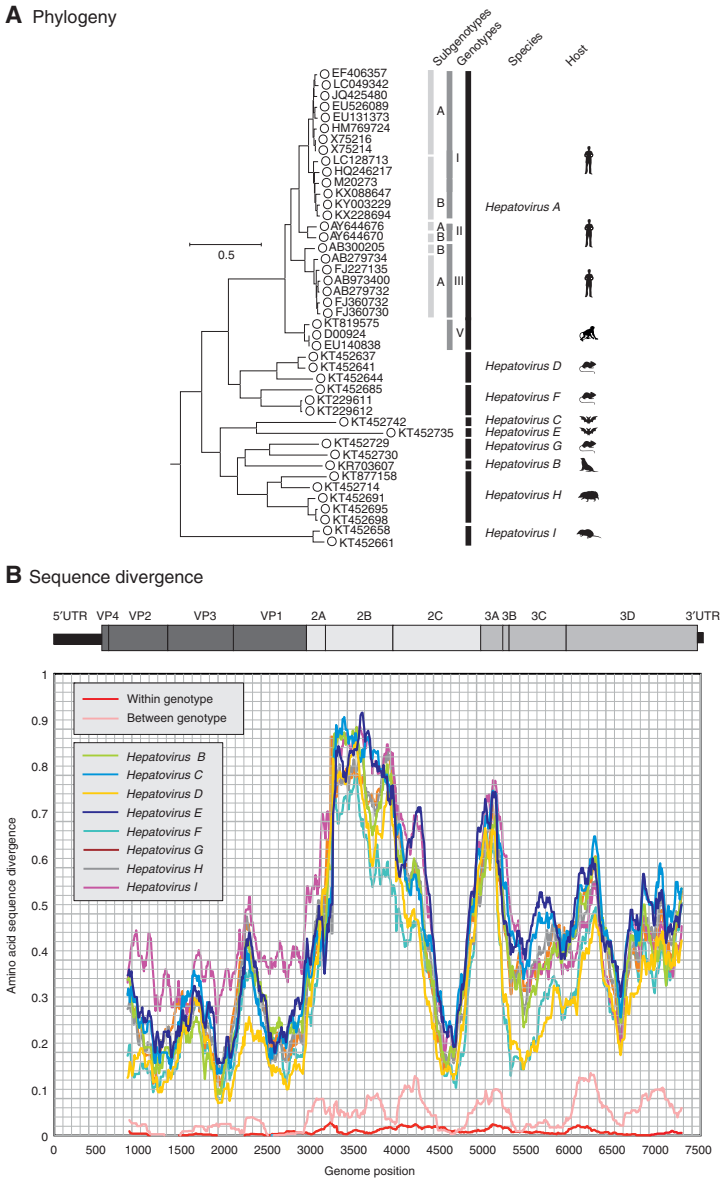

Figure 1. Phylogeny and sequence divergence of hepatitis A viruses (HAVs). (*A*) Phylogenetic analysis of the complete coding sequences of representative variants of HAV (species A) and of all available sequences of other nonhuman hepatoviruses infecting other mammalian species, labeled as in the key. (The coding region spans positions 723–7407 in the HAV-MBB sequences, M20273 [Paul et al. 1987].) The tree was constructed by maximum likelihood using an optimal substitution model (general time reversible and γ distribution). Robustness of branches was indicated by bootstrap resampling supported by ≥70 from 100 replicate samples. The tree was rooted using sequences from the most closely similar genus of picornaviruses (*Tremovirus*: KT880668, AY275539, KF979338, AJ225173, and AY517471). The host origins of the different species are indicated diagrammatically and correspond to the following species: *Hepatovirus B*, seals (*Phoca vitulina vitulina*); *Hepatovirus C*, bats (*Miniopterus cf. manavi*); *Hepatovirus D*, rodents (*Microtus arvalis*, *Myodes glareolus*); *Hepatovirus E*, bats (*Lophuromys sikapusi*); *Hepatovirus F*, rodents (*Marmota himalayana*, *Sigmodon mascotensis*); *Hepatovirus G*, bats (*Rhinolophus landeri*, *Coleura afra*); *Hepatovirus H*, hedgehogs, tupias, and bats (*Erinaceus europaeus*, *Tupaia belangeri chinensis*, *Eidolon helvum*); and *Hepatovirus I*, shrews (*Sorex araneus*). (*B*) Scan of amino acid sequence divergence between HAV and hepatovirus species B–I, and comparison with between and among genotype divergence of HAV (red and pink lines). The distances represent mean values for available sequences of each species and were calculated for sequential fragments spanning >90 codons, incrementing by nine codons between data points.

among hosts, it has recently been shown that host range may be restricted by incompatibilities in the interaction of the virus and the host innate immune system (Hirai-Yuki et al. 2016). HAV is unable to replicate in mice because it is unable to evade the cytosolic pattern recognition receptor, MAVS (see Hirai-Yuki et al. 2018), suggesting that major jumps in host species may require substantial adaptive changes in the virus. It is very likely that a much wider range of HAV-like viruses will be found in other mammalian species in the future and animal-associated hepatoviruses may conceivably represent zoonotic sources of infections in humans.

HAV Diversity and Genotypes

Most information on diversity within hepatovirus species is available for *Hepatovirus A*. HAV shows a modest degree of genetic diversity, with an average of ~10% nucleotide sequence divergence (2.2% amino acid sequence divergence) among coding region sequences of available (nearly) complete genome sequences. Somewhat higher estimates are obtained once epidemiologically linked HAV variants are excluded (12% and 3.7%, respectively). Using sample dates to estimate substitution rates of HAV over time, it was concluded from Bayesian analysis of complete genome sequences that the common ancestor of all variants belonging to *Hepatovirus A* might have existed 2000–3000 years ago, while the subset of HAV variants infecting humans were somewhat less diverse and may have a more recent common ancestor (~1500 years ago) (Kulkarni et al. 2009). Underpinning these historically relatively remote dates was a substitution rate of $1.7–2.0 \times 10^{-4}$ substitutions per site per year (SSY) for whole-genome sequences (Kulkarni et al. 2009) and 9.8×10^{-4} SSY for VP1 (Moratorio et al. 2007). These rates are lower than estimates for VP1 genes of other picornaviruses, for example, $4–14 \times 10^{-3}$ SSY for foot and mouth disease virus (genus *Aphthovirus*) (Cottam et al. 2006) and 13.5×10^{-3} for EV-A71 (genus *Enterovirus*) (Brown et al. 1999) and indeed most other RNA viruses. The reasons for such a low substitution rate in HAV are not known. Putting these estimates into historical context, whether the dating of the divergence of currently circulating strains of HAV to some time after the collapse of the Roman Empire and before the rise of Islam has any epidemiological or historical correlates remains undetermined.

Phylogenetic analysis of all available HAV VP1 sequences on GenBank (June 14, 2017) shows clustering of variants into the previously assigned genotypes (gt)I–III infecting humans and IV and V infecting nonhuman primates (Fig. 2) (Robertson et al. 1992; Costa-Mattioli et al. 2002). The original assignments were based on a 168-nucleotide sequence at the VP1/2A boundary, but the subsequent use of whole VP1 sequences produces a more robust phylogenetic tree and has led to the incorporation of what was originally described as gtVII from Sierra Leone as a member of gtII (Lu et al. 2004). Variants of HAV infecting humans are all assigned to gtI–III and are phylogenetically distinct from nonhuman primate HAV strains, assigned as gtIV, V, and VI (Fig. 2; no VP1 sequence is available for the macaque-derived gtVI). Although sampling of primates is highly limited, current evidence indicates that a wide range of Old World monkey (OWM) species may carry simian strains of HAV. gtIV was isolated from a cynomolgus macaque in the Philippines (Nainan et al. 1991), whereas variants classified as gtV have been found in three different OWM species and locations (African green monkey, Kenya; olive baboon, Uganda; rhesus macaque, India) (Nainan et al. 1991; Arankalle and Ramakrishnan 2009; Bennett et al. 2016). gtVI originated from a cynomolgus macaque originally from Indonesia (Costa-Mattioli et al. 2002).

The phylogenetic clustering of sequences into different genotypes is supported by differences in sequence divergence between and within genotype groupings. Analyzing available (near-) complete genome sequences, pairwise nucleotide distances between gtI–III range from 12.2% to 21.9% (mean, 18.2%), whereas diversity within genotypes ranges from 0.3% to 6.5% (mean, 4.3%). Mean distances among subtypes IA/IB, IIA/IIB, and IIIA/IIIB are intermediate (9.3%, 9.6%, and 11.8%, respectively).

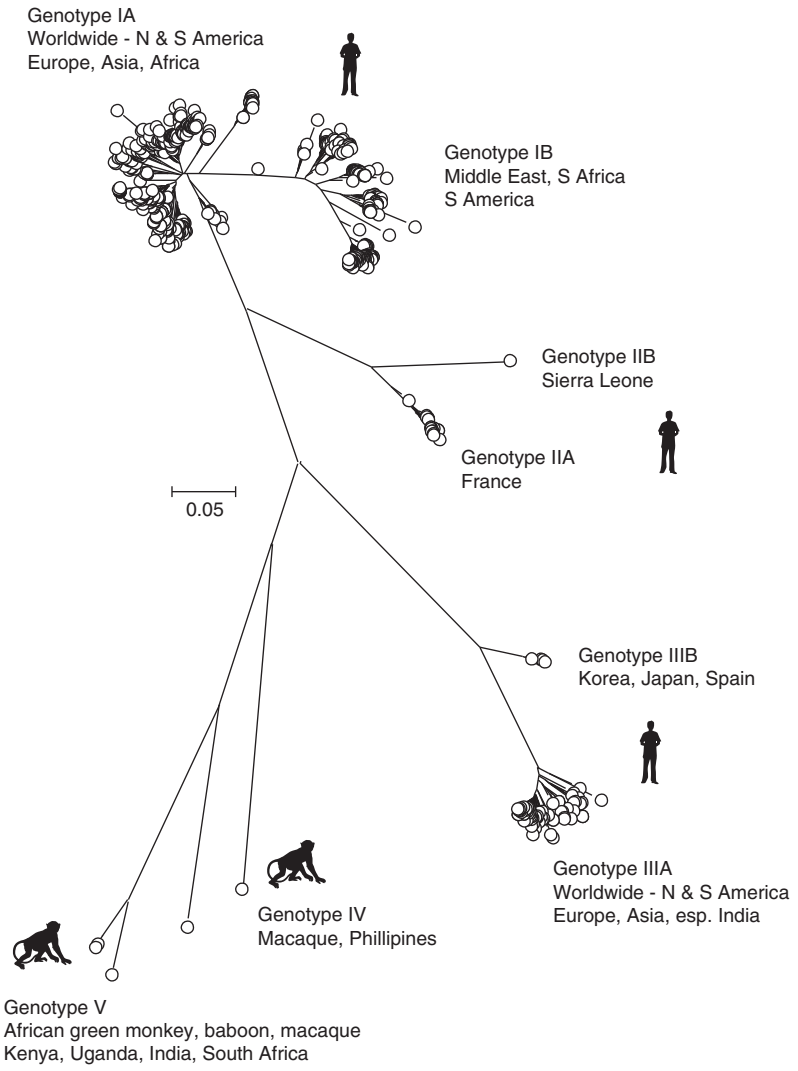

Figure 2. Unrooted phylogenetic analysis of the 625 available hepatitis A virus (HAV) sequences in the VP1 region. Positions 2220–3234 numbered as in Fig. 1. The tree was constructed by neighbor joining of maximum composite likelihood distances as implemented in the program, MEGA6 (Tamura et al. 2013). Bootstrap resampling was performed as described in Figure 1. Sequences were selected for analysis based on being >90% complete in the VP1 coding region and lacking internal stop codons.

These ranges are comparable to those determined previously for different subgenomic regions, such as VP1 (Endo et al. 2007).

Genotype Distributions

Geographically and numerically, infections with gtIA predominate worldwide, with a smaller number of gtIB and IIIA sequences represented in the VP1 dataset (Fig. 2). gtIA predominates in almost all countries where surveillance data is available, and includes North and South America, Europe, and large areas of Asia and Africa (Robertson et al. 1992; Costa-Mattioli et al. 2003; Nainan et al. 2006). gtIB circulates extensively in the Middle East (Normann et al. 2008; Nejati et al. 2012) and South Africa (Taylor 1997), and has been detected among environ-

mental surveillance samples in Brazil. gtII was identified among samples from France and Sierra Leone in the 1990s (Robertson et al. 1992), but has only been rarely reported since. Although less frequently detected than gtI, gtIIIA, and gtIIIB, variants of HAV are also distributed globally, being reported throughout the United States, Europe, and Asia (Robertson et al. 1992; Nainan et al. 2006; Endo et al. 2007). The underlying reasons for the unequal frequencies of infections with different HAV genotypes and their somewhat different geographical distributions are uncertain.

Biological Significance of Hepatovirus Diversity

There is very limited information currently available on the existence of possible biological differences among viruses belonging to different hepatovirus species. They are all hepatotropic, judging from the detection of high levels of viral RNA and replication intermediates in the livers of seals, bats, rodents, hedgehogs, and shrews (Anthony et al. 2015; Drexler et al. 2015; Yu et al. 2016). There is, however, some preliminary evidence for a broader tissue distribution in bats (Drexler et al. 2015). There is substantial amino acid sequence divergence among capsid genes of members of different species (Fig. 1B), greater than 15%–20% over most of P1, although with some variability among genotypes. This degree of divergence is consistent with the general lack of serological cross-reactivity described among rodent, shrew, hedgehog, and bat sera and HAV antigens (Drexler et al. 2015). The strong serological cross-reactivity of a small number of bat sera could potentially be attributable to their previous exposure to HAV or a close relative.

Compared with the extensive divergence among sequences of viruses belonging to different hepatovirus species, variants of HAV (*Hepatovirus A*) show only minimal amino acid sequence divergence within and between genotypes. It is generally considered that human genotypes of HAV are a single serotype (Lemon et al. 1992); even the relatively divergent HAV gtIB and V showed almost identical neutralization profiles with panels of monoclonal antibodies (Brown et al. 1989; Crevat et al. 1990). Vaccines for HAV may thus be equally effective across the range of genotypes documented to date.

There is similarly little evidence for major differences in clinical outcomes of infections with different HAV genotypes, as might be expected from their high degree of genetic conservation. However, severe fulminant hepatitis associated with a particular genotype IA variant been described in a number of Japanese patients, suggesting the potential existence of strain-associated differences in pathogenicity (Fujiwara et al. 2001; Miura et al. 2017). A cohort study of Korean HAV-infected patients reported that gtIII may be associated with higher levels of biochemical markers of liver damage and lower platelet counts than gtIA, even though the overall disease severity (duration of hospital stay, liver failure, mortality) were comparable (Kim et al. 2013).

DIVERSITY AND TAXONOMY OF HEV AND RELATED VIRUSES

When hepatitis E was first described as an enterically transmitted epidemic in New Delhi in 1955, following fecal contamination of drinking water (Viswanathan 1957), the causative agent was presumed to be a picornavirus. Subsequent studies of virus particles revealed it to be more like a calicivirus, but once a complete genome sequence was obtained (Tam et al. 1991; Huang et al. 1992; Tsarev et al. 1992), the virus was reassigned to its own genus *Hepatitis E-like viruses* (renamed *Hepevirus* in 2004, and *Orthohepevirus A* in 2015), and placed in its own family, *Hepeviridae*, in 2009.

Like HAV, HEV has a nonenveloped virion and a positive-sense RNA genome, but unlike members of the *Picornaviridae*, it encodes a single capsid protein and its genome comprises multiple, separately transcribed genes. A short 5′-noncoding region is followed by a long open reading frame (ORF)1 that encodes methyltransferase, Y, papain-like cysteine protease, X, helicase, and RNA-dependent RNA polymerase domains (see Kenney and Meng 2018). ORF1 is immediately followed by ORF2, encoding the

capsid protein, while overlapping the 5′-end of ORF2 is another, shorter reading frame, ORF3, which encodes a protein with multiple functions, including one possibly acting as an ion channel. At the 3′-end is a short noncoding region of variable length followed by a poly(A) tail.

Orthohepevirus Species

The current taxonomy of viruses within the genus *Orthohepevirus* is based on the phylogenetic analysis and distribution of sequence distances for subgenomic regions of the virus genome (Purdy et al. 2015; Smith et al. 2015b). For example, phylogenetic analysis of a conserved region of the capsid protein supports the assignment of viruses into four species, named *Orthohepevirus A–D*, together with additional unassigned variants (Fig. 3). HEV, in the species *Orthohepevirus A*, includes isolates from humans, pigs, and deer. At present, the only hepeviruses known to infect humans are those belonging to *Orthohepevirus A*. Human infection is normally asymptomatic or very mild, with few individuals developing an acute, self-limiting hepatitis. Severe or fulminant hepatitis is a rare outcome except in pregnant women in which mortality can be significant. Infection can become chronic when immune function is suppressed. Infection of pigs is asymptomatic, but HEV-infected dolphins had elevated liver transaminase levels and signs of malaise (Montalvo Villalba et al. 2017).

Related viruses with a similar genome structure have been isolated from adult chickens with an epidemic disease variously described as big liver and spleen disease or hepatitis-splenomegaly syndrome and associated with weight loss, increased mortality, and decreased egg production (Payne et al. 1999; Haqshenas et al. 2001, 2002; Huang et al. 2002). These avian HEVs are members of the species *Orthohepevirus B*. More recently, additional viruses have been detected in the rat (Johne et al. 2010a,b), musk shrew, ferret, and mink (all members of the species *Orthohepevirus C*) and in bats (*Orthohepevirus D*) (Drexler et al. 2012).

Additional variants with similarities to members of the *Orthohepevirus* genus, but that do not appear to belong to these four species (Fig. 3), have been described from moose (Lin et al. 2014), fox (Bodewes et al. 2013), kestrel (Reuter et al. 2016a), and little egret (Reuter et al. 2016b), although these have yet to be formally classified into individual species. An incomplete virus genome obtained from a metagenomic study of sewage (hepalivirus) may represent an additional group of viruses (Ng et al. 2012). It seems likely that diversity within the *Orthohepevirus* genus will be revealed to be even more extensive as studies are extended to a wider range of host species.

A more distantly related virus isolated from salmonids (Hedrick et al. 1991; Batts et al. 2011) has been assigned to the species *Piscihepevirus A*, the sole member of the genus *Piscihepevirus*.

HEV Diversity and Genotypes

Sequence analyses of *Orthohepevirus A* isolates from humans around the world and from different host species have revealed the presence of distinct variants that have been classified into several genotypes (gt) and subtypes (Fig. 4). These comprise HEV gt1–4 that have been detected in humans (Lu et al. 2006), gt5 and gt6 that have only been isolated from pigs (Takahashi et al. 2014), gt7 obtained from dromedary camels (Woo et al. 2014) (although there is also one report of human infection) (Lee et al. 2016), and gt8 reported from Bactrian camels (Woo et al. 2016). Numerous subtypes have been described within gt1–4 (Lu et al. 2006), but it is difficult to provide consistent criteria that discriminate viruses that are members of the same subtype from those of different subtypes (Smith et al. 2013a). A particular difficulty applies to variants first isolated from rabbits (Zhao et al. 2009), but now shown to also infect humans (Izopet et al. 2012; Abravanel et al. 2017), and that include characteristic insertions in ORF1. These viruses group phylogenetically with gt3 isolates, but are more divergent from human gt3 variants than these are from each other (Smith et al. 2013a) and are currently considered as belonging to gt3. A similar difficulty applies

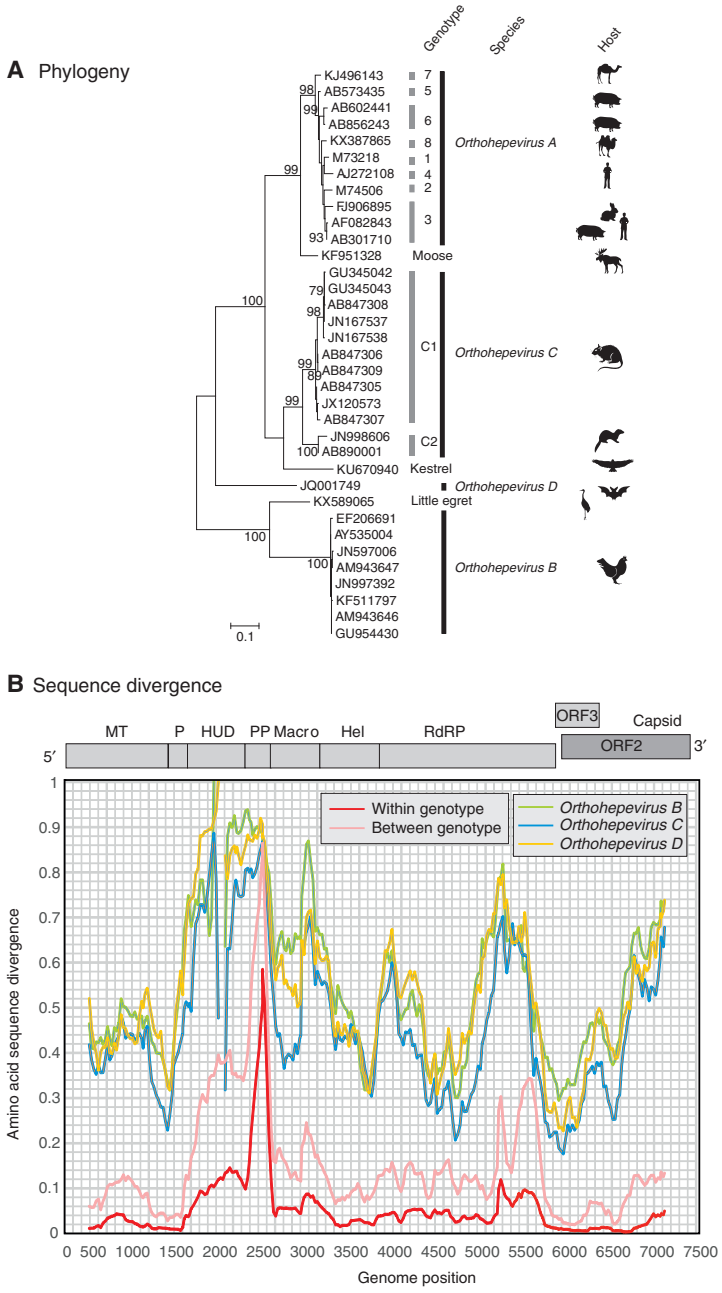

Figure 3. Phylogeny and sequence divergence of *Orthohepevirus* species. (*A*) Phylogenetic relationships of *Orthohepevirus* species. A conserved region of the capsid protein (nucleotide positions 5485–6497, numbered according to M73218) was aligned for representative isolates and the amino acid sequence used to generate a maximum likelihood tree based on the LG model with a γ distribution of evolutionary rates among sites as implemented in the program MEGA6 (Tamura et al. 2013). Branches supported in >70% of bootstrap replicates are indicated. Genotypes, virus species, and host species are indicated. (*B*) Scan of amino acid diversity across the *Orthohepevirus* genome. The sequences used to produce Figure 3A were analyzed as described in Figure 1B.

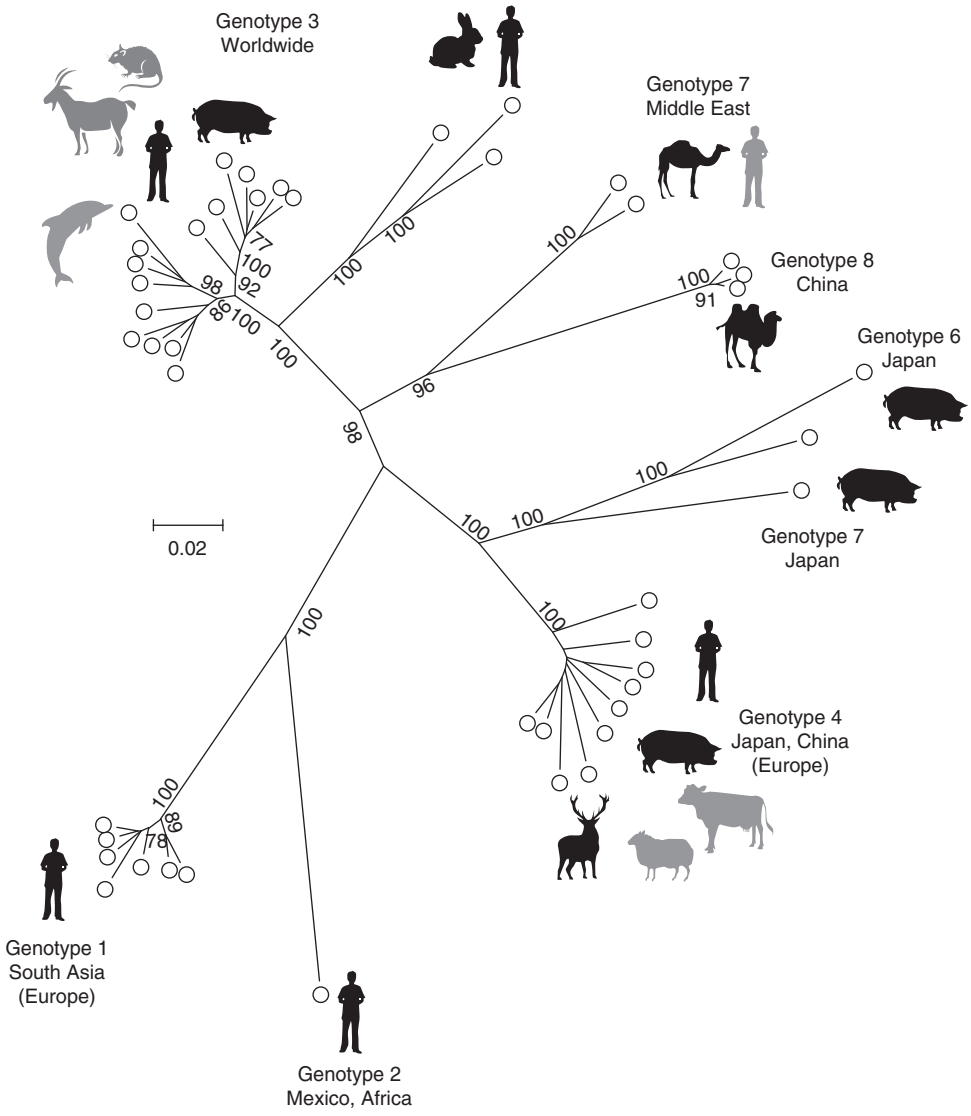

Figure 4. Unrooted phylogenetic analysis of coding sequences of representative members of *Orthohepevirus A*. Concatenated open reading frame (ORF)1 and ORF2 coding sequences (excluding the hypervariable region) were aligned and used to produce a maximum likelihood tree using a JTT model (with frequencies) and a γ distribution of rate differences among sites, including invariant sites as implemented in the program MEGA6 (Tamura et al. 2013). Branches supported in >70% of bootstrap replicates are indicated. Sequences used were 1a_M73218, 1b_D11092, 1c_X98292, 1d_AY230202, 1e_AY204877, 1f_JF443721, 1_FJ457024, 2a_M74506, 3a_AF082843, 3b_AP003430, 3c_FJ705359, 3e_AB248521, 3_EU360977, 3f_AB369687, 3_EU723513, 3_KJ873911, 3g_AF455784, 3h_JQ013794, 3i_FJ998008, 3j_AY115488, 3_AB290312, 3_AB369689, 3_JQ953664, 3_AB290313, 3ra_FJ906895, 3_JQ013791, 3_KJ013415, 4a_AB197673, 4b_DQ279091, 4c_AB074915, 4d_AJ272108, 4e_AY723745, 4f_AB220974, 4g_AB108537, 4h_GU119961, 4i_DQ450072, 4_AB369688, 5a_AB573435, 6a_AB602441, 6_AB856243, 7a_KJ496143, 7_KJ496144, 8_KX387865, 8_KX387866, and 8_KX387867. Host species are indicated by icons, with less frequent or uncertain species shaded in gray.

to gt5 and 6, which could alternatively be considered as subtypes of a single genotype.

A recurring problem in the description of HEV subtypes has been the fact that several were first defined on the basis of subgenomic sequences (Lu et al. 2006). A result of this has been uncertainty about the relation of these variants to viruses sequenced in other genomic regions; there are several examples in the literature in which the same subtype name has been used to identify phylogenetically distinct viruses or where different names have been used for viruses that belong to the same subtype. To address this problem, a list of reference complete genome sequences for each subtype has been proposed by the ICTV *Hepeviridae* study group and others with an interest in the variation of the *Orthohepevirus* species (Smith et al. 2016b).

Sequence variability across the HEV genome is relatively constant with few sites of insertion/deletion and a strong bias against nonsynonymous substitution. However, there is a region of unusual nucleotide and amino acid variability within ORF1 between the papain-like cysteine protease and X domains, termed the hypervariable region, which includes an intrinsically disordered region (Purdy et al. 2012) composed of 20%–30% proline and which evolves by both mutation and duplication (Smith et al. 2012). Another notable region is where ORF2 and ORF3 overlap in different reading frames leading to a marked suppression of genetic variability. Finally, attempts to grow HEV in cell culture have led to the identification of HEV strains in which different human coding sequences have been incorporated in frame with ORF1; these can produce viruses that show an enhanced replication ability in vitro compared with that of the virus strains before passage (Nguyen et al. 2012; Shukla et al. 2012).

Diversity within other *Orthohepevirus* species is much less well characterized. Four closely related genotypes have been described within *Orthohepevirus B*; two divergent genotypes within *Orthohepevirus C* are confined to rodents and mustelids, respectively, and it seems likely that multiple genotypes exist within *Orthohepevirus D*.

Genotype Distributions

The genotype distribution of human isolates of *Orthohepevirus A* is still incompletely known, with sparse information about virus diversity in many parts of the world, but particularly in Africa where the genotype(s) responsible for epidemic and sporadic infections remains unknown for several countries (Kim et al. 2014). gt1 is associated with epidemics of enterically transmitted hepatitis in the Indian subcontinent and Africa, with cases elsewhere apparently deriving from recent travel to those regions. gt2 has been reported from Mexico and Africa, where it also associated with epidemic spread, but very few isolates are known. To date, gt1 and 2 have only been detected in humans. gt3 is widely distributed in Europe, Africa, North and South America, and in Northeast and Southeast Asia. In these regions, acute (non-travel-associated) infection occurs sporadically with a strong bias toward older men (Lewis et al. 2008), although serological evidence suggests a more general risk of subclinical infection in the whole population, with 25% of blood donors in the Netherlands and Austria having evidence of past infection by the age of 50 (Slot et al. 2013; Fischer et al. 2015). gt4 occurs in Northeast Asia with only occasional reports in Europe (Colson et al. 2016), possibly reflecting the recent introduction of infected pigs from Japan (Nakano et al. 2016). These genotype distributions are mirrored by those in domestic pigs, wild boar, and deer in these regions, the presumption being that human infection results from transmission from these animal reservoirs through consumption of undercooked meat. Such transmission has been shown directly in a few cases (Takahashi et al. 2004; Colson et al. 2010) and suggested by epidemiological studies (Said et al. 2014). This simple picture has been complicated in recent years by the detection of HEV gt3 in goats (Di Martino et al. 2016), rats (Kanai et al. 2012), and, unexpectedly, in bottlenose dolphins (Montalvo Villalba et al. 2017); gt4 has been detected in cows (Hu and Ma 2010; Huang et al. 2016) and sheep (Wu et al. 2015). That patterns of transmission are more complicated than they might seem is suggested by a study of virus diversity among pigs in India.

This revealed that all isolates belonged to gt4, whereas human isolates from the same region were all gt1 (Arankalle et al. 2002). In most cases of nonepidemic hepatitis E, the source of human infection is unknown; addressing this should be a priority for future research.

A complication to this description is evidence that the distribution of virus genotypes can change over relatively short time scales. The first isolates of HEV identified in China before the year 2000 were gt1, but this genotype now represents <10% of human infections, with gt4 predominating (Liu et al. 2012). This change may partially reflect improvements in detection methods and interest in HEV.

However, there is also evidence for changes in the distribution of variants within a genotype. For example, in the period 2003–2005, subtype 3f represented 90% of infections in Southwestern France, but by 2012–2014, this had decreased to 65% with the frequency of subtype 3i (previously described as 3c) rising from 5% to 25% (Lhomme et al. 2015). Similarly, a new subtype of gt3 (3c) was introduced to the United Kingdom from about 2008, where within 5 years it became the predominant source of infections (Ijaz et al. 2014). This rapid shift in virus variants may reflect changes in food sourcing and processing, although specific events have yet to be identified. For example, the new variant was not obviously derived from pigs from the United Kingdom; the most closely related, although still distinct, viruses were isolated from a German wild boar and from Italian and French pigs (Grierson et al. 2015).

Biological Significance of HEV Diversity

gt1 and 2 of HEV appear to differ from gt3 and 4 in their ability to be transmitted by the fecal–oral route; only the former genotypes are associated with epidemic spread in the Indian subcontinent and Africa, despite the presence of the other genotypes in these regions. Similarly, although HEV gt3 can be readily detected in human sewage (see references in Smith et al. 2016a), epidemiological studies of HEV infection in Europe do not usually report working with water or contact with untreated sewage as a risk factor. Another difference is that infection with gt1 has been associated with an increased risk of mortality in pregnant women (Jin et al. 2016), but no such association has yet been reported for gt3 or 4, despite the increased awareness of HEV infection as a leading cause of liver disease with a potential impact on pregnancy (Renou et al. 2014; Tabatabai et al. 2014).

There have been occasional reports of HEV genotypes or variants associated with severe outcomes of infection (Takahashi et al. 2009; Jeblaoui et al. 2013). However, a study of virus diversity in hepatitis patients with one or more clinical sign of overt disease, compared with that in blood donors revealed no difference in the distribution of virus subtypes between these two groups for separate studies in France, Germany, the Netherlands, or the United Kingdom (Smith et al. 2015a). An assumption made in this study, that blood donors were asymptomatic, is qualified by the results of a follow-up of HEV-infected U.K. blood donors (Tedder et al. 2016). Although most individuals were healthy at the time of donation, 10% had some signs of illness (fatigue, dark urine, pale stools, or diarrhea), whereas for another 20% these symptoms developed subsequently, with some having elevated transaminase levels. Among these blood donors, there was evidence that those infected with viruses of subtypes 3a, 3b, 3c, 3h, 3i, and 3j (mostly 3c), had a lower virus load and less severe disease than those infected with subtypes 3e, 3f, and 3g. Nevertheless, it does not seem to be the case that virus strains that cause overt hepatitis are distinct from those that result in asymptomatic or relatively mild infections.

A rare outcome of HEV infection is progression to fulminant hepatitis, and there have been suggestions that this might be related to particular genotypes, subtypes or strains of virus (Inoue et al. 2009; Pujhari et al. 2010; Miyashita et al. 2012; Mishra et al. 2013). However, a survey of such reports concluded that fulminant hepatitis could occur after infection with any genotype of HEV, and that once epidemiological linkage of viruses was accounted for, there was no evidence for an association with particular subtypes or with particular mutations or strains (Smith and Simmonds 2015).

There have been few reports of mixed infections with different subtypes of HEV (Moal et al. 2012; Smith et al. 2013a) and it is not yet known whether there is any effect on disease outcome.

CONCLUDING REMARKS

Our understanding of the diversity of HEV has passed through phases of expansion and confusion to what may now be a period of stability in which most virus isolates can be assigned unambiguously to a particular genotype and subtype. In combination with diagnostic methods that are capable of detecting viruses of different genotypes, it should now be possible to address two important questions that relate to HEV variation. First, it should be possible to establish the source of human HEV infections by phylogenetic analysis of virus genomes by relating the subtypes and variants found in human populations with those present in contaminated foods, farmed animals, and environmental sources, with conclusive identification of sources obtained by comparison of hypervariable region sequences. Second, increased awareness of HEV as a major source of acute hepatitis (Kokki et al. 2016) together with more frequent analysis of virus genome sequences may reveal whether particular virus variants are associated with specific disease outcomes. For example, a not-infrequent outcome of HEV infection is the development of neurological symptoms such as Guillain–Barré syndrome, neuralgic amyotrophy, and meningoencephalitis (McLean et al. 2017), but it is unknown whether specific virus variants are responsible for this pathology. Similarly, although the usual treatment for HEV infection is ribavirin, there are currently no reports of the influence of virus genotype on treatment outcomes; this contrasts with hepatitis C virus where the influence of virus genotype on the efficacy of interferon and antiviral treatment has been a fertile topic of research for three decades.

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