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TRANSMISSION AND EVOLUTION OF TICK-BORNE VIRUSES

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Abstract

Ticks transmit a diverse array of viruses such as tick-borne encephalitis virus, Powassan virus, and Crimean-Congo Hemorrhagic fever virus that are reemerging in many parts of the world. Most tick-borne viruses (TBVs) are RNA viruses that replicate using error-prone polymerases and produce genetically diverse viral populations that facilitate their rapid evolution and adaptation to novel environments. This article reviews the mechanisms of virus transmission by tick vectors, the molecular evolution of TBVs circulating in nature, and the processes shaping viral diversity within hosts to better understand how these viruses may become public health threats. In addition, remaining questions and future directions for research are discussed.

Introduction

Tick-borne viruses (TBVs) are highly focal infections that persist in nature by continuous transmission among vector ticks and wild animal hosts [1]. Although the natural history varies considerably for each virus, at their core, they all require a permissive environment that supports the spatial and temporal overlap of the virus, vector, and vertebrate host. These viruses often remain undetected until humans encroach upon the natural transmission cycle, become infected, and develop clinical illness leading to their identification. In recent decades, a number of established TBVs have emerged as public health concerns including tick-borne encephalitis virus (TBEV) in Europe and Asia [2], Crimean-Congo hemorrhagic fever virus (CCHFV) in Asia and Africa [3], and Powassan virus (POWV) in North America (Table 1) [4]. Meanwhile, new TBVs are continually being discovered including severe fever with thrombocytopenia syndrome virus or Huaiyangshan virus in East Asia [5], and Heartland and Bourbon viruses in the U.S. [6,7]. These trends are driven by the proliferation of ticks in many regions of the world and by human encroachment into tick-infested habitats. In addition, most TBVs are RNA viruses that mutate faster than DNA-based organisms and replicate to high population sizes within individual hosts to form a heterogeneous population of closely related viral variants termed a mutant swarm or quasispecies [8]. This population structure allows RNA viruses to rapidly evolve and adapt into new ecological niches, and to develop new biological properties that can lead to changes in disease patterns and virulence [9]. The purpose of this paper is to review the mechanisms of virus transmission among vector ticks and vertebrate hosts and to examine the diversity and molecular evolution of TBVs circulating in nature. This article also describes recent research on viral genetic changes occurring during tick-borne transmission to better understand how these viruses interact with their hosts and emerge as health problems.

Taxonomy of tick-borne viruses

TBVs comprise a diverse array of viral entities that are classified into six virus families: *Flaviviridae*, *Bunyaviridae*, *Orthomyxoviridae*, *Rhabdoviridae*, *Reoviridae*, and *Asfarviridae* (Table 1). The most important TBVs belong to the family *Flaviviridae* and *Bunyaviridae* and include numerous viral agents that cause encephalitis or hemorrhagic fever in humans. Virus families differ in many fundamental characteristics including nucleic acid type, morphology, and replication strategy that are the basis for their classification into distinct viral families. Details of their genome organization and replication process are beyond the scope of this article.

The inclusion of TBVs into multiple virus families suggests that this mode of transmission arose independently in several different viral lineages as a successful evolutionary strategy for infecting new hosts. All of the viruses listed in Table 1 are considered true arthropod-borne viruses (arboviruses) because they replicate both within ticks and vertebrate hosts. Next generation sequencing techniques, however, have revealed a much larger diversity of viruses in ticks that are not amenable to isolation by traditional techniques like cell culture and suckling mice inoculation [10,11]. Many of these viruses may prove to be tick-specific viruses similar to the recent discovery of insect-specific viruses within mosquitoes [12]. TBVs that cause human and animal disease appear to represent a small fraction of the tick virome. The remainder of this review will focus on the transmission and evolution of members of the family *Flaviviridae* because they represent the best studied and well-characterized TBVs to date. However, it is worth noting that ticks also transmit segmented viruses (*Bunyaviridae* and *Orthomyxoviridae*) that may evolve by segment reassortment in addition to random mutation.

Evidence for genetic reassortment has been inferred for CCHFV with variable mutation rates between segments and possible genetic recombination within segments [13].

The family *Flaviviridae*, genus *Flavivirus* represents a diverse group of viruses and includes a number of important mosquito- and tick-borne pathogens such as dengue virus, TBEV, West Nile virus (WNV) and Zika virus. These viruses are approximately 40-50 nm in diameter and consist of a host-cell derived lipid bilayer membrane surrounding a nucleocapsid core containing a single strand of positive-sense RNA. By phylogenetic analysis, flaviviruses cluster into groups defined by the arthropod vector: mosquito-borne, tick-borne, no known vector, and insect-specific flaviviruses [14,15]. The tick-borne flaviviruses may be further subdivided into the mammalian and seabird associated groups, and Kadam virus that forms a third evolutionary lineage (Figure 1) [16]. Tick-borne flaviviruses known to cause disease in humans and domestic animals are all members of the tick-borne encephalitis sero-complex and include TBEV, louping ill virus (LIV), Omsk hemorrhagic fever virus, Kyasanur Forest Disease virus (KFDV), and POWV. The designation of these viruses was historically based on clinical symptoms, geography, and serological criteria; however, genetic analyses do not support some species assignments. Grard et al 2007 proposed taxonomic changes that involved synonymizing LIV and other sheep and goat encephalitis viruses with eastern and western TBEV into a single species- TBEV [16]. In addition, Alkhurma hemorrhagic virus is considered a subtype of KFDV [17] and Deer tick virus (DTV) is recognized as a subtype of POWV [18,19].

Virus transmission by tick vectors

Both *Argasidae* (soft-ticks) and *Ixodidae* (hard-ticks) can transmit viruses; however, the vast majority of TBVs of human and agricultural importance are transmitted by hard-ticks [20].

Hard-ticks have three distinct life-stages; larvae, nymph and adults with each stage dependent on blood from a vertebrate host for either molting or oogenesis. Consequently there are repeated opportunities for TBV transmission which can occur through a number of mechanisms.

Horizontal virus transmission to ticks occurs when a naïve tick feeds upon an infected vertebrate host. During feeding, blood and virus are pumped into the gut after which virions are able to infect midgut epithelial cells. Because ticks digest bloodmeals intracellularly, it is unclear how viruses gain access into midgut cells. However, it is thought that some viruses utilize species-specific receptors or co-factors while others passively enter digestive cells during blood uptake [21-25]. Once a midgut infection has been established, viruses must disseminate to distal tissues in order to be transmitted. Again, little is known about this process, but it has been hypothesized that some viruses escape the midgut prior to molting and infect hemocytes circulating within the hemolymph [21]. Molting is a highly proteolytic process in which tissues undergo histolysis and become extensively re-organized [26]. Hemocytes are resistant to histolysis and thereby provide a protected environment enabling infection of distal tissues upon completion of molting [21,24]. Alternatively, virions may escape the midgut during molting enabling rapid dissemination to distal tissues during tissue re-organization. Future studies should focus on elucidating the mechanisms and dynamics of virus dissemination within ticks.

Once infected, ticks remain infected for life and can transmit the virus to other ticks or vertebrate hosts [27,28]. It has been demonstrated that numerous TBVs can be transmitted vertically from adult females to their offspring, including LIV, TBEV, and CCHFV [24,29]. While vertical transmission efficiency appears to be low, it is undoubtedly important for the persistence of many TBVs in nature. The mechanisms surrounding this mode of transmission are completely unknown and require further investigation. During horizontal transmission to

vertebrates, ticks expectorate saliva along with virus while feeding. The saliva contains a plethora of pharmacologically active molecules with anti-hemostatic, anti-inflammatory and immunomodulatory properties [30,31]. These saliva-associated molecules significantly alter host physiology and appear to facilitate infection of and pathogenesis within the vertebrate host [30,32]. In addition, these molecules enhance the efficiency of co-feeding transmission [33-35]. This mode of transmission occurs when a naïve tick acquires an infection after feeding in close proximity to an infected tick [34]. Co-feeding transmission is a form of tick to tick horizontal transmission in which the vertebrate host merely serves as a meeting place. This form of transmission has been demonstrated for TBEV and Thogoto virus among others [33,34]; however, the process is not completely understood.

In addition to virus transmission by tick-bite, vertebrate hosts can acquire infection by other means such as ingestion of infected ticks [36], consumption of unpasteurized dairy products from infected animals [37], the butchering and consumption of TBV infected meat [38], and blood transfusions [39]. Clearly TBVs are very opportunistic and can exploit multiple transmission routes.

Molecular phylogenetics of tick-borne flaviviruses

Phylogenetic approaches have been used to investigate the genetic diversity, evolution and dispersal patterns of tick-borne flaviviruses circulating in nature [40-43]. These viruses were shown to form a highly asymmetric phylogenetic tree with a relatively constant rate of branching [44]. The high proportion of deep nodes within the tick-borne flavivirus tree reflects the survival of ancient viral lineages. An earlier analysis found that tick-borne flaviviruses formed a genetic cline across Eurasia with the greatest distances among isolates in East Asia and progressively

smaller distances among isolates in Western Europe and the British Isles [45]. The authors concluded that these viruses originated from East Asia 2,500 years ago before spreading westward. However, a more recent analysis has challenged this hypothesis and concluded that tick-borne flaviviruses expanded northward into central Asia about 7,000 years ago before spreading outwards from a central point into East Asia and Europe [42]. The inclusion of genomic sequences from more virus strains suggests that these viruses dispersed much earlier than prior estimates.

Numerous TBEV strains have been sequenced and analyzed to reveal a genetically diverse group of viruses dispersed across Eurasia. TBEV comprises at least three distinct genetic lineages termed the Western, Siberian, and Far Eastern subtypes [46,47]. The Western subtype of TBEV is transmitted by *Ixodes ricinus* in Europe and groups with LIV from the British Isles. The Far Eastern and Siberian subtypes group together as sister clades and are maintained by *Ixodes persulcatus* ticks in east and central Asia. This corresponds to an earlier classification scheme that split TBEV into Central European encephalitis virus and Russian spring-summer encephalitis virus based on antigenic relationships, geographic location and disease severity in humans [48]. In 2014, Kovalev and Mukhacheva proposed that TBEV diverged rapidly into these subtypes when adapting to new vector species rather than by gradual evolution [49]. This process could be facilitated by a combination of hybrid tick species and co-feeding transmission.

Powassan virus has been documented in Canada, the U. S. A., and eastern Russia and is the only known member of the tick-borne encephalitis serologic complex in North America. Phylogenetic analyses indicate that POWV consists of two genetic lineages, POW (lineage 1) and DTV (lineage 2), each with a distinct natural history [41,50]. The POW lineage is maintained in an enzootic cycle involving mainly *Ixodes cookei* and *Ixodes marxi* and medium-

sized mammals, such as groundhogs, and skunks, whereas the DTV lineage has been isolated primarily from *Ixodes scapularis*. POWV appears to evolve gradually in small, stable foci as shown by the persistence of distinct viral subclades in locations in Connecticut and Wisconsin [51,52]. Human cases of POWV encephalitis have become increasingly prevalent in regions of the northeastern and northcentral US where *Ixodes scapularis* is the dominant vector suggesting that these cases were caused by the DTV lineage [53].

Viral population diversity

Viruses exist within and between hosts as a collection of unique viral variants [54]. This is especially true for RNA viruses which are the majority of the TBVs. These complex populations arise because viruses exist as large populations, have short generation times and encode for RNA-dependent RNA polymerases that lack proofreading capabilities [54]. This population structure enables them to explore sequence space and consequently emerge, adapt and persist in new or changing ecological niches [55]. In recent years, there has been a proliferation of studies examining the processes involved in shaping the evolution of arboviruses; however, these studies have been primarily restricted to viruses transmitted by mosquitoes [56-60]. The limited amount of data available on the population structure and diversity of TBVs derives from work on POWV.

Earlier work evaluated POWV diversity within field-collected *Ixodes scapularis* ticks. POWV populations were shown to exist as a quasispecies within individual ticks, but complexity of the populations was extremely low [61]. This data significantly differed from the observed complexity of WNV populations in naturally infected mosquitoes [62]. The discrepancy observed between these two arthropod vectors could be explained by differences in the life

history traits and physiology of mosquitoes and ticks. It was hypothesized that this lack of diversity may be the result of POWV populations undergoing severe genetic bottlenecks during transstadial transmission [61]. Recently, we experimentally tested this hypothesis in the laboratory. BALB/C mice were intraperitoneally inoculated with POWV and offered to *Ix. scapularis* larvae and nymphs. Subsequently, viral populations from mice and pre- and post-molt ticks were analyzed by RNA-Seq. We observed that POWV populations rapidly diversify within the mammalian host (Figure 2) (Grubaugh et al., unpublished data). These findings were in stark contrast to those observed of WNV in its vertebrate host, birds [59,62]. During horizontal transmission, POWV populations were subject to severe transmission bottlenecks similar to WNV [59,60]. Once in ticks, POWV populations gradually accumulated diversity as a result of RNAi-mediated targeting (Grubaugh et al., unpublished data). While similar to RNAi-mediated diversification of WNV populations in mosquitoes, the degree of diversification was significantly less [56,63]. In order to examine the impact of transstadial transmission on POWV populations, ticks infected as larvae were analyzed as nymphs as well as adults. Interestingly, signs of genetic bottlenecks associated with transstadial transmission from one life stage to another were not observed (Figure 2) (Grubaugh et al. unpublished data). These studies are the first to closely examine the intrahost population dynamics of a TBV and provide key insights into the observed slow, long-term evolutionary trends of POWV. Further, while commonalities exist, these findings demonstrate that each arbovirus is subject to its own unique set of forces shaping their evolutionary trajectory.

Despite these advances much remains unknown about the evolutionary forces shaping POWV populations. Aforementioned, ticks can become infected by both vertical transmission and co-feeding transmission, however this study did not evaluate the effects of these modes of

transmission on the evolution of POWV populations (Figure 2). Further, because whole ticks were assessed in the above study, it is unknown if the observed population dynamics are representative of what is actually being transmitted in the saliva. Similarly, while horizontal virus transmission to ticks was evaluated, it is unknown what may be occurring during transmission from ticks to mice. Finally, laboratory mice were used in the study as opposed to the most likely natural host, *Peromyscus leucopus* [4]; it is unknown how this difference may have affected the results. Clearly much remains to be determined about how POWV and other TBVs evolve in the context of their transmission cycles.

Conclusions

The TBVs represent a large and diverse group of viruses, many of which adversely affect human and livestock populations, yet their transmission and evolution are poorly understood. In recent years, there have been advances in the molecular phylogenetics of tick-borne flaviviruses; however, the same cannot be said for the vast majority of other TBVs and continued research is needed. In addition, the mechanisms and kinetics by which TBVs infect and disseminate within ticks and how they are transmitted horizontally, vertically and through co-feeding are little understood. Further research dissecting these processes is greatly needed. While molecular phylogenetics provides insights into how virus species or genera evolve, it is unable to address the micro-evolutionary forces shaping virus populations in and between vectors and hosts. This area of research has been largely ignored. In order to fully understand the evolutionary potential of these viruses, future research efforts will be needed to address these shortcomings.

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Table 1. Select tick-borne viruses listed by virus family

Family	Characteristics ^a	Virus	Geographic Location	Disease	Tick vectors ^b
<i>Flaviviridae</i>	Enveloped, spherical, ssRNA(+)	Tick-Borne Encephalitis	Europe, Northern Asia	Encephalitis (humans)	<i>Ix. ricinus</i> , <i>Ix. persulcatus</i>
		Powassan	North America, Russia	Encephalitis (humans)	<i>Ix. cookei</i> , <i>Ix. scapularis</i>
		Louping Ill	Great Britain, Ireland	Encephalitis (sheep)	<i>Ix. ricinus</i>
		Kyasanur Forest Disease	India	Hemorrhagic fever (humans)	<i>Ha. spinigera</i>
		Omsk Hemorrhagic Fever	Russia	Hemorrhagic fever (humans)	<i>De. reticulatus</i>
<i>Bunyaviridae</i>	Enveloped, spherical, segmented ssRNA(-)	Crimean-Congo Hemorrhagic Fever	Africa, Asia, southern Europe	Hemorrhagic fever (humans)	<i>Hy. marginatum</i> , <i>De. marginatus</i>
		Nairobi Sheep Disease	Africa	Fever, diarrhea (sheep, goats)	<i>Rh. appendiculatus</i>
		Huaiyangshan	East Asia	Hemorrhagic fever (humans)	<i>Ha. longicornis</i>
		Heartland	Central United States	Hemorrhagic fever (humans)	<i>Am. americanum</i>
<i>Orthomyxoviridae</i>	Enveloped, spherical, segmented ssRNA(-)	Bourbon	Central United States	Hemorrhagic fever (humans)	Unknown
<i>Rhabdoviridae</i>	Enveloped, bullet-shaped, ssRNA(-)	Sawgrass	Eastern United States	Unknown	<i>De. variabilis</i>
<i>Reoviridae</i>	Non-enveloped, icosahedral, dsRNA	Colorado Tick Fever	Western United States	Fever (humans)	<i>De. andersoni</i>
<i>Asfarviridae</i>	Enveloped, icosahedral, dsDNA	African Swine Fever	Africa, southern Europe, Caucasus region	Fever (pigs)	<i>Or. moubata</i> , <i>Or. erraticus</i>

a. ssRNA(+)= single-stranded positive-sense RNA, ssRNA(-)= single-stranded negative-sense RNA, dsRNA= double-stranded RNA, dsDNA= double-stranded DNA.

b. Am.= Amblyomma, De.= Dermacentor, Ix.= Ixodes, Ha.= Haemaphysalis, Hy.= Hyalomma, Or.= Ornithodoros, Rh= Rhipicephalus.

Figure Legends

Figure 1. Phylogenetic tree of tick-borne flaviviruses based on maximum likelihood analysis of nucleotide sequences of the complete viral polyprotein. Labels include virus name and corresponding GenBank accession number. Number at nodes indicate bootstrap support values .

Figure 2. Model of POWV Intrahost Population Dynamics. Within mice, POWV populations accumulate diversity. In late summer, *Ix. scapularis* larvae may imbibe a bloodmeal from an infected mouse. During horizontal transmission to ticks, populations are subject to a severe transmission bottleneck leading to founder's effect and genetic drift within the newly infected tick. The engorged ticks will molt thereby transstadially transmitting the virus to the next life stage; bottlenecks are relaxed during this process. The infected nymphs will overwinter and, in the spring of the subsequent year, horizontally transmit the POWV populations back to a naïve host and potentially to another naïve co-feeding tick. For both scenarios the presence and/ or severity of these potential transmission bottlenecks are unknown. Again, the ticks will molt while POWV populations remain stable and the newly emerged adults will acquire a final bloodmeal from a large mammal host in the autumn. The large mammalian host may become infected, but they do not contribute to the transmission cycle and represent dead-end hosts. The following spring adults will lay their eggs and potentially transmit POWV virus to their offspring; however, the effects of vertical transmission on POWV populations remains unknown.