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# HCV-specific cellular immune responses in subjects exposed to but uninfected by HCV

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# Exploring the Possibility of Arthropod Transmission of HCV

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Hepatitis C virus (HCV) is a major cause of chronic hepatitis, cirrhosis, and liver cancer occurring in up to 3% of the world's population. Parenteral exposure to HCV is the major mode of transmission of infection. Once established, infection will persist in up to 85% of individuals with only a minority of patients clearing viremia. Egypt has possibly the highest HCV prevalence in the world where 10–20% of the general population are infected with HCV. Endemic HCV appears to be concentrated in the tropics and sub-tropics where there are higher biting rates from insects. The question as to whether a bridge vector transmission is possible, via arthropods, both between humans and/or from an animal reservoir to humans is explored. Mechanical transmission, as opposed to biological transmission, is considered. Mechanical transmission can be an efficient way of transmitting an infection, as effective as biological transmission. Probability of transmission can increase as to the immediate circumstances and conditions at the time. Several factors may enhance mechanical transmission, including high levels of microbes in the vector, frequent biting, the close proximity, and contact between vectors and recipients as well as high density of insects. HCV has been isolated from bodies or heads of mosquitoes collected from the houses of HCV-infected individuals. The possibility of enzootic cycles of HCV tangential transmission via bridging vectors, such as, arthropods needs to be further investigated and possible animal reservoirs, including domestic rural epizootic cycles for HCV infection, requires further research with particular initial emphasis on equine infections. ***J. Med. Virol.***



**KEY WORDS:** HCV; mechanical transmission; biological transmission; *arbovirus*; arthropod transmission; mosquito; *flavivirus*; enzootic cycles; bridging vectors; animal reservoirs; tangential transmission; rural epizootic; urban endemic cycle

## INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic hepatitis, cirrhosis, and liver cancer occurring in up to 3% global prevalence of the world's population [Alter 1999; Thomas 2013; Lee et al., 2014]. Parenteral exposure to HCV is the major mode of transmission of infection and once established, infection will persist in up to 85% of individuals with only a minority of patients clearing viremia. Egypt has possibly the highest HCV prevalence in the world where 10–20% of the general population are infected with HCV [Mohamoud et al., 2013]. The infection is the leading cause of hepatocellular carcinoma (HCC) and chronic liver disease in the country [Nafeh et al., 2000; Habib et al., 2001]. A single HCV subtype of 4a contributes to 90% of Egyptian HCV isolates. The probability of transmission may be enhanced by the extremely high population prevalence of HCV and whose spread can be reconstructed to have occurred in the 1930s–1950s during a period that coincides with targeted extensive parenteral antischistosomal injection campaigns and may represent the world's largest iatrogenic transmission of blood-borne pathogens. This intensive transmission established a large reservoir of chronic HCV infection, responsible for the high prevalence of HCV infection and current high rates of transmission [Darwish et al., 1996; el-Zayadi et al., 1997; Frank et al., 2000; Pybus et al., 2001, 2003, 2007, 2009].

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Although animal reservoirs are not the focus of this paper, a consideration of this aspect supports the discussion of the subject as a source of arthropod transmission of HCV. The characterization of new *Hepaciviruses* that infect animal species has greatly contributed to our understanding of the origins of HCV infection in humans [Burbelo et al., 2012]. Is it possible that the human infection of HCV is acquired zoonotically from a non-human reservoir of pathogens? The direct ancestor of HCV is not known, but it is genetically most closely related to *Hepaciviruses* that exist in horses [Lyons et al., 2012, 2014; Tanaka et al., 2014] also bats are an ancient evolutionary natural reservoir for some *Hepaciviruses*. Indeed, bats are a major reservoir host for several viruses that infect humans [Quan et al., 2013; Schountz, 2014].

It should be noted that other viruses, such as, Ebola (EV), Human Immune-Deficiency Virus (HIV), and Severe Acute Respiratory Syndrome (SARS) are all infectious to humans due to a host shift where the pathogen originates from a different host species. Host shifts often are accompanied with a substantial change in virulence with relatively benign infections in one host becoming fatal in others. High viral loads often result in high levels of virulence, and this may determine the transmission rate of the virus [Longdon et al., 2015].

Clinical hosts (horses, cows, and pigs) may spread the virus by direct contact or indirectly via mechanical transmission by arthropods and animal handlers or veterinarians. However, the source of infection and natural reservoir of some viruses are uncertain, and many theories have been proposed [Monath, 1999].

In the *Flavividae* family, the genus *Flavivirus* can be broadly divided into three groups; first the dual host viruses; second are the insect-specific *Flaviviruses* that infect mosquitoes but do not infect vertebrates or vertebrate cell lines; third vertebrate-only *Flavivruses* (no known vector group) [Kuno, 2004].

*Flaviviruses* often infect multiple vertebrate hosts and are transmitted by multiple vector species but there is little evidence, so far, of an animal reservoir for viruses closely related to HCV, however, several recent studies have discovered hepatic and *Pegivirus* genera in small wild animals such as rodents and bats, which are related to HCV [Drexler et al., 2013; Hemachudha et al., 2013; Kapoor et al., 2013]. Domestic animals, such as, horses that contract non primate *Hepacivirus* (NPHV) and dogs that contract canine *Hepacivirus* (CHV) are also susceptible to infection by newly observed HCV related viruses [Bukh, 2011; Kapoor et al., 2011; Lyons et al., 2012; Tanaka et al., 2014]. The horse is the natural host for NPHV, which is very close in RNA sequence to HCV. Genus-specific features including hepatotropism, with other key viral peptides and some homologous regions of HCV genotypes 1–7 were found in a portion of immunoreactive horses tested [Kapoor et al., 2011; Burbelo et al., 2012].

It is thought that NPHV infection may be the origin of human infection [Simmonds, 2013]. Domestic horses are most unlikely to be the only mammalian species (other than human or tamarins) infected with *Hepaciviruses* and there is clearly much to be learned in short term from more extensive screening. Horses found to be positive for *Hepacivirus* sequences by PCR appeared to remain well but some had nominal indicators of liver inflammation, after liver function tests [Lyons et al., 2012, 2014; Kapoor et al., 2015].

Avian Encephalomyelitis (AE), caused by a viral infection, is an example of a *Picornavirus*, harboring an HCV-like internal ribosome biding site (IRES) element within its genome, and thus, its classification within the *Hepatovirus* genus may need to be reassessed in light of these findings and may indicate the possibility of an avian animal reservoir for HCV as well as AE [Bakhshesh et al., 2008; Belsham, 2009].

Recent advances in sequencing technologies have greatly enhanced our abilities to identify novel microbial sequences and recognize zoonotic reservoirs for emerging infections [Scheel et al., 2015].

West Nile virus (WNV) is an infection caused by a *Flavivirus* and demonstrates an example of an arboviral zoonotic cycle. The animal reservoir of the virus consists of birds and its vector is a mosquito of the *Culex* (*Cx*) genus. WNV initially affected Africa, part of central and southern Europe, the Middle East, and India but is spreading to the West. It remains to be seen if co-infection of insect-specific *Flaviviruses* will change the vector competence of mosquitoes for pathogenic *Flaviviruses* [Doyle et al., 2011; Huang et al., 2014; Huhtamo et al., 2014; Yan-Jang et al., 2014]. Another arboviral *Flavivirus* is the Zika (ZIKV) virus, which is transmitted via a mosquito (*Aedes*) vector with some recent examples of sexually transmitted pathogens in humans [Schuler-Faccini et al., 2016], including anecdotal evidence from Assoc. Prof. Brian Foy of hematospermia in ZIKV-infected patients.

In the case of EV and Marburg hemorrhagic fevers, the identification of natural reservoir species has been sought (bats and rats are suspected hosts), neither hosts nor arthropod vectors have been definitively identified, although Leroy et al. [2009] strongly suspects that fruit bats were linked to the EV outbreak in Luebo, Democratic Republic of Congo, in 2007 [Feldmann and Geisbert, 2011]. Other virus examples are the Crimean-Congo hemorrhagic fever, African swine fever, vesicular stomatitis viruses (VSVs) [Monath, 1999]. Interesting host switches to consider are the yellow fever (YF) and dengue (DENV) viruses as two arboviral pathogens that have adopted humans as their amplification hosts thus enabling urban disease. DENV is currently the most prevalent mosquito-borne virus pathogen in humans, also a *Flavivirus*, transmitted by *Aedes aegypti* and *Aedes albopictus* and earlier forms of DENV was transmitted among nonhuman primate reservoir host by arboreal mosquitoes [Weaver, 2005; Simmons et al., 2012].

In an enzootic cycle, a vector borne exposure happens when an insect transmits a pathogen from an animal reservoir to another animal. The vector transmits the pathogen via a mechanical mechanism or a biological one. In a mechanical transmission, the virus is transported by one animal to another and does not replicate or develop in the vector. In the case of biological transmission, the vector takes up the microbial agent through a blood meal from an infected animal, replicates, and develops it but then regurgitates the pathogen onto or injects it into a susceptible animal. The most common hematophagus vectors of pathogens are arthropods such as fleas, kissing bugs, biting flies, lice, ticks, horseflies, fleas, and mosquitoes. Bed bugs have been investigated and suspected of being vectors of pathogens but this has not yet been proven.

The arthropods that transmit between animal species, including humans, are known as bridging vectors. Tangential transmission of human infection HCV infection can arise from random crossovers of these enzootic cycles where a tangential transmission can occur between bridge vectors and humans. This can happen when humans enter sylvatic enzootic environments. It may be possible for domestic animals with a rural epizootic cycle, such as horses to enter the cycle (Fig. 1).

When a mosquito bites, it injects saliva through one channel. The saliva functions as a lubricant to help the mosquito feed easier. The blood that a mosquito sucks as a meal flows in a completely separate tube than the channel that saliva is injected and only in one direction, toward the mosquito. It has been considered, so far, that it is biologically unlikely for infected blood to be spread to another person when the insect feeds off an uninfected individual. Biological transmission occurs when the pathogen replicates or develops within the mosquito, prior to transmission, as is the case with Malaria (Anopheles). This is considered, by some, not to happen with HCV virions in mosquitoes by the World Health Organization (WHO). Thus WHO considered that blood infected with HCV from one person is not injected into another human causing HCV infection. However, in contrast to biological transmission, mechanical transmission occurs when the pathogen is transmitted without amplification and development within the insect via contaminated blood remaining on the insect's tubular mouthparts [Mullenes and Durden, 2009; Baldacchino et al., 2014]. HCV virions have been found to be resilient for up to 6 weeks on inanimate surfaces at room temperature [Paintsil et al., 2014] and for several days when dried and exposed to the environment at room temperature [Kamili et al., 2007]. Therefore, HCV may remain infectious on the proboscis of an arthropod for a period of time, enabling it to infect the next mammal that it feeds from. HCV RNA was detected in the heads of symbiotic mosquitoes collected from homes of HCV positive patients at 3 and 6 hr after feeding [Hassan et al., 2003]. Another research group did not establish HCV viral replication in *Cx. quinquefasciatus* species of mosquito and concluded that biological transmission did not occur in this species. However, the experiments in China established, that HCV replication did not occur and did not demonstrate viral transmission but that HCV viral RNA was detectable on the mosquitoes during the experiments [Chang et al., 2001]. In experiments that fed mosquitoes blood infected with HCV, the virus RNA was

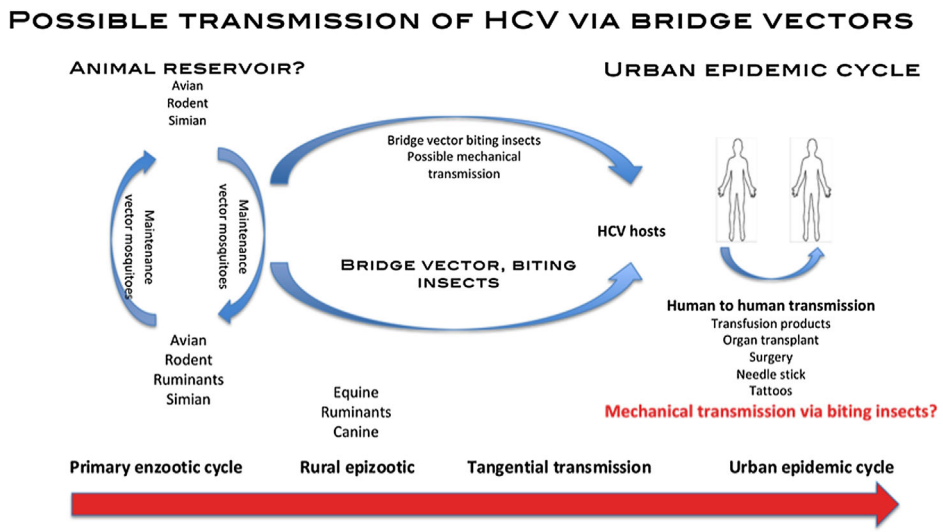


Fig. 1. Possible animal reservoir infected with HCV, which vector bridge biting insects transmit to domestic animals and humans, possibly continuing to transmit from human to human in areas with endemic disease and high population of bridge vectors (adapted from Weaver and Barrett [2004]).

detected in the guts of the insects 3–8 days after being fed HCV RNA positive blood [Ting-Tsung et al., 2001; Hassan et al., 2003].

Mosquito AP61 cells, inoculated with HCV-positive plasma, were examined for the presence of the viral genome at different times post infection, using an RT-PCR method. Binding of HCV to cells was estimated by HCV RNA detection in cells 2 hr after inoculation and in the last wash of these cells. All the cells studied were able to bind HCV but only AP61 cells provided evidence of replication and production of infectious virus. Virus RNA was detected during 28 days post-infection in four successive virus passages. CD81 molecules, a putative HCV receptor, were detected by cytofluorometric analysis [Germi et al., 2001]. Several cell surface molecules have HCV binding properties and are considered to serve as receptors facilitating viral entry into cells. The large extracellular loop (LEL) of CD81 has been shown to bind the HCV envelope protein E2 with several critical residues for the CD81–HCV–E2 interaction [Houldsworth et al., 2014]. These findings give evidence that both mechanical and biological transmission of the virus may be possible [Germi et al., 2001].

Blanc and Gutiérrez [2015] compared non-circulative transmission of plant viruses with mechanical transmission of animal viruses and proposes that these processes have much more in common than previously thought. Blanc and Gutiérrez [2015] describes the sophisticated process of vector specificity in non-circulative plant viruses including the identification of virus-specific receptors in a newly identified structure within the maxillary stylets as well as in the foregut. Non-circulative plant viruses comprise the majority of plant viruses, and can be either non-persistent, where transmission must occur within minutes or hours, or semi-persistent, where the virus is retained by the vector for hours to days but transmissibility is lost when the vector moults [Weaver and Simon, 2015].

Interestingly, areas endemic for *Plasmodium falciparum* (mosquito borne), hepatitis B virus (HBV), and HCV overlap in many parts of sub-Saharan Africa and is something that may need further investigation to ascertain if this is a coincidence and provide more evidence as to whether mosquitoes transmit HCV in these areas [Vall Mayans et al., 1990; Ouwe-Missi-Oukem-Boyer et al., 2011].

Huff [1965] stated, in his Charles Franklin Craig lecture, "When we look back on the history of malaria and its control we are struck by the repetition of failures made as the result of premature generalizations. To stop the spread of malaria it first appeared to be only necessary to eradicate Aiwpheles." Chamberlain and Sudia [1961] addresses the importance of mechanical transmission of pathogens, with the vector carrying the infection on its mouthparts and recognized that mosquitoes, in this way, can transmit viruses. Furthermore, he recognized that infection from a vector could be categorized as biological or mechanical but that premature generalization about the mechanisms of pathogen transmission should be avoided. Day, Fenner, and Huff have also previously discussed mechanical transmission of pathogen via arthropod vectors and in a parasitical review by Day, mechanical transmission is categorized into three regions of simple, delayed, and modified [Day, 1955; Day et al., 1956; Fenner et al., 1956; Huff, 1965].

About 14,000 infectious microorganisms are associated with mosquitoes and 600 are shared between animals and humans and some pathogens tend to be specific to a mosquito genus or species. *Aedes aegypti* female mosquitoes are capable of the mechanical transmission of the Lumpy Skin Disease Virus (LSDV) from infected susceptible cattle, 2–6 days post-infective feeding. A recent paper suggests that there is currently limited knowledge about the mechanical transmission of viruses and that more needs to be understood about the restriction transmission modes to a given host type [Blanc and Gutiérrez, 2015]. Chihota et al. [2001] sug-] suggests that mosquito species are competent vectors [Lubinga et al., 2013]. Genetic variations in both vector and virus strains and their interactions can profoundly influence the efficiency of transmission to the host thus strongly influencing vector competence in a three-dimensional way [Scott and Simon, 2015; Vasilakia and Tesh, 2015].

Although mosquitoes are an example of the many hematophagus organisms able to host pathogens and are known to carry many infectious diseases from several different classes of microorganisms, it is important to consider other hematophagus species that may be candidates for HCV transmission and have that has been proved to transmit other infections.

Bed bugs have been suspected of transmitting infectious agents; over 40 microorganisms have frequently been considered as strong candidates [Burton, 1963; Goddard and deShazo, 2009; Adelman et al., 2013]. In contrast to mosquitoes or ticks, the literature evidence for pathogen transmission by bed bugs (*Cimex lectularius*), is very heterogeneous and sometimes incomplete [Delaunay et al., 2011]. Furthermore, bed bugs have also been found in association with bat and bird populations [Calisher et al., 2006; Balvin et al., 2012].

Although there is currently no evidence that bed bugs and Stomoxys flies can transmit HBV to humans, HBV has frequently been detected in wild bed bugs [Godard and deShazo, 2009]. In the laboratory, it has been detected up to 2 months after an infectious meal or after direct injection into the bedbug, it has been found in feces, and transstadial transmission has been demonstrated. These members of the triatominea, hematophagus family have also been proved to be competent vectors for the parasitic worm, Chagas disease (American trypanosomiasis) [Salazar et al., 2015]. However, bed bugs (*C. lectularius* and *Cimex hemipterus*) and

mosquitoes (*Ae. Aegypti*) were found not to act as vectors to transmit HIV in in vitro experiments, but generalization should be made cautiously as different insect species and other viruses may associate differently [Jupp and Lyons, 1987].

Ticks have also been shown to transmit pathogens to humans and pathogens were detected in saliva samples of both *A. hebraeum* and *R. appendiculatus* ticks where mechanical/intrastadial and transstadial passage of the virus was demonstrated and confirmed [Lubinga et al., 2013].

Tabanid flies are also blood feeding and important vectors of pathogens to human and livestock, such as, surra, anthrax, and *Loa loa* [Mullenes, 2009], belonging to the *Tabanidae* family of large Brachyceran flies of which there are 4,400 worldwide species and 144 genera [Evenhuis et al., 2008]. In Egypt, the *Tabanidae* family was studied by Kröber [1925]. Currently, Tabanid flies are represented by 30 species and one variety within 5 genera according to the list of Steyskal and El Bialy [1967] and Gawhara et al. [2010]. Interestingly, when considering that HCV-like infections exist in horses, it may be possible for Tabanids such as horse flies to transmit human and animal disease agents both biologically or mechanically [Krinsky, 1976; Foil et al., 1987, 1988a, b]. Tabanids are highly mobile, engage in interrupted feeding and have large mouthparts [Foil et al., 1987]. It has been thought that mechanical transmission is of minor epidemiological importance for transmitting pathogens and that biological vectors carry much more relevance with this regard [Cam, 1996]. However, although the impact of this is difficult to assess, in specific circumstances, mechanical transmission can be an efficient way of transmitting an infection and as effective as biological transmission. Probability of transmission can increase depending on the immediate circumstances and conditions at the time [Desquesnes et al., 2009, 2013]. There are several factors that can enhance mechanical transmission including interrupted feeding, quantity of blood meal residues on mouthparts, sensitivity to host defensive reactions, and the tendency to switch hosts, biting intensity, and quantity [Desquesnes et al., 2009, 2013]. Other factors include, high levels of microbes in the vector, frequent biting, and the close proximity and contact between vectors and recipients and high density of insects.

When considering the role of Tabanids as viral vectors, interrupted feeding is an important factor, increasing the probability of mechanical transmission between hosts [Magnarelli and Anderson, 1980]. Tabanids have been shown to be capable of mechanically transmitting pathogens like Equine Infectious Anaemia Virus (EIAV) [Foil et al., 1989; Foil and Hogsette, 1994].

Adding to the complexity of this discussion, it should be considered that much research is required to investigate vector competence of various mosquito species and different mosquito-borne pathogens. WNV has been isolated from more that ten mosquito species in India but a limited number of them have been investigated for their efficient transmission to humans by *Cx. quinquefasciatus* mosquitoes [Sudeep et al., 2015]. The British Isles, alone, has about 30 endemic mosquito species, several with seasonal abundance and other eco-behavioral characteristics predisposing them to serve as potential WNV bridge vectors from birds to humans [Medlock et al., 2005].

Endemic HCV appears to be concentrated in the tropics and sub-tropics. Human populations in these regions experience higher biting rates due to an abundance of many varieties of arthropods. As previously discussed, the rest of the human pathogenic *Flaviviruses* are vector borne and 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]. Pybus et al. [2009] write extensively on this subject suggesting the possibility of arthropod transmission of HCV.

The possibility of arthropod transmission of HCV from an animal reservoir via bridging vectors such as mosquitoes appears to have some evidence to support the concept but there need to be studies developed to confirm if arthropods can transmit the virus from human to human. Further analysis of arthropods for HCV RNA from family homes where a member is infected with HCV may elucidate this concept. The detection of NPHV in horses and associating the same genotype of virus with that found in arthropods would also clarify whether transmission is possible between species from an animal reservoir. Further assessment is required to determine, more accurately, the epidemiological role of Tabanids in the mechanical transmission of pathogens and carry out vector surveillance for high populations of Tabanids identifying HCV virions/RNA in these species. Although mechanical transmission may act as a mechanism for disease, biological transmission may also be possible if the virus is found to be viable in arthropod cells.

In order to ascertain if arthropods are involved in HCV infection, in endemic areas for HCV infection, determination of HCV RNA in arthropods in these areas and establishing the presence of HCV RNA in domestic animals would indicate if there is a rural enzootic cycle. An investigation to further establish the existence of HCV virions in horses and other domestic animals, as an animal reservoir for the virus, and the analysis of arthropods associated with these animals would be an important step forward in this area of research.

Investigating the mutant spectra of the HCV quasispecies may be an indication of the intra-epidemic evolutionary dynamics of the virus as viral evolution and transmission intensity may be related [Hapuarachchi et al., 2016].

Reducing the opportunity of blood feeding from arthropods by treating standing water thus reducing larvae as well as mosquito nets to reduce night-time biting may also be important considerations, especially in the delta region of Egypt.

Although there is currently no concrete evidence that arthropods play a role in the transmission of HCV, exploring the possibility that humans can be infected with HCV by bites from an arthropod, as some other arboviruses are transmitted, is an important issue to investigate in countries with hyperendemic HCV infection, like Egypt, with high populations of arthropods. Further considerations of widespread international travel and climate change are also important factors to consider in this issue.

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