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Investigating the endemic transmission of the hepatitis C virus

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Abstract

The hepatitis C virus (HCV) infects at least 3% of people worldwide and is a leading global cause of liver disease. Although HCV spread epidemiologically during the 20th century, particularly by blood transfusion, it has clearly been present in human populations for several centuries. Here we attempt to redress the paucity of investigation into how long-term endemic transmission of HCV has been maintained. Such transmission not only represents the ‘natural’ route of infection but also contributes to new infections today. As a first step, we investigate the hypothesis that HCV can be mechanically transmitted by biting arthropods. Firstly, we use a combined bioinformatic and geographic approach to build a spatial database of endemic HCV infection and demonstrate that this can be used to geographically compare endemic HCV with the range distributions of potential vector species. Second, we use models from mathematical epidemiology to investigate if the parameters that describe the biting behaviour of vectors are consistent with a proposed basic reproduction number (R0) for HCV, and with the sustained transmission of the virus by mechanical transmission. Our analyses indicate that the mechanical transmission of HCV is plausible and that much further research into endemic HCV is needed.

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1. Introduction

The hepatitis C virus (HCV) is an important viral pathogen of humans, infecting an estimated 120–180 million people globally (CDC, 1998) and causing 3–4 million new infections each year. HCV causes substantial morbidity and mortality worldwide – chronic infection can lead to liver damage (cirrhosis) and hepatocellular carcinoma, resulting in 8000–10,000 deaths per year in the United States alone. HCV is a positive-sense single-stranded RNA virus, taxonomically classified as the sole member of the genus Hepacivirus in the family Flaviviridae. It therefore shares genomic similarities with the flaviviruses, a genus of about 70 species that contains several important human pathogens, including Dengue virus and West Nile virus (Table 1). The vast majority of flaviviruses are transmitted by arthropod vectors, either mosquitoes or ticks, within which they are able to replicate (Gaunt et al., 2001).

HCV is a genetically diverse virus that is classified by phylogenetic analysis into six major genotypes (denoted 1–6), each of which contains many different subtypes (denoted alphabetically, 1a, 2a, 2c, 3d, etc.; Simmonds et al., 2005). The majority of HCV infections worldwide are caused by a subset of subtypes, notably subtypes 1a, 1b, 2a, 2b, 2c and 3a (Simmonds, 2004). These strains are both highly prevalent and globally distributed and consequently have been termed ‘epidemic’ subtypes (Smith et al., 1997; Pybus et al., 2001). Their existence stems from the recent transmission history of HCV, a blood-borne infection that remained unidentified until 1989. During the 20th century, the ‘epidemic’ strains rode a rising tide of human behaviours that inadvertently promoted the rapid global transmission of HCV: blood transfusion, use of blood products, haemodialysis, non-sterile administration of medicines by injection and intravenous drug use. Unsurprisingly, most research interest has been directed towards...
the development of drug, vaccine or preventative strategies against these epidemic subtypes because they cause the bulk of HCV morbidity. Following the identification of the virus in 1989, transmission via blood transfusion and blood products all but ceased.

These factors may explain why few attempts have been made to understand the apparent long history of HCV transmission in some regions, particularly Africa and South-East Asia. Many HCV strains show an 'endemic' pattern of transmission, characterised by a relatively low prevalence and by a high virus genetic diversity in a geographically restricted area (e.g. Mellor et al., 1995; Jeannel et al., 1998; Candotti et al., 2003; Ndjomou et al., 2003). Phylogenetic analyses using molecular clock methods estimate that the genotypes of HCV are in the region of 500–2000 years old (Smith et al., 1997; Pybus et al., 2001), indicating that endemic strains have been present and circulating in human populations for centuries before the introduction of medical injections, surgery and transmissions. The 'epidemic' subtypes of HCV were therefore originally endemic strains that became associated, most likely by chance, with efficient transmission networks during the 20th century (see Pybus et al., 2005).

Previous explanations for the long-term “endemic” transmission of HCV have proposed a panoply of culturally or religiously conditioned routes of transmission, including circumcision, ritual scarification, female circumcision and genital mutilation, as well as acupuncture (e.g. Shepard et al., 2005). While all these possibly contribute to HCV transmission to some degree in their respective locations of practice, the maintenance of HCV endemcity over many centuries and across continents, cultures and religions warrants a more widespread and ubiquitous mechanism.

We suggest two potentially ubiquitous routes of endemic HCV transmission. The first comprises the combined effects of domestic, sexual, vertical and intra-familial transmission. Sexual and vertical transmission of HCV are well studied, but not currently thought to contribute greatly to transmission. About 5% of children of infected mothers are perinatally infected with HCV (Shepard et al., 2005) and the per-year risk of transmission among individuals in long-term monogamous partnerships is just 0–0.6% (Terra-ult, 2002). Very little is known about other, presumably varied, blood-to-blood contact events in the home that might lead to HCV transmission. The global genetic diversity of vertically transmitted viruses should geographically mirror that of the human populations within which they have evolved, as has been suggested for GB virus C. However, HCV does not show any such evidence (although little co-evolution would be expected if HCV has only recently entered human populations).

The second ubiquitous route of endemic HCV transmission is by vector. As we show later, endemic HCV appears to be concentrated in the tropics and sub-tropics, where human populations are subject to higher biting rates by a wide range of abundant arthropods. Furthermore, the rest of the human pathogenic flaviviruses are vector borne (Table 1). HCV has been isolated from bodies or heads of mosquitoes collected from the houses of HCV-infected individuals (Chang et al., 2001; Hassan et al., 2003) and from mosquitoes experimentally fed with infected blood (Silverman et al., 1996; Bellini et al., 1997; Chang et al., 2001; Hassan et al., 2003). These experimental studies have failed to demonstrate that HCV replicates in mosquitoes, although one in vitro investigation reports that HCV can bind to and replicate within the mosquito AP61 cell line (Germs et al., 2001). Evolutionary analysis also indicates that HCV is unable to replicate in arthropod vectors; the unconstrained molecular evolution of the HCV envelope gene is much more consistent with virus replication in just one host species (Woelk and Holmes, 2002). However, this does not preclude the mechanical transmission of HCV on the mouthparts of biting arthropods. Mechanical transmission is important in the epidemiology of several viruses (Carn, 1996) and is non-specific, with single or multiple vector taxa contributing to transmission. In addition to transmission via mouthparts, pathogens could remain intact and infectious in an insect’s foregut and be regurgitated into a new host at a later bloodmeal.

The possibility of mechanical transmission has also been considered for other blood-borne human viruses, particularly human immunodeficiency virus (HIV) and the hepatitis B virus (HBV). Jupp and Lyons (1987) report HIV survival for several hours in bedbugs (Cimex sp.), but did not observe onward virus transmission, and also found

<table>
<thead>
<tr>
<th>Virus</th>
<th>Animal reservoir</th>
<th>Vector</th>
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<tr>
<td>Dengue viruses 1-4</td>
<td>Non-human primates</td>
<td>Aedes mosquitoes</td>
</tr>
<tr>
<td>Japanese encephalitis virus</td>
<td>Domestic pigs, wild birds</td>
<td>Culex mosquitoes</td>
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<td>Kyasanur forest disease virus</td>
<td>Porcupines, rats and mice</td>
<td>Forest tick (Haemaphysalis spinigera)</td>
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<td>Murray Valley encephalitis virus (MVEV)</td>
<td>Birds</td>
<td>Culex mosquitoes</td>
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<tr>
<td>St. Louis encephalitis virus</td>
<td>Birds</td>
<td>Culex mosquitoes</td>
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<tr>
<td>Tick-borne encephalitis virus (TBEV)</td>
<td>Many mammals</td>
<td>Ixodes ticks</td>
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<tr>
<td>West Nile virus</td>
<td>Forest monkeys</td>
<td>Culex mosquitoes</td>
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<tr>
<td>Yellow fever virus</td>
<td>Mainly birds but many mammals as well</td>
<td>Aedes, Haemagogus and Sabethes mosquitoes</td>
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<td>Omsk hemorrhagic fever virus</td>
<td>Rodents, especially muskrats and voles</td>
<td>Ixodid ticks (of the genus Dermacentor)</td>
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<td>Rocio virus</td>
<td>Rodents (mice) and wild birds</td>
<td>Psorophora and Aedes mosquitoes</td>
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<td>Zika virus</td>
<td>Other primates</td>
<td>Aedes mosquitoes</td>
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no survival of HIV virions in *Aedes aegypti* mosquitoes. In contrast, Eigen et al. (2002) reported that infectious HIV can be regurgitated by the stable fly *Stomoxys calcitrans*. For HBV, several studies indicate that the virus can survive for some time in bedbugs and mosquitoes (e.g. Fouche et al., 1990; Silverman et al., 2001). Irrespective of these experimental results, mechanical transmission of HIV and HBV is not documented and even if it does occur, does not contribute appreciably to infection. However, HBV and HIV are transmitted effectively through sex, whereas HCV has no known efficient transmission route prior to the 20th century.

Here we aim to redress the comparative lack of investigation into the endemic transmission of HCV. Firstly, we combine bioinformatic and geographic approaches to estimate, to our knowledge, the first high-resolution global maps of endemic HCV infection. Second, we use epidemiological models to test the plausibility of the hypothesis that HCV can be mechanically transmitted by arthropod vectors.

2. The geographic distribution of endemic HCV infection

Several studies have found restricted geographic areas in which multiple uncommon and genetically divergent HCV subtypes are found (e.g. Mellor et al., 1995; Jeannel et al., 1998; Candotti et al., 2003; Ndjoumou et al., 2003), suggesting a long duration of continuous HCV transmission in these locations. Here we collate and systematically analyse this information to estimate the spatial distribution of endemic HCV infection. Although geographic plots of HCV genotype distributions have been made before (see http://hcv.lanl.gov/), these have not made the crucial distinction between epidemic and endemic strains, nor have they contained spatial information below the national level. We further use these distributions in a preliminary, proof-of-principle demonstration of how the geographic distributions of candidate vector species can be correlated with the distribution of endemic HCV.

In order to identify genetically divergent strains, we first obtained viral gene sequences from the HCV Sequence Database (Kuiken et al., 2005). Database sequences that met the following criteria were retained: (i) the sequence was at least 200 nucleotides long, (ii) the sequence represented either the Core, E1 or NS5B genes. These genes were chosen because they are frequently sequenced, phylogenetically informative, and commonly used to assign sequences to subtypes and (iii) the sequence did not belong to one of the globally distributed ‘epidemic’ subtypes; specifically, subtypes 1a, 1b, 2a, 2b, 2c, 3a. No sequences from genotype 5 were collected because it has limited genetic diversity and, as yet, no detectable regions of long-term endemic transmission (Verbeeck et al., 2006). In addition, subtype 4a strains from Egypt and subtype 4d strains from France were excluded. These strains have not spread globally, but did spread locally very quickly during the 20th century as a result of medical injections (Frank et al., 2000) or injecting drug use (Morice et al., 2001) and therefore represent ‘local epidemic’ subtypes rather than endemic transmission. The remaining collated sequences were grouped according to genotype and gene region and aligned by hand. Phylogenetic analysis was used to establish or confirm the subtype assignment of each sequence. Maximum likelihood phylogenies were estimated under the HKY + Γ nucleotide substitution model using the maximum likelihood approach implemented in PAUP* (see http://paup.csit.fsu.edu for details).

We then collated all available geographic information for each HCV strain, starting with the HCV sequence database (Kuiken et al., 2005), which provides the country of sampling for many sequences. However, this information is missing for many entries and is imprecise for large countries such as India or China. In addition, many genetically divergent strains sampled in ‘developed’ countries (e.g. North America, Europe) were obtained from recent immigrants. We therefore undertook a systematic search of the primary literature for more precise information about the location and immigrant status of sampled individuals. If the literature was uninformative, or if sequences had been submitted to the database without an accompanying paper, we attempted to contact the sequence authors directly, many of whom provided further information. Here, we considered the immigrants’ country of origin to be a better estimate of the location of HCV infection than the country of sampling (even when the country of infection was not conclusively known) for three reasons: (i) HCV prevalences are typically higher in the country of origin, (ii) genetically divergent endemic strains are known to be concentrated in Africa and Asia and (iii) immigrant strains usually group phylogenetically with strains sampled from the immigrant’s country of origin.

The locations (cities, districts, regions) obtained were geopositioned in decimal degrees using digital gazetteers (GEOnet names server, available from http://earth-info.nga.mil/gns/html/; Alexandria Digital Library, available from http://www.alexandria.ucsb.edu; Encarta 2006 Map Library, Microsoft). Our complete data set contained the spatial coordinates of nearly 1500 HCV sequences. For 23 locations, only country-level information was available, in which case the geographical centre of the country was used. The bias arising from this will depend upon country size, but should be small overall, since only eight of these 23 locations were very uncertain (corresponding to the centres of Egypt, India, Saudi Arabia, Thailand, Vietnam, Democratic Republic of Congo, Ghana and Nigeria). Lastly, we classified all sequences into four groups according to their immigration status: (1) non-immigrant, (2) confirmed immigrant (in which case the individual’s country of origin was the location used), (3) possible or probable immigrant (in which case the individual’s country of sampling was the location used) and (4) no information about immigrant status.

The methods described above – for identifying endemic strains and for determining the location and immigration
status of infected individuals – are rule-based and therefore may occasionally misclassify or mis-position strains (discussed below). However, the advantages of this approach are that it is repeatable, consistent, and amenable to future improvements. A spreadsheet containing the complete genetic and geographic information for each strain is available on request.

Fig. 1 shows the mapped locations of endemic HCV. Fig. 1a shows the number of sequences obtained for each sampling location, which was highly variable. A particularly large cohort of sequences from the UK was unfortunately without information on the immigrant status of the sampled individuals. Fig. 1b colours the sequence locations according to HCV genotype. Northern European countries (UK, Netherlands, France, Belgium) contain a wide variety of genotypes, almost certainly as a result of the immigration to those countries of infected individuals from Africa and Asia. More unexpectedly, Java also contains a variety of ‘endemic’ genotypes. This may reflect either local, epidemic transmission (Smith et al., 1997) or a past influx of strains to Java resulting from the region’s role as a historical centre of global trade.

As previously noted (Smith et al., 1997), endemic genotype 6 strains are found in South East Asia and genotype 4 strains are concentrated in Central Africa and the Middle East, particularly in Egypt. Endemic genotype 1 and 2 strains are found most often in West Africa; their presence on other continents is likely the result of migration. Genotype 3 strains are characterised by a South Asian distribution, but are also reported in China and Japan. Fig. 1c shows the locations coloured according to the immigration status of the sequences. Confirmed immigrants were originally from either Africa or Asia. Most of the other sequences from western countries were classified as probable immigrants or had no information on immigration status.

Two previously unrecognised patterns can be discerned from Fig. 1. Firstly, the distribution of endemic HCV in Africa is equatorial and covers the entire width of the continent, not just west-central Africa as previously discussed. Second, endemic HCV appears to be evenly distributed through South Asia and the Indian subcontinent, in addition to being common in South-East Asia.

There are some outliers to the above patterns. Firstly, subtype 2k is notably distributed among several countries that were part of the former Soviet Union, perhaps due to migration from Africa followed by the distribution of blood or blood products throughout the former Soviet bloc. Second, a cohort of infections from southern France contains multiple genotype 2 strains (Cantaloube et al., 2005). It is not clear how this situation arose, but our rules classified these as endemic because the infected individuals appeared to be non-immigrant. However, undocumented migration or multiple contamination of blood supplies are possible explanations, in which case genotype 2 should not be considered endemic to France. Third, there are a very large number of sequence locations from Egypt. Egypt has a uniquely high HCV prevalence, resulting from the widespread 20th-century use of injectable anti-schistosomiasis treatments (Frank et al., 2000). Genetic analysis suggests that HCV was present at low prevalence in Egypt around the 1920s (Pybus et al., 2003), so it seems reasonable to include Egypt as a location of historical infection. However, Egyptian sequence locations are perhaps over-represented in our analysis because they are very common in the HCV database, and some should perhaps be properly classified as ‘local epidemic’ strains (as was subtype 4a).

As a first step in identifying correlates of endemic HCV infection, we overlay the locations of endemic HCV onto the estimated range distributions of several well-known disease vectors (Fig. 2). This includes four mosquito groups studied in previous experimental investigations of HCV mechanical transmission, plus representatives of the ticks and biting flies. The match between endemic HCV and vector distribution is generally good for all vector species with a tropical distribution. This is perhaps unsurprising in the context of the global distribution of infectious disease – human disease diversity increases towards the equator and this trend is very strong for indirectly transmitted viruses (Guernier et al., 2004), possibly because arthropod diversity also follows the same latitudinal tendency.

Of course, correlation is not causation and a geographic match does not demonstrate that mechanical HCV transmission occurs. However, the absence of a reasonable match to any species would cast doubt on the plausibility of mechanical transmission. Observed matches could also result from confounding factors that are correlated with both geography and endemic HCV transmission. Most obviously, any traditional practice or treatment against vector-borne disease that also increases HCV infection risk could explain an observed match. We provide these plots here to illustrate that our spatial distribution of endemic HCV (Fig. 1) can be used to explore potential risk factors for endemic HCV. In future studies other proposed risks, such as cultural practices like tattooing or scarification, could be compared with the geographic distribution of endemic HCV in a similar way.

Fig. 1. The global geographic distribution of endemic hepatitis C virus (HCV) strains, mapped using the methods described in the main text. The Pacific contains no data points and is not displayed. (a) The number of sequences per location is shown proportional to the area of the grey circles. (b) Sequence locations are coloured according to the genotype of HCV sequences found there. Entirely overlapping points of different colours have been moved slightly apart to improve clarity. Red, genotype 1; black, genotype 2; green, genotype 3; blue, genotype 4 and yellow, genotype 6. (c) Sequence locations coloured according the immigration status of the sequences found. As above, overlapping points have been moved slightly apart. Red, non-immigrant individual; yellow, confirmed immigrant individual (mapped to country or region of origin); blue, probable immigrant (mapped to country of sampling); green, no information on immigration status.
3. Models of vector-borne transmission

A handful of experiments have considered the hypothesis that HCV could be transmitted by vectors (Silverman et al., 1996; Bellini et al., 1997; Chang et al., 2001; Hassan et al., 2003). Here we attempt to gain greater insight into this hypothesis by considering epidemiological models of the transmission of vector-borne pathogens.

Standard epidemiological models for insect-borne transmission of micro-parasites focus on the fundamental epidemiological parameter \( R_0 \) – the basic reproduction number (Anderson and May, 1991). \( R_0 \) is defined as the number of secondary infections resulting from the introduction of a single infectious case in an entirely susceptible population. It combines the intrinsic transmissibility of an infection with the potential for infectious contacts in the population. Because \( R_0 \) is not a function of prevalence, it can be compared among outbreaks and among different diseases. When \( R_0 < 1 \) each case gives rise to less than one new infection and the infection dies out. If \( R_0 > 1 \) then each case infects more than one individual on average, resulting in an initially exponentially growing epidemic that later slows once equilibrium prevalence is approached.

For a vector borne disease, \( R_0 \) is a function of several variables that describe the nature of the infection in the vector and host species, the density and biting behaviour of the vector, and the transmissibility of the infection. Table 2 provides a definition and biological interpretation of each variable and Eq. (1) shows how they are combined to calculate \( R_0 \):

\[
R_0 = \frac{ma^2 \beta^2 zD}{\mu + s}
\]  

Eq. (1) is derived from the models presented by Randolph (1998) and Anderson and May (1991) for insect-borne transmission, with one minor modification to make the model specific to the process of mechanical transmission: we have introduced parameter \( s \), the viral clearance rate in vectors. This represents the rate at which infectivity in the vector is lost through time because viral replication within the vector is not possible. This decay in infectivity was observed in experiments during which HCV infected blood was fed to mosquitoes (e.g. Silverman et al., 1996; Hassan et al., 2003). Furthermore, we simplify the model by assuming that the transmission coefficient from host to vector is equal to that of vector to host.

To use this model we need values for each model parameter. We obtained parameter estimates and ranges from the literature, using mosquitoes as a model system for our exploration (see Table 2). Mosquitoes were chosen because previous experimental studies have focused on mosquitoes, and because the biting behaviours and abundances of mosquitoes are better studied than those of other arthropods. However, mosquitoes may not be biologically well suited to be HCV mechanical vectors, a point considered in detail in Section 4. We were able to find values for all the model parameters except \( \beta \), the transmission coefficient, which represents the probability per bite of transmission from infected host to uninfected mosquito, or vice versa. \( \beta^2 \) is therefore the probability that an infection transmits from one human to another through two bites of the intermediary vector. This transmission coefficient is obviously specific to the pathogen transmitted and therefore it is unsurprising that no HCV-specific value was found.

Eq. (1) and Table 2 can be combined to suggest a range of transmission coefficient values consistent with the endemic mechanical transmission of HCV. By substituting the estimated parameter values from Table 2 into Eq. (1) we obtain \( R_0 = 22,850 \beta^2 \). If the most extreme values from Table 2 are substituted instead, then we obtain minimum and maximum results of \( R_0 = 4\beta^2 \) and \( R_0 = 2,265,450 \beta^2 \), respectively. The only paper to date that has estimated \( R_0 \) values for endemic HCV reported that \( R_0 = 1.68 \) for genotype 4 and \( R_0 = 1.21 \) for genotype 6 (Pybus et al., 2001). \( R_0 \) values close to one are to be expected given the low prevalence generated by endemic transmission. By substituting the average of these two \( R_0 \) values into Eq. (1), we estimate that \( \beta^2 = 0.000063 \) (maximum possible \( \beta^2 = 0.36 \); minimum possible \( \beta^2 = 6.4 \times 10^{-7} \)). This calculation indicates that only very low transmission probabilities need to be assumed for the hypothesis of mechanical HCV to be realistic. We estimate that if only one in 16,000 host-to-host transmission events actually transmits the virus, then long-term endemic transmission will be maintained.

The parameter values for mosquitoes used above may not accurately reflect those for other potential vectors, and there is likely to be substantial uncertainty associated with each estimate. However, \( R_0 \) may actually be higher for biting flies, given their longer survival and higher rate of switching among hosts (Randolph, 1998). If so, this would permit even lower transmissibilities to be consistent with the maintenance of endemic infection. Additionally, it is possible that multiple vector species are capable of mechanically transmitting HCV, especially given the much lower level of biological specificity required for pathogen/mechanical vector associations compared with pathogen/
Fig. 2 (continued)
biological vector associations. The contribution of each mechanical vector species to incidence, when combined, will therefore further reduce the transmission coefficients necessary to sustain endemic transmission. It therefore seems reasonable to consider that our transmission coefficient values are conservative.

Lastly, the above calculations explore mechanical transmission as the sole route of infection, and do not take into account other potential routes, such as sexual, perinatal and general intra-familial transmission. The low transmission coefficients obtained stem from the high frequency of biting events combined with the very long duration of infectiousness in humans. This combination of factors can also be used to argue for the likelihood of sexual HCV transmission among long-term partners. Hence it may be possible to perform a calculation similar to that above to investigate whether sexual and vertical transmission alone are capable of sustaining HCV transmission. However, the exposure rate will generally be higher for mechanical transmission but transmission coefficients are likely greater for sexual transmission. If multiple routes contribute to endemic HCV infection then the transmission coefficients of each route can again be reduced. Given the short duration of HCV infectiousness on mechanical vectors, it is likely that most mechanical transmission will occur in the home and hence will at present be classified as intra-familial or domestic transmission.

4. Discussion

Our epidemiological model results are likely conservative because more than one vector species could contribute to mechanical transmission. In contrast, viral replication within a vector requires specific viral adaptations that restrict the range of vectors available. Viral switching among vector species is exceptionally rare in the evolution of the flaviviruses and much less frequent than changes in host species range (Garrett-Jones and Shidrawi, 1969; Molineaux and Gramiccia, 1980). To date there has been little evolutionary consideration of mechanical transmission as a viral life-history strategy. If mechanical transmission is spread among many casual vectors, so that no one vector species contributes sufficiently to sustain transmission by itself, then there is no selective advantage to the virus in evolving the capacity to replicate within a specific vector. Furthermore, we would expect mechanical transmission to be a beneficial transmission strategy only when host infectiousness is prolonged and potential vector species richness is high, for example in the tropics.

If HCV is mechanically transmitted, then culprit vectors should not necessarily be restricted to the biological vectors of the related flaviviruses. Factors that make certain insects more likely mechanical vectors than others include the tendency to bite two or more hosts in close succession, the volume of blood carried on mouthparts and the probability of blood being regurgitated into a new host. In our epidemiological model, we used mosquitoes as an example merely because much more is known about their behaviour and distribution. However, other taxa, particularly the tabanid, stable and tsetse flies, could be more likely candidates. The bites of many blood-sucking flies are painful or irritating, prompting the provoked host to drive them away, often, in dense populations, to another host. Rapid host switching increases the probability of an infectious virus surviving on the vector until it reaches a new host. In addition, larger
vectors have lower feeding persistence – the tenacity with which a vector engages a single host – and may therefore switch hosts more readily (Foil, 1989). Furthermore, vector size is correlated with the size of biting parts, the amount of blood left on those and the size of the biting wound. The volume of blood with which a mosquito can inoculate a new host (having been interrupted whilst feeding on a previous host) is reported to be less than a picolitre (Booth, 1987), ruling out transmission of HIV. Greater volumes of blood are thought to be carried by flies such as S. calcitrans (Prullage et al., 1993).

Other candidate vectors include the less mobile triatomid bugs, bedbugs, fleas and lice. Ticks seem unlikely suspects as most species have little mobility and feed only once per life-stage, with very long durations between blood meals (Randolph, 1998), during which HCV is unlikely to survive (Krawczynski et al., 2003). An important task for future work is to combine the global geographic distributions of multiple biting arthropods, in order to estimate the potential for the mechanical transmission of blood-borne diseases in different parts of the world.

The prevalence of biologically transmitted pathogens in vectors can be low even though the pathogen may live in the vector for many days (Anderson and May, 1991). The low transmission coefficients we propose, and the likely rapid decay of infectious HCV within a mechanical vector, suggest that prevalence of HCV infection amongst vectors will be exceedingly low. Hence, the probability of detecting infectious vectors and observing host-to-host transmission in cross-sectional field studies may be remote and experimental tests of mechanical HCV transmission (of the scope and size of previous studies) will be unlikely to generate positive results. Therefore, the current absence of documented cases of mechanical HCV transmission should not, as yet, be interpreted as a rejection of the hypothesis that it occurs.

Although our analyses are indirect and our results circumstantial, we conclude that they suggest that the mechanical transmission of endemic HCV is, at the least, a plausible hypothesis that warrants greater consideration. Despite primarily considering mechanical transmission here, we recognise that intra-familial transmission is also an important factor that requires further research. We hope our analyses will encourage greater interest in endemic HCV transmission, which not only represents the ‘natural’ mode of transmission to which the virus must have become adapted over several centuries, but is also an ongoing infection route in parts of the world today.

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