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Insect-specific viruses and their potential impact on arbovirus transmission

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Arthropod-borne viruses (arboviruses) are the causative agents of significant morbidity and mortality among humans and animals globally. In the past few years, the widespread adoption of next generation sequencing and metagenomics has led to a new era of virus discovery, where many novel viruses have been documented, exhibiting a restricted host-range in mosquitoes. They represent a wide-range of insect-specific viruses within the families of *Bunyaviridae*, *Flaviviridae*, *Mesoniviridae*, *Reoviridae*, *Rhabdoviridae*, *Togaviridae*, and the newly recognized taxon of *Negeviruses*. Collectively, their discovery has opened new vistas about the extent of viral diversity and evolution, their influence on vector competence and ability of their insect hosts to transmit human pathogens (e.g. arboviruses), and their potential development as biological control agents or novel vaccine platforms.

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Introduction

During the past decade, advances in sequencing technology (next generation sequencing and metagenomics) and phylogenetics have identified a wide range of novel RNA viruses associated with hematophagous insects [1••,2••,3,4,5••,6]. It is now apparent that biting insects harbor a diverse range of RNA viruses, many of which are not commonly known vertebrate pathogens. The RNA viruses of hematophagous insects can be conveniently divided into two broad groups, based on their host range: first, the insect viruses that infect vertebrates and second, the insect viruses that do not. RNA viruses that infect both biting insects and vertebrates are generally classified as arthropod-borne viruses (arboviruses) and include agents such as dengue (DENV), yellow fever (YFV), chikungunya (CHIKV) and West Nile (WNV) viruses. These dual-host viruses typically undergo replicative cycles in both their insect and vertebrate hosts and include many important human and veterinary pathogens. The second group of RNA insect viruses are the insect-specific viruses (ISVs). ISVs infect insects and insect cells, but they do not replicate in vertebrates or vertebrate cells. The focus of this chapter will be on the ISVs, and their interaction with their insect hosts.

Most of the known ISVs that have been described from hematophagous insects are associated with mosquitoes. The predominance of mosquito-associated viruses may simply be a reflection of sampling bias, since most of the known ISVs were initially detected during arbovirus surveillance studies; and these types of studies have generally focused on mosquitoes and mosquito-borne viruses of public health and veterinary importance. A second reason for bias is the availability of a stable and highly susceptible mosquito cell line (*Aedes albopictus* C6/36) for virus isolation. The C6/36 clone has a defective RNAi response after infection to arboviruses infection [7,8]; and this defect could account for its increased susceptibility to ISVs. There are no comparable cell lines available for culicoid midges, phlebotomine sandflies or other biting flies. It seems probably that some of these latter biting insects also harbor ISVs, but they have been under sampled and relatively few have been tested. Consequently, this review will focus on the ISVs of mosquitoes identified in the past four years.

Bunyaviridae

The family *Bunyaviridae* currently encompasses five genera (*Hantavirus*, *Nairovirus*, *Orthobunyavirus*, *Phelebovirus* and *Tospovirus*), a classification based on structural, genetic and antigenic characteristics [9]. The first four genera include viruses that infect vertebrates, including humans, whereas the fifth genus contains viruses that are known to cause disease in plants, not animals [9]. Exemplifying the great diversity of species classified within this family, the discovery of Gouléako (GOLV) and Cumuto (CUMV) viruses, during arbovirus surveillance study in Côte d'Ivoire [10] and Trinidad and Tobago [11], suggests a new novel genus evolutionarily related to but distinct from the genus *phlebovirus*. More recently, a unique clade defined by the restricted host range Herbert (HEBV), Tai (TAIV) and Kibale (KIBV) viruses, forms a phylogenetically sister group to the genus *Orthobunyavirus*

[6], whereas Jonchet (JONV) and Ferak (FERV) viruses are probably two equally novel sister genera [5••].

Flaviviridae

Biologically, viruses in the genus *Flavivirus* can be divided into three broad groups, based on their arthropod and vertebrate host associations [12,13]: first, dual-host viruses which are maintained in an alternating arthropod/vertebrate cycle [14], which includes most of the flavivirus pathogens; and second, insect-specific flaviviruses (ISFs) which naturally infect mosquitoes and replicate in insect cells but do not infect vertebrates or vertebrate cell lines [15]; and third, vertebrate-only flaviviruses [also known as 'no known vector' (NKV) group, which naturally infect and are transmitted between vertebrates but have no known arthropod-association [12]. Some of the bat salivary gland viruses (i.e. Rio Bravo) and rodent-associated flaviviruses (i.e. Modoc) are examples of this group [16]. Viruses in the NKV group infect vertebrates (including sometimes humans), but they do not infect arthropods or insect cells [13]. The ISFs form two distinct phylogenetic and antigenic subgroups or clades: first, one clade includes viruses, such as the cell fusing agent (CFAV) [17,18], Culex flavivirus (CxFV) [19–22], Aedes flavivirus (AeFV) [23,24••,25–27], Kamiti River (KRV) [28,29], and others (reviewed in [30••]), which forms a distinct clade apart from the main flavivirus tree of vertebrate pathogens; and second, a second clade, which currently includes Aripo [JA Auguste *et al.*, unpublished], Barkedji (BJV) [31], Chaoyang (CHAOV) [32–34], Donggang (DONV) [N Vasilakis and RB Tesh, unpublished], Ilomantsi (ILOV) [35], Lammi (LAMV) [36], La Tina [N Vasilakis and RB Tesh, unpublished], Marisma (MMV) [37,38], Nanay (NANV) [39], Nhuhirim (NHUV) [40] and Nounané (NOUV) [41] viruses (and reviewed in [30••]), which are antigenically and phylogenetically related to and fall within the dual-host group of flavivirus pathogens in close proximity to WNV, DENV and YFV.

Reoviridae

Members of this family are classified into two subfamilies, *Spinareovirinae* and *Sedoreovirinae*, based on their core structures, and both have a wide geographic distribution and wide host range, as they have been isolated from fungi, plants, insects, ticks, arachnids, fish, marine protists, crustaceans, mammals, and birds [42]. Phylogenetic analysis of a single isolate, *Aedes pseudoscutellaris* reovirus (APRV), a host-restricted virus isolated from the AP61 mosquito cell line in 2005, indicates that it represents a distinct genus (genus *Dinovernavirus*) within the subfamily *Spinareovirinae* [43]. A second member of this genus, the host-restricted Fako virus (FAKV), isolated in 2010 during a surveillance study in Cameroon, has the closest genetic and structural relationships are with reoviruses in the genera *Cypovirus* and *Oryzavirus* [44]. Cimodo virus (CMDV) is also a unique ISV that belongs to the family *Reoviridae*. It was isolated in Côte d'Ivoire and probably defines a new genus within the subfamily *Spinareovirinae*, closely related to myco-virus and coltivirus [45].

Rhabdoviridae

The family Rhabdoviridae is comprised of a diverse group of non-segmented, negative-sense RNA viruses that infect a wide range of vertebrates, invertebrates and plants [46], distributed across 17 recognized and proposed genera [47••]. Vector-borne rhabdoviruses are present in 12 of the 17 groups, and most prevalent in the dimarhabdovirus supergroup. Two host-restricted rhabdoviruses in mosquitoes, Arboretum (ABTV) and Puerto Almendras (PTAMV) viruses, were isolated from pools of female *Ochlerotattus fulvus* and *Psorophora albigenu* mosquitoes, during an arbovirus surveillance in Peru, in 2009 [48]. Experimental infections *in vitro* failed to produce detectable CPE in vertebrate cell lines, and similarly with *in vivo* studies, both viruses failed to produce illness and/or death in newborn mice. Since both viruses have only been detected once in mosquito pools, a comprehensive assessment of host range remains to be determined through surveillance and experimental studies.

Togaviridae

The genus *Alphavirus* within the family *Togaviridae* consists of 29 species grouped into 10 complexes based on antigenic and/or genetic similarities [49•,50]. Alphaviruses exhibit a wide host range that includes vertebrates such as fish, equids, birds, amphibians, reptiles, rodents, pigs, humans, and nonhuman primates as well as insect hosts, including mosquito species encompassing at least six genera and also ticks and lice (reviewed in [51]). While the fish alphaviruses exhibit a restricted host range (replication only in fish), the only known host-restricted alphavirus of mosquitoes is Eilat virus (EILV) [52], which was isolated during a two-year (1982–1984) arbovirus survey of the Negev desert in Israel, from a pool of *Anopheles coustani* mosquitoes [53••].

Mesoniviridae

The newly recognized family Mesoniviridae consists of single-stranded RNA, positive-sense RNA viruses belongs within the order Nidovirales [54]. Till date, members of this family have only been isolated from various pools of mosquitoes within the family Culicidae [3,55••,56–60] and appear to have an extensive geographic distribution. The family *Mesoniviridae* includes a single genus *Alphamesonivirus* with seven recognized species: *Alphamesonivirus-1* includes the two closely related viruses, Nam Dinh (NDiV) [55••], Cavally (CavV) [56], and the recently characterized NDiV Ngewotan [3] and NDiV Houston [3]; *Alphamesonivirus-2-4* includes the previously described African mesoniviruses, HanaV, MenoV, and NseV, respectively [57]; *Alphamesonivirus-5* includes the four Bontag Baru (BBaV) isolates sampled in the early 1980s in Indonesia [3]; *Alphamesonivirus-6* includes the single isolate of Karang Sari (KSaV) virus,