

Hepacivirus cross-species transmission and the origins of the hepatitis C virus

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Just 5 years ago the hepatitis C virus (HCV) — a major cause of liver disease infecting >3% of people worldwide — was the sole confirmed member of the *Hepacivirus* genus. Since then, genetically-diverse hepaciviruses have been isolated from bats, dogs, cows, horses, primates and rodents. Here we review current information on the hepaciviruses and speculate on the zoonotic origins of the viruses in humans, horses and dogs. Recent and direct cross-species transmission from horses to dogs appears plausible, but the zoonotic origins of HCV in humans remain opaque. Mechanical transmission by biting insects, notably tabanids, could, in theory, connect all three host species. Much further work is needed to understand the transmission and zoonotic potential of hepaciviruses in natural populations.

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Current Opinion in Virology 2016, **16**:1–7

This review comes from a themed issue on **Emerging viruses: interspecies transmission**

Edited by **Christian Drosten** and **Yi Guan**

For a complete overview see the [Issue](#) and the [Editorial](#)

Available online 28th October 2015

<http://dx.doi.org/10.1016/j.coviro.2015.10.002>

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Introduction

Our understanding of the genetic diversity and evolution of the hepaciviruses has undergone recent and rapid change. Just five years ago the genus *Hepacivirus* (family *Flaviviridae*) contained only one confirmed member, the hepatitis C virus (HCV), a human pathogen that is estimated to infect more than 185 million people worldwide and is a leading global cause of liver disease [1]. Today, genetically-diverse hepaciviruses are known to infect a range of different mammal species in nature, including bats [2^{**}], primates [3^{*}] and rodents [4^{**},5^{**},6^{**}]. Novel hepaciviruses have been also detected in several domesticated animals, specifically, dogs [7,8^{*}], cows [9^{*},10^{*}] and horses [11–17]. In this article we will discuss questions about hepacivirus ecology and epidemiology arising from these recent discoveries. For example, how do

hepaciviruses move among host species, how is hepacivirus transmission sustained in natural populations, and how and when did HCV originate in humans?

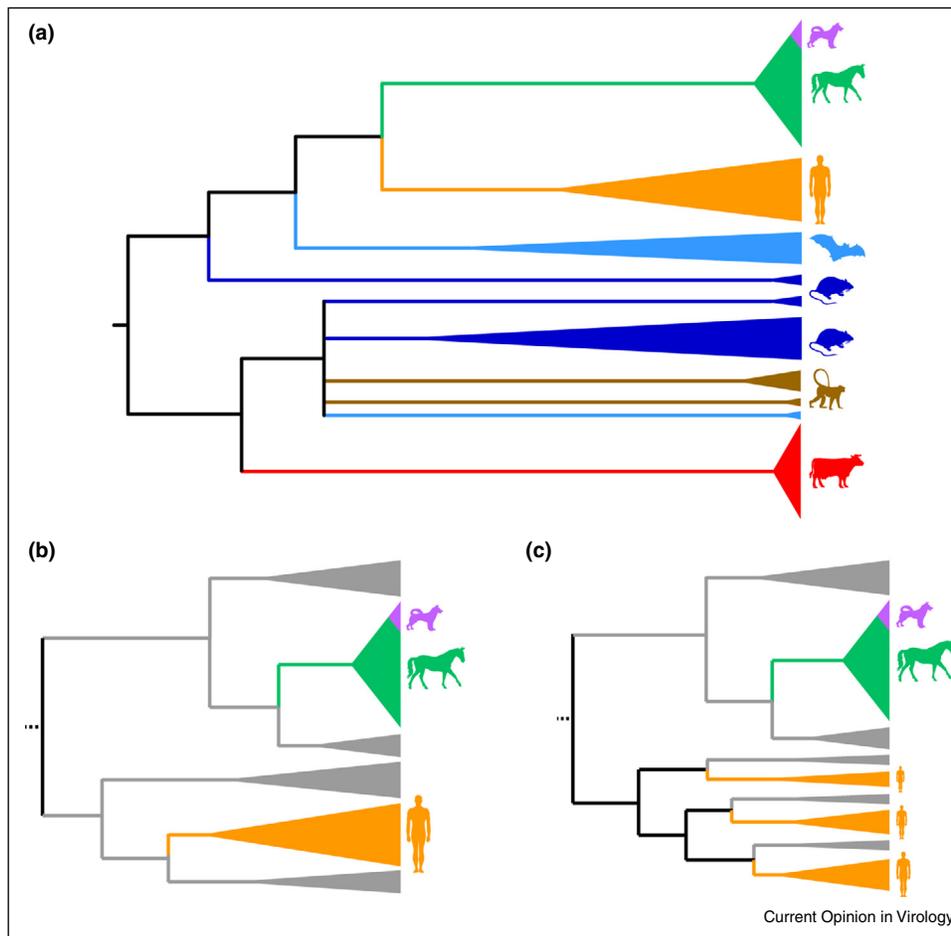
To date, phylogenetic analysis of the hepaciviruses has focused on the highly-conserved NS3 protein [18^{**}], which encodes a viral helicase and a serine protease that antagonises the host innate immune response [19]. Phylogenies estimated from this region demonstrate that viral lineages isolated from different hosts are interspersed with each other (Figure 1a). Since the arrangement of viral lineages does not match the branching order of the host phylogeny [18^{**}], this suggests an evolutionary history of hepacivirus cross-species transmission. The lineage most closely related to HCV comprises the equine and canine hepaciviruses (EHV and CHV; Figure 1a). However, the genetic distance between EHV/CHV and HCV is substantial so there is no reason to suppose that HCV originated directly from horses or dogs. Instead, it seems more likely that both HCV and the EHV/CHV lineage arose from independent cross-species transmissions of hepaciviruses from one or more as-yet unidentified source species (Figure 1b).

Evolution and transmission of equine and canine hepaciviruses

The close genetic similarity of hepaciviruses isolated from dogs and horses makes the EHV/CHV lineage particularly interesting in the context of cross-species transmission. CHV was discovered before EHV, in 2011, among domestic dogs in North America with respiratory disease [7]. Despite several subsequent surveys [11,12,20,21,22^{*}] no further direct evidence of CHV in dogs was found until El-Attar *et al.* [8^{*}] reported a panel of CHV sequences from dogs kennelled in the UK. Hepaciviruses in horses have been comparatively easier to find and EHV sequences have now been reported from all continents except Africa [11–17].

Phylogenetic analysis of available EHV and CHV NS3 gene sequences provides some insight into the evolutionary history of the lineage (Figure 2a). EHV sequences are more diverse than CHV isolates and the viruses from dogs all belong to a single well-supported cluster (posterior probability = 0.9) within the broader EHV diversity (Figure 2a). One isolate, NZP1, has been deliberately omitted from Figure 2a. NZP1 was detected in a commercial horse serum pool from New Zealand [11] and its complete genome (accession number JQ434001) is >99% identical to that of CHV (accession number JF744991).

Figure 1



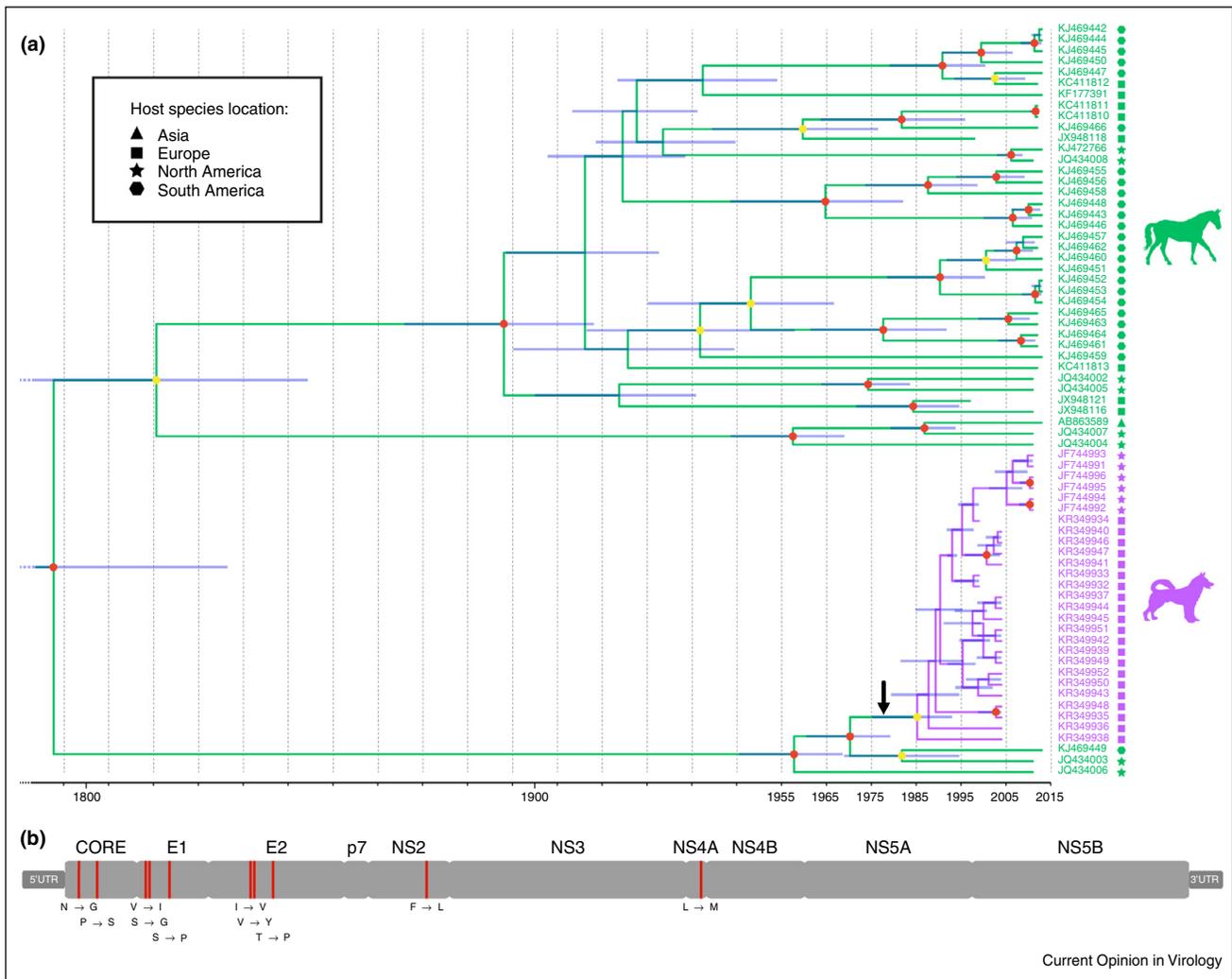
Simplified view of the hepacivirus phylogeny based on current data, and possible zoonotic scenarios for HCV and the EHV/CHV lineage. **(a)** Cladogram illustrating the branching order and host range of known hepaciviruses. Uncertain nodes have been collapsed into a polytomy. Silhouettes denote the host species of each viral lineage: dogs (purple), equids (green), humans (orange), bats (light blue), primates (brown), rodents (dark blue) and bovids (red). Triangles represent the relative genetic diversity of each virus lineage and are coloured according to host species. **(b)** and **(c)** illustrate two possible scenarios for the zoonotic origins of HCV and the EHV/CHV lineage. In both scenarios, HCV and EHV/CHV are postulated to arise from independent cross-species transmissions of hepaciviruses from one or more unidentified source species (grey triangles). In **(b)** these unidentified viruses are paraphyletic with respect to HCV, indicating that all HCV in humans arose from a single ancestral transfer event. In **(c)** the unidentified viruses fall within the genetic diversity of HCV, indicating that HCV genetic diversity arose from two or more separate cross-species transmissions.

How an apparently “canine-like” virus came to be in a horse serum pool is unclear. Scheel *et al.* [16] note that EHV is common in horse sera that is sometimes used as a cell culture additive, so the potential for contamination will need to be considered should CHV or EHV be grown in cell culture.

Molecular clock analysis indicates that the canine virus sequences from North America and the UK share a common ancestor in the 1980s, and that the CHV cluster shares a common ancestor with EHV around 1970 (Figure 2a). The short time between these two divergence dates increases the likelihood that the CHV cluster was the result of direct cross-species transmission of EHV

to dogs, as opposed indirect transfer via a third unsampled species, as has been proposed for parvoviruses from cats and dogs [23]. Whether this event represents the only hepacivirus zoonosis from horses to dogs remains to be seen. Lyons *et al.* [22^{*}] reported that a dog in frequent contact with a EHV-viraemic horse later tested seropositive for hepacivirus, suggesting that other cross-species transmission events may have occurred. Figure 2b illustrates the ten amino acid changes that are inferred to occur on the phylogeny branch separating CHV and EHV in Figure 2a (denoted by arrow). Interestingly, 8 of these 10 changes are located in the hepacivirus structural genes Core, E1 and E2 (Figure 2b). Thus the CHV/EHV lineage represents a potentially attractive model system

Figure 2



(a) Molecular clock phylogeny of current CHV (purple) and EHV (green) NS3 gene sequences (1329 nt long). The sampling location and accession number of each sequence is shown. Red and yellow circles at internal nodes indicate Bayesian posterior probabilities >0.95 and >0.70, respectively. Blue horizontal bars denote the 95% credible region for the age of each node (see timescale below the phylogeny). A black arrow indicates the branch leading to the CHV cluster. The phylogeny was estimated using the Bayesian MCMC approach in BEAST [46] under a GTR + G + I substitution model and a log-normal relaxed clock model. A strong normal prior distribution (mean = 0.0009; std. dev. = 0.00002) was placed on the clock rate parameter. The prior represents the estimated clock rate of HCV evolution for the same genome region [24]. Mean pairwise genetic distances of EHV and CHV sequences are 0.22 and 0.013, respectively. **(b)** Hepacivirus genes and genome structure. Vertical red bars and annotations below indicate the approximate position of the 10 amino acid changes that are inferred to occur on the branch ancestral to the CHV cluster (black arrow).

in which to study the molecular and ecological determinants of viral zoonoses among commensal species.

The phylogenetic timescale in Figure 2a was calibrated using a rate of evolution estimated for the NS3 gene of HCV [24] and should be interpreted cautiously, because the evolutionary rate of the EHV/CHV lineage may differ from that of HCV. That said, if the timescale is approximately correct then transmission from horses to dogs via some form of veterinary intervention is at least

plausible, since HCV is known to be efficiently propagated via infected blood or blood products [25], and the number of interventions in animal shelters and kennels will have grown substantially during the twentieth century [26]. For example, horse sera can be used in the production of tissue culture-grown rabies or canine distemper vaccines. Other theoretically possible routes of transmission include respiratory transmission among co-housed animals, or the consumption of horse meat or offal by dogs [18•].

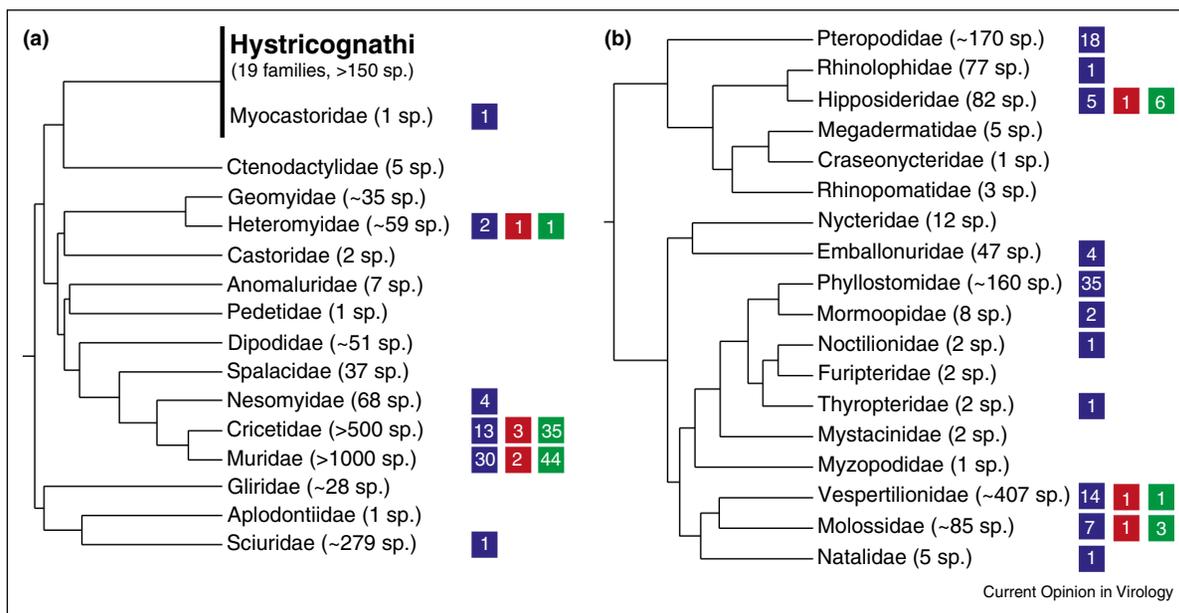
Origins of the hepatitis C virus

In contrast to CHV, we have little to no evidence about the origins of either EHV or HCV. Two lines of argument point towards rodents (and, to a lesser extent, bats) as plausible sources of both lineages. First, the genetic diversity of currently-known hepaciviruses is greater in rodents than in any other host group [4**,5**,6**]. Although this conclusion is sensitive to undersampling of virus diversity, it would be wrong to conclude that hepaciviruses from bats and rodents are particularly diverse because those species have been extensively surveyed; to date only ~2% of rodent species (out of ~2258) [4**,5**,6**] and ~8% of bat species (out of ~1150) [2**,5**] have been screened for hepaciviruses (Figure 3). Further, these species represent only 6 of the 33 families in the order Rodentia and 11 of the 18 families in the order Chiroptera (Figure 3). Comprehensive sampling is further limited by the huge population sizes and wide geographic distributions of many small mammal species. Hence sampling of hepaciviruses from rodents and bats may be no better than that of other host groups, and the viruses already discovered likely represent just a small fraction of the diversity and host range of the virus genus. Second, the development of human agriculture generated a new ecological niche that has since been occupied by rodent and bat species, some of which shelter in barns and stables and, in some cases, consume food intended for livestock [27], thereby

providing ecological opportunities for cross-species transmission. Hepaciviruses are present in bovids as well as equids and have been found in cattle from Europe [10*,12,14] and Africa [9*]. Further, there is direct evidence that commensal rodents carry hepaciviruses, as two novel hepacivirus lineages were detected among a sample of 133 Norway rats (*Rattus norvegicus*) captured in New York city [6**].

The arguments for the origins of EHV outlined above apply equally to HCV. Although HCV causes sustained infections in experimentally-inoculated chimpanzees [28] this may simply reflect the genetic similarity of humans and chimps and no HCV-like viruses have been reported from natural populations of chimpanzees [29]. In addition to the unknown zoonotic source of HCV, there is further uncertainty concerning the number of cross-species events that might have given rise to the virus in humans [30,31]. HCV contains an unusual level of genetic diversity for a single virus species and is classified into seven equally-distinct genotypes that differ at 30–35% of nucleotides across the viral coding region [32]. Consequently the divergence among HCV genotypes is greater than the threshold genetic distance used to distinguish individual virus species in the related *Flavivirus* genus [33]. Further, prior to the twentieth century, the different genotypes of HCV appear to have existed in restricted geographic areas for at least several hundred years: West

Figure 3



Sampling of hepaciviruses in rodents and bats. Phylogenies of (a) extant rodent families and (b) extant bat families are based on the results of Blanga-Kanfi *et al.* [47] and Telling *et al.* [48], respectively. For clarity, the large rodent suborder Hystricognathi (in bold) has been collapsed into a single lineage. Estimated number of species in each rodent/bat family are shown next to family name (data from <http://animaldiversity.org> [49]). Blue boxes are shown adjacent to rodent and bat families that have been screened for hepaciviruses; numbers inside blue boxes show how many species in each family have been screened. Red boxes show the number of rodent or bat species in each family within which hepaciviruses have been actually been found. Green boxes show the number of hepacivirus sequences discovered to date in the corresponding rodent or bat family.

Africa for genotypes 1 and 2; Central Africa for genotype 4, the Indian subcontinent for genotype 3 and Southeast Asia for genotype 6 [34–38]. All these observations are consistent with the hypothesis that each HCV genotype arose from cross-species transmission from separate zoonotic sources in different locations (Figure 1c). However, there is no direct evidence to reject the alternative hypothesis that HCV originated from a single zoonosis and that its genotypes diverged within human populations (Figure 1b). The problem may be resolved through the continued discovery of novel hepaciviruses. If, in future, novel hepaciviruses are found that group phylogenetically within the current diversity of HCV then a scenario of multiple zoonotic origins is likely (Figure 1c). Conversely, if substantial future hepaciviral diversity is uncovered, yet none falls within the HCV clade, then a single origin hypothesis is supported (Figure 1b; [30]).

Reservoir species and possible routes of transmission

At present we can only speculate on the route of transmission by which a hepacivirus present in a reservoir population (commensal bats or rodents, perhaps) might transfer to humans, horses and cattle. HCV is a blood-borne virus and the majority of people currently carrying it are thought to have been infected through injections or historical blood transfusion. EHV can be similarly transmitted via direct inoculation [39] and the high prevalence of EHV among some racehorses (e.g. [13*]) suggests that at least some EHV transmission among horses may occur via parenteral exposure. In nature, humans and horses could be exposed to hepaciviruses from rodents or bats through faecal contamination of foodstuffs and bedding, or via fomites or aerosols, but there is no evidence that transmission by these routes does, or does not, occur. EHV RNA was not detected in a cohort of 172 people with occupational exposure to horses [40].

One further possible mechanism for hepaciviral zoonosis is transmission via biting arthropods, which could act as either mechanical (non-replicative) or biological (replicative) vectors. Although there are no reports of HCV transmission caused by biting insects, mathematical modelling suggests that mechanical transmission will be exceptionally difficult to observe directly, because high biting rates and a long duration of chronic infection can combine to maintain transmission indefinitely even when the per-bite probability of infection is exceptionally small [36]. It is now known that EHV, like HCV, can establish long-term chronic infection in its host [41]. Pybus *et al.* [36] discussed current theories for the maintenance of endemic HCV in human populations prior to the twentieth century, and explored whether mechanical insect transmission could play an important role. They concluded that this hypothesis was most feasible for insects such as the Tabanidae (horse flies, deer flies) that cut open skin to feed, carry larger volumes of blood on

mouthparts, and actively switch hosts when interrupted during feeding [42].

Tabanids are known to be competent mechanical vectors of several viruses of livestock [43], most notably the retrovirus equine infectious anaemia virus (EIAV). Tabanid transmission of EIAV was first suspected a century ago [44] and was evident from the seasonality and geographic distribution of disease cases [42]. If EHV were transmitted by biting insects then this pattern would not be seen, because the symptoms of acute EHV infection in horses (like those of HCV in humans) seem to be mostly sub-clinical [45*]. The hypothesis of insect-mediated transmission could, in theory, provide a single explanation for EHV transmission among horses, endemic HCV transmission in humans, and the origin of both viruses via cross-species transmission from a reservoir species. Unlike questions concerning HCV transmission, it should be possible to directly test hypotheses of EHV transmission by transferring varying numbers of biting insects, with and without delay, from viraemic to unexposed horses. Much further work, whether experimental, epidemiological or exploratory, will be needed to explain the transmission of the hepaciviruses in natural populations. Until then our understanding of the ecology and threat to human health of these viruses will remain primitive.

Acknowledgments

This work was supported by the European Research Council under the European Commission Seventh Framework Programme (FP7/2007-2013)/European Research Council grant agreement 614725-PATHPHYLODYN.

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