

# A SURVEY OF THE RELATIONSHIPS AMONG THE VIRUSES NOT CONSIDERED ARBOVIRUSES, VERTEBRATES, AND ARTHROPODS

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**Summary.** – No single group of organisms demonstrates more extensive and diverse associations with animal viruses than the phylum *Arthropoda*. Compared with the well-recognized relationship found in arboviruses, however, most of the atypical arthropod-vertebrate relationships of the viruses normally not considered arboviruses have received much less attention, as they remain in the marginal areas of interest for most researchers in animal virology, veterinary medicine, medical entomology, and invertebrate pathology. However, this comprehensive review of the information gathered from several branches of virology by profession reveals highly valuable information potentially useful in the fields of research ranging from investigations of the mode of transmission of poorly understood or emerging viral diseases to studies of the evolution of biological transmission of animal viruses by arthropod vectors. The observations and data obtained for the animal virus relationships with arthropods and vertebrates outside the boundaries of arboviruses, in turn, can be used to re-examine more closely the definition of arboviruses. With increasing number of reports challenging one of the basic tenets of the definition of arbovirus (requirement of viremia in vertebrate host) and others describing virus-host relationships that complicate the definition of arbovirus, the accumulated information clearly demonstrates the difficulty of defining the boundaries of arboviruses.

**Key words:** animal virus; arthropod; vertebrate; arbovirus; virus-host relationship

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## 1. Introduction

The viral relationship with both vertebrate and arthropod is best recognized in arboviruses. However, other types of associations of animal viruses with these two phyla of hosts either *in vivo* under natural conditions or *in vitro* under artificial conditions have also been documented. Generally, these poorly-studied or “unusual” associations of the animal viruses normally not recognized as arboviruses fall in the

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**Abbreviations:** HIV-1 = human immunodeficiency virus 1-; IgM = immunoglobulin M; LCMV = Lymphocytic choriomeningitis virus; TSV = Taura syndrome virus; VSV = Vesicular stomatitis virus

marginal or overlapping areas in each of or between the subdivisions of virology by profession, such as human virology, veterinary virology, medical entomology, arbovirology, and invertebrate pathology. Due to relative lack of interest or attention, few have attempted to review systematically the relationships among those viruses, vertebrates, and arthropods.

A collection of accumulated, published documents compiled for this review, however, clearly reveals fascinating sources of data on the complexity of virus-host relationship that are useful in various branches of virology. Furthermore, by studying these virus-host relationships, one can examine more closely the true boundaries of arboviruses.

The aims of this review are first to survey the poorly recognized relationships by virus family or group and to discuss the validity of *in vitro* replication of viruses in “unnatural” or “unusual” hosts and the importance in public health and veterinary medicine. With this background information, discussion is then focused on virus-host relationship in the context of the evolution of biological transmission by arthropods and re-examination of the boundaries of arboviruses. Excluded from this review are *in vitro* expression studies of recombinant animal virus genes in insect virus vectors (such as baculovirus and parvovirus) and animal virus replication studies performed in vertebrate-insect hybrid cells. The abbreviations and virus classification used are according to the International Committee on Taxonomy of Viruses (van Regenmortel *et al.*, 2000).

## 2. Survey of the arthropod relationships of the viruses not considered arboviruses

The reports of virus isolation and/or observation of transmission under natural conditions and of laboratory experiments *in vivo* or *in vitro* using arthropods or arthropod cell culture were examined by virus family or group.

### 2.1 Family Arenaviridae

Arenaviruses, except for Lymphocytic choriomeningitis virus (LCMV), do not replicate in vector cell lines (Pudney *et al.*, 1982). Nevertheless, some arenaviruses have been isolated from arthropods, such as Junin virus from ticks, Pichinde virus from mites and ticks, Tacaribe virus from mosquitoes, and Amapari virus from mites (Childs and Peters, 1993; Downs *et al.*, 1963; Pinheiro *et al.*, 1966). LCMV replicates not only in tick and mosquito cell cultures (Pudney *et al.*, 1982; Řeháček, 1965) but was found to be transmitted trans-stadially in ticks or to guinea pigs by mosquito under laboratory conditions (Coggeshall, 1939; Milzer, 1942; Hoogstraal *et al.*, 1966). Among unclassified viruses, Quarantil virus was proposed to be an arenavirus

(Zeller *et al.*, 1989). The virus, like other unclassified virus, Johnston Atoll virus, has been well known to be transmitted by ticks and replicates in tick cell cultures quite well (Varma *et al.*, 1975). Furthermore, this virus has been isolated from febrile humans, pigeons, cattle egrets, and other animals.

### 2.2 Family Bunyaviridae

Members of the genus *Hantavirus* are known to be directly transmitted by persistently infected rodent hosts. Recently, evidence of viral transmission by ectoparasites, in particular chiggers and Ixodid ticks, has been obtained in both Asia and North America (Dong *et al.*, 1991; Zhuge *et al.*, 1998; Hauck *et al.*, 2001). Among bunyaviruses found in bats, Kaeng Khoi virus was isolated from louse on bats or from diseased bats. Mojui dos Campos virus, which is occasionally isolated from bats, has close relatives among *Nyando* and *Bunyamwera*-complex arboviruses (Williams *et al.*, 1976; Osborne *et al.*, 2003; Wanzeller *et al.*, 2002).

### 2.3 Family Flaviviridae

The members of the genus *Flavivirus* can be basically classified into three groups in terms of host association: (i) mosquito viruses that cannot replicate in vertebrate cells and that play no role in vector-borne transmission (such as Cell fusing agent virus and Kamiti River virus (Stollar and Thomas, 1975; Crabtree *et al.*, 2003); (ii) strictly vertebrate viruses without a vector (no-vector group); (iii) vector-borne vertebrate viruses that replicate in ticks, mosquitoes, or both. Although the first two groups are not called arboviruses based on the definition, nonetheless, they are inseparable from the third group because all three groups share the same genome organization and numerous amino acid motifs and conserve a monophyletic relationship (Kuno *et al.*, 1998). As for the viruses in other genera, Bovine viral diarrhea virus of the genus *Pestivirus* is known to readily replicate in mosquito cells (Potts *et al.*, 1989). On the other hand, a report of replication of Hepatitis C virus (genus *Hepacivirus*) in mosquito cells *in vitro* (Germi *et al.*, 2001) requires corroborations, since similar attempts by others failed

### 2.4 Family Nodaviridae

An RNA virus isolated from mosquito *Culex tritaeniorhynchus* in the 1960s turned out to represent an entirely new group of viruses (Scherer and Hurlbut, 1967). The dichotomy of the host range of this family of viruses into the genus *Alphanodavirus* (exclusively viruses of insects) and the genus *Betanodavirus* (all viruses of fishes) somewhat resembles the division of the genus *Vesiculovirus* of the family *Rhabdoviridae* into fish and insect virus groups. Although members of the genus *Alphanodavirus* do not

satisfy natural biological transmission cycle, Nodamura-virus, for example, can be transmitted by *Aedes aegypti* at least under laboratory conditions. Furthermore, the virus replicates in both insects and vertebrates, causing paralysis or mortality in both kinds of hosts. Another nodavirus, Flock house virus, which was originally isolated from a coleopterous insect, is unique in that it also multiplies in mammalian cells, plants and yeast. Furthermore, it can orally infect mosquitoes (Dasgupta *et al.*, 2003).

### 2.5 Family Reoviridae

Among the large number of viruses in the genus *Orbivirus* are found interesting examples of arthropod relationships. An orbivirus designated JKT-7400 was isolated from *Culex* mosquitoes in Indonesia. Infection of mosquitoes and rabbits (however, without replication) with this virus induces carbon dioxide sensitivity and mortality, respectively. Furthermore, the virus replicates in a mosquito cell culture (Vazeille *et al.*, 1988). Another orbivirus isolated from *Culex pipiens* mosquito in Israel, Netivot virus, replicates in both Vero and mosquito cell cultures but kills adult mosquitoes (Tesh *et al.*, 1986).

### 2.6 Family Retroviridae

Some of large DNA viruses, such as baculoviruses of insects, have been recognized to spontaneously integrate transposons of the host. According to a study, the genome of a field strain (as well as vaccine strain) of Fowlpox virus has an almost entire infectious genome of an avian retrovirus, Reticuloendotheliosis virus, integrated in it (Hertig *et al.*, 1997). Because insects including mosquitoes are involved in mechanical transmission of Fowlpox virus, concomitant transmission of this retrovirus by insects has become an important subject in veterinary medicine. Transmission of other retroviruses, in particular, Equine infectious anemia virus, also heavily depends on mechanical transmission by insects. Interestingly, this virus is known to replicate well in mosquito cells *in vitro* (Breaud *et al.*, 1976).

### 2.7 Family Rhabdoviridae

Five of seven genera of this family of viruses replicate in vectors. It has been argued that rhabdoviruses evolved in arthropods and were originally maintained by vertical transmission (Peters, 1991). Among the members of the genus *Vesiculovirus*, a few unclassified viruses (Kotonkan, Obodhiang, and Rochambeau viruses) have been the focus of evolutionary interest in bridging the gap between arboviruses and rabies virus group because they were isolated from vectors in the field and, furthermore, replicate in mosquito cells *in vitro* (Buckley, 1973). Among the genus *Lyssavirus*, Mokola virus is a human pathogen that replicates

in mosquitoes *in vivo* and *in vitro*. Rabies virus does not replicate in vectors, although, in one experiment, it survived for up to 34 days in nymphal argasid ticks (Bell *et al.*, 1957). Thus, the report of a mutant of rabies virus adopted in *Drosophila melanogaster* (Plus and Atanasiu, 1966) needs to be corroborated. Furthermore, although infectious virions are not generated in mosquito cell culture, when rabies virus-inoculated mosquito cell cultures were maintained for long periods, synthesis of nucleocapsid and envelope protein was detectable (Seganti *et al.*, 1991). Similar synthesis of hemagglutinin without production of infectious virions has been also reported in a mosquito cell culture inoculated with an influenza A virus (White, 1987). In the genus *Novirhabdovirus* (all fish viruses), a few of them, such as Spring viremia of carp virus and Pike fry virus, like Sigma virus (an insect rhabdovirus), replicate and induce carbon dioxide sensitivity in *Drosophila melanogaster* flies (Bussereau *et al.*, 1975).

## 3. Arthropod viruses

Some so-called “insect viruses”, such as baculoviruses, replicate only in insect cells, and their replication in mammalian cells has never been exactly proven, even though rare observations of a baculovirus or invertebrate Iridescent virus 6 (family *Iridoviridae*) replication in poikilothermic vertebrate cells were reported (Ignoffo, 1973; McIntosh and Kimura, 1974; Granados, 1976). Like many baculoviruses, some densovirus (family *Parvoviridae*) of insects also have been used in biological control of insect pests. Although they do not replicate in mammalian cells, if transfected as infectious clone, viral genome of at least one virus can integrate into mammalian cell chromosome (El-Far *et al.*, 2004).

Some insect viruses may have an indirect relationship with vertebrates. For example, the envelope protein of the tick-borne Thogoto virus (an arbovirus) has nearly 30% amino acid sequence identity with the envelope protein (gp64) of baculoviruses (Morse *et al.*, 1992). According to a report, this could be interpreted to represent either the transfer of the envelope protein gene between double-stranded DNA virus and a negative-sense single-stranded RNA virus or independent acquisition by each of these viruses from a shared, conserved host gene (Pearson and Rohmann, 2002).

A small number of intriguing epidemiological observations of antibodies to insect viruses in vertebrate blood samples include reports of antibody to a picorna-like insect virus (*Gonometa podocarpi* virus of moth) in several mammals (cattle, deer, horse, and pig) in East Africa (Longworth *et al.*, 1973), and IgM antibody to Cricket paralysis virus in human blood (Scotti and Longworth, 1980; Moore *et al.*, 1981). Detection in human of antibody to another insect virus (*Darna trima* virus), a member of the

family *Tetraviridae*, in parts of Malaysia (where it was sprayed as bioinsecticide against caterpillars) was also reported (MacCallum *et al.*, 1979). The biological or medical significance of all these observations is unknown in the absence of human illness.

Nevertheless, generalization of the lack of pathogenicity in mammalian hosts of other viruses associated with arthropods, including those associated with *Drosophila* cell cultures, is not warranted because few serious studies have been undertaken and because some insect viruses and human-pathogenic viruses are phylogenetically inseparable. The aforementioned genus *Flavivirus* of the family *Flaviviridae* is a good example.

The picornavirus superfamily is an extremely diverse group of both aquatic and terrestrial viruses including polio and foot-and-mouth disease viruses. Taura syndrome virus (TSV) is a virus closely related to the Cricket paralysis virus in the tentative family *Dicistroviridae* and is known to be a serious pathogen of one of the most popularly consumed marine shrimps by humans. According to a recent report, the virus was found to readily replicate in human and monkey cells *in vitro* (Audelo del Valle *et al.*, 2003).

#### 4. Validity of the results of unusual viral replication in arthropod cell culture

In the past, replication of animal viruses in cell cultures derived from irrelevant (unnatural) hosts has been occasionally reported, but it was received with skepticism by some concerned researchers. Beside the obvious artificial aspect of *in vitro* conditions that bypass natural routes of viral entry and infection *in vivo*, other factors, such as modification in virus traits through repeated laboratory passages or purely accidental physico-chemical matching of cell membrane receptors and intracellular replicative mechanism of selected cell cultures favorable for unnatural viral replication, have been considered responsible for producing “odd” results. Also, the fact that some animal viruses that have nothing to do with arthropods under natural conditions nonetheless survive in cell culture without replication (Zhang *et al.*, 1994) has been cited as another difficulty distinguishing true replication from mere persistence in arthropod cell culture. Therefore, nearly all available publications worldwide on the replication of animal viruses in cell cultures derived from unnatural arthropod hosts were re-examined for this review; and the following conclusions were obtained.

(i) Specificity of viral replication in cell culture is not absolute, and replication of some viruses (such as Human coxsackie virus A 10, Human enterovirus 68 or 70, Mammalian orthoreovirus serotypes 1 and 2) in unnatural mosquito cells (White, 1987)) remains puzzling. However, the number of viruses demonstrating such unusual

replication without any plausible explanation is actually very small. (ii) For some reason still poorly understood, replication of some unnatural vertebrate viruses in vectors (either *in vitro* or *in vivo*) has been demonstrated in the first few passages. But, if these viruses were passaged repeatedly further in the same arthropod cell cultures or vectors, infectious titers of many of them either sharply dropped or disappeared in the next few more passages (Hurlbut and Thomas, 1960; Singh and Paul, 1968). However, because, in most of those unusual reports viral replication was attempted only once and further passage has been rarely performed, from the published data alone it is difficult to ascertain if those viruses could be adaptable to arthropod cells. (iii) For other vertebrate viruses, such as Bovine viral diarrhea virus and picornaviruses, replication in mosquito cells may be partly attributable to possible sharing of replicative mechanism (including shared cellular receptors for the viruses), because the former is a member of the family *Flaviviridae* which contains many vector-borne arboviruses and picornaviruses are extremely successful viruses of a superfamily that have adapted very well to both aquatic and terrestrial hosts. The replication of Vaccinia virus in mosquito cell culture (White, 1987) similarly appears unusual. However, some insect poxviruses (genus *Entomopoxvirus*) share a similar cell entry and uncoating mechanism as well as biochemical strategy for replication with some poxviruses of vertebrates, except that the former are defective in late gene expression in vertebrate cells while the latter are defective in proteolytic processing of late viral proteins in insect cells (Li *et al.*, 1997, 1998).

#### 5. Significance in Public Health and Veterinary Medicine

When an outbreak of emerging viral disease with unknown natural mode of transmission occurs, investigation is routinely launched in many directions, including the possibility of arthropod involvement. In fact, historically, such an activity has been conducted repeatedly during nearly all major emerging disease outbreaks such as poliomyelitis, type B hepatitis, Burkitt's lymphoma, Kawasaki disease, acquired immunodeficiency syndrome, Norwalk virus infection, type C hepatitis, Ebola fever, **Hendra (??? Such a virus was not found in ICTV Taxonomy)** viral disease outbreak, and more recently severe acute respiratory syndrome.

Some of the classical epidemiological investigations regarding the role of arthropods in viral infections in humans, such as poliomyelitis transmission studies (Sabin and Ward, 1941), provide invaluable lessons and strategies for epidemiological investigations that are still useful today. For many other viral diseases, even after the etiology and major transmission mechanism were more or less established,

arthropods remained a focus of investigation among some researchers to elucidate alternative routes of transmission.

For example, regarding the transmission of Hepatitis B virus, although no convincing evidence in support of arthropod involvement has ever been found, under laboratory conditions the virus or virus antigen has been found to persist in mosquitoes and other arthropods for long periods. The virus was also found in mosquitoes caught in nature. In some mosquitoes, even vertical transmission of this virus was reported. But, the biological significance of such reports has never been clearly proved. Interpretation of the report of the replication of Hepatitis C virus in mosquito cells (Germi *et al.*, 2001) was also controversial, since others obtained negative results (Bellini *et al.*, 1997; Chang *et al.*, 2001). Similar problems exist in resolving other puzzling questions, such as the possibility of hepatitis C infection by tick bite (Wurzel *et al.*, 2002).

Similarly, despite almost unanimous failure to prove involvement of arthropods in Human immunodeficiency virus 1 (HIV-1) transmission, interests in this possibility persisted among a small number of investigators. It was suggested that insects could be reservoirs of HIV-1 because viral genome could be integrated as provirus in cultured insect cells (including mosquito and *Drosophila*) and because the viral genome was detectable in insects captured under natural conditions in Africa (Becker *et al.*, 1986). Furthermore, mechanical transmission of HIV-1 by biting flies (such as stable flies) from a subspecies of chimpanzee to human was proposed to be possible (Eigan *et al.*, 2002). Also, the fact that the virus could survive in a tick for more than 10 days and that adult female ticks would imbibe as much as 70 times the amount of blood mosquitoes imbibe on average were considered significant for their potential role in natural transmission (Humphrey-Smith *et al.*, 1993).

Insect cell culture has been increasingly used for efficient production of certain biologicals and other biosynthetic products. Genetically-modified *Drosophila* cell culture, for example, has been used for production of HIV-1 gp 120 envelope glycoprotein (Culp *et al.*, 1991), fish antifreeze protein (Rancourt *et al.*, 1990), Hepatitis B virus surface antigen for vaccine (Deml *et al.*, 1999), and others. However, cell lines derived from *Drosophila* flies have been long recognized to harbor a variety of viruses representing at least several families of RNA viruses as well as other unclassified viruses.

Regarding the aforementioned TSV that was reported to replicate readily in human cells *in vitro*, safety of the consumption of some highly popular shrimps on dinner table needs to be seriously investigated. All these reports raised a question of the potential vertebrate host range of arthropod viruses.

The importance of mechanical transmission of the viruses of veterinary medicine and wildlife microbiology has been investigated for many years, with the myxomatosis in rabbits

as the best known example. Other similarly transmitted viruses of veterinary importance include Porcine respiratory and reproductive syndrome virus, Hog cholera virus, Infectious bursal disease virus, Bovine leukemia virus, a capripox virus causing lumpy skin disease of cattle, and Equine infectious anemia virus (Krisinsky, 1976; Foil and Issel, 1991; Carn, 1996). Mechanical transmission of Rabbit hemorrhagic disease virus, a calicivirus, was also confirmed recently (Asgari *et al.*, 1998). The impact of mechanical transmission by insects, such as horse flies, cannot be underestimated, since their home (flight) ranges sometimes exceed 6 km. Furthermore, some of the mechanically-transmitted DNA viruses persist for a long period. For example, transmission of Fowlpox virus by mosquitoes was demonstrated as long as 41 days after vector contact with the virus (Carn, 1996). As for **Hendra virus ???** transmission, the role of *Ixodes holocyclus* in transmitting the virus from flying fox to horse or other animals (Barker, 2003) is still a mere conjecture.

## 6. Evolution of biological transmission of animal viruses

The evolution of biological transmission of animal viruses by arthropod vectors has been the subject of continuous interest among some researchers. Although theoretically both ancestral viruses in vectors and those in vertebrate hosts could be considered the origins of the extant arboviruses, most researchers speculated that the adaptation of arthropod viruses to vertebrate hosts is a mechanism by which arboviruses evolved (Andrewes, 1957; Porterfield, 1980; Reeves, 1983). Furthermore, for the flavivirus group that comprises both no-vector and vector-borne groups, Porterfield (1980) assumed that no-vector viruses emerged secondarily from vector-borne viruses through degenerative process by losing vector association, while some vector-borne viruses continued to adapt to different hosts. At least so far as the latter part of this theory is concerned, it is supported by our previous phylogenetic result demonstrating evolutionary transition of flaviviruses from tick-borne to mosquito-borne group (Kuno *et al.*, 1998).

As evolution is an on-going process, primordial forms of virus-arthropod-vertebrate relationship must be constantly evolving wherever or whenever the three elements meet, even though in most of such encounters biological transmission may never evolve because all necessary requirements under optimal conditions are rarely met. Still, it is highly conceivable that some of the virus-host relationships observed today may represent transitional stages in terms of the evolution of biological transmission. Thus, it is worth speculating if some of the host relationships of viruses presented in this review, such as LCMV,

nodaviruses, Quarantilla virus, a few unclassified viruses of the genus *Vesiculovirus* and Netivot virus, are in such intermediate stages either toward acquiring biological transmission or at degenerative stages from it. If the answer is neither one, are they the cases of purely coincidental and partial resemblance to biological transmission that have nothing to do with evolution of this mode of transmission?

## 7. Definition of arbovirus

The term “arbovirus” derived from a laboratory jargon coined by the investigators in California in the 1940s emphasized a set of common ecological denominators and was never meant to be a taxonomically valid group. Replication in both vector and vertebrate hosts, transmission between vertebrate hosts by blood-feeding of vectors, and presence of viremia in vertebrate hosts for vectors to acquire virus, were the three tenets of the 1967 definition. The revised definition of 1985 incorporated direct transmission and vertical transmission in vectors as alternative modes of transmission (WHO Scientific Group, 1985).

Among the above tenets of the definition, the requirement of viremia in vertebrate hosts has been challenged lately. Virus transmission between infected and non-infected vectors through co-feeding on the same vertebrate host without viremia has been demonstrated for Thogoto virus, Tick-borne encephalitis virus, Crimean-Congo hemorrhagic fever virus, Bluetongue virus, Louping ill virus, Vesicular stomatitis virus (VSV), and West Nile virus (Jones *et al.*, 1987; Alekseev and Chunikhin, 1990; Labuda *et al.*, 1993; Gordon *et al.*, 1993; Katz *et al.*, 1994; Jones *et al.*, 1997; Mead *et al.*, 2000; Lawrie *et al.*, 2004). Another complication in classifying some flaviviruses as arboviruses is found in the no-vector group that has 13 members and Tamana bat virus in tentative status, according to the current classification (van Regenmortel *et al.*, 2000). The Arbovirus Catalogue (ASTMH, 1985) and its current online version (<http://www2.ncid.cdc.gov/arbovat/index.htm>) list 5 viruses (Apoi virus, Carey Island virus, Jutiapa virus, Sal Vieja virus, and San Perlita virus) as possible arboviruses, despite the fact that vector association has never been proved for any of those viruses. To make the definition of arbovirus status more complicated, among the phylogenetically clustered mosquito-borne group of flaviviruses (Kuno *et al.*, 1998), no vector has ever been identified thus far for several viruses (i.e., Cacipacore virus, Entebbe bat virus, Sokuluk virus, and Yokose virus).

In addition to the above complications, strict application of the definition of arbovirus also leaves the status of too many viruses (including popularly studied VSV – New Jersey serotype) tentative or inconclusive, as evidenced by the fact that in nearly 550 viruses listed in the Arbovirus Catalogue and others added later, only about 20% of them are fully

confirmed arboviruses. While many in the remaining 80% of the viruses (excluding those that are definitely not arboviruses) currently classified in either “probable” or “possible” status are most likely arboviruses, given a variety of difficulties in conducting a satisfactory field investigation (which often takes many years of arduous work), including a substantial decline in financial support for long-term field-oriented studies in recent years, the exact arbovirus status of many animal viruses will not be settled for many more years to come. Collectively, both the ambiguity of classification of arbovirus status and the complexity of arthropod-vertebrate relationships demonstrated by some animal viruses that are not considered arboviruses contribute to the difficulty of defining the boundaries of arboviruses.

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