Future VIROLOGY

Review

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Revisiting new tick-associated viruses: what comes next?

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Tick-borne viral infections continue to cause diseases with considerable impact on humans, livestock, companion animals and wildlife. Many lack specific therapeutics and vaccines are available for only a few. Tick-borne viruses will continue to emerge, facilitated by anthroponotic factors related to the modern lifestyle. We persistently identify and are obliged to cope with new examples of emerging tick-borne viral diseases and novel viruses today. Many new strains have been detected in vertebrates and arthropods, some causing severe diseases likely to challenge public and veterinary health. This manuscript aims to provide a narrative overview of recently-described tick-associated viruses, with perspectives on changing paradigms in identification, screening and control.

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It has been over 100 years since ticks have been described as a source of viruses with potential to infect vertebrates and cause outcomes with considerable public health impact [1,2]. Several pathogenic viruses have been characterized since then, and we persistently identify novel viruses and are obliged to cope with new examples of emerging tick-borne viral diseases today. Through the 20th century, viruses transmitted by ticks continued to cause diseases with considerable morbidity, and mortality among humans, livestock, companion animals and in wildlife. Specific therapeutic agents are still lacking for these infections and vaccines are available for only a few. Therefore, disease prevention has to rely primarily on understanding vector dynamics and subsequent control measures [2]. It is likely that tick-borne viruses will continue to emerge, further aided by several anthroponotic factors related to the modern lifestyle [3,4]. However, recent technical advancements and relevant data accumulation are expected to alleviate the paucity of effective strategies for detection, control and prevention.

Technological innovations leading to the commercial availability of massively parallel sequencing equipment and subsequent development of methods (frequently called as next generation or deep sequencing) enabled an unprecedented pace of sequence information to be produced, leading to the characterization of microbial populations and their association with diseases [5]. These methods have now been widely applied to investigate key information gaps in vector-borne infections with considerable success and have become indispensable tools in studying virally induced diseases. Viral genomes can now be efficiently sequenced without the requirement of an laboratory-grown isolate and the subsequent availability of genome data have facilitated a better understanding of structural aspects associated with biological behavior as well as genetic relationships among strains. Moreover, the diversity of viral populations could be explored in depth, leading to the characterization of viral communities associated with particular conditions or hosts, including ticks. Many novel virus strains have also been detected in vertebrate and arthropod specimens, some causing severe diseases likely to challenge public and veterinary health [6–8]. Early recognition and detailed characterization of the pathogenic viruses is, therefore, critical for mitigation and control of the associated diseases. In addition, understanding the interactions between the emergent viruses and vector ticks provides new opportunities for the identification of potential targets for future control strategies [2,3]. This manuscript was intended to provide an overview of recently-described tick-associated viruses, with perspectives on changing paradigms in screening, identification and control.

Future Medicine

Ticks as virus vectors

Ticks cause damage to their vertebrate host directly via blood feeding during which they transmit a wide variety of viruses as well as bacteria, viruses and helminths [9]. Viruses constitute a major group among pathogenic microorganisms transmitted by ticks and several tick species are involved in virus transmission. The interactions between the tick, its host and pathogens is complex and beyond the scope of this review, but a greater understanding of the physiological and immune-mediated processes in ticks during infection and transmission will obviously provide unique opportunities for intervention and must be explored in detail [10,11].

Tick-borne viruses has adapted to replicate in the diverse and contrasting environments of the vertebrate host and the tick vector [12]. Therefore, they exhibit complex relationships due to these co-evolutionary processes and viral transmission and replication is closely-coordinated with the tick feeding cycle [11]. Ticks exhibit a multi-stage lifecycle including larva, nymph and adult stages, with significant variations in duration and blood meal requirement among species. Tick-borne viruses may infects the ticks in various stages, forming a persistent infection without detrimental effects to the tick [2,12]. Ticks also become infected horizontally, following blood meal from an infected mammalian host. Of particular interest is the co-feeding mechanism, that describes virus transmission during feeding of multiple ticks in close proximity, without infection in the host. Following the blood meal, the viruses enter vector host cells in the presence of appropriate receptors. Subsequently, they replicate in the midgut, access the hemolymph and disseminate to various tissues with peak concentrations in the salivary glands and reproductive organs. Vertical (or transovarial) pathogen transmission between tick generations is also possible and has been documented for particular tick-borne viruses as well [2,10,11].

Several factors influence the introduction and establishment of tick-borne infections within defined regions and disease emergence/reemergence as isolated cases or outbreaks. Tick-borne viruses basically produce zoonotic infections with the causative agents maintained in natural cycles involving tick vectors and animal hosts [2,10]. Human beings are occasional hosts for ticks and are frequently considered as dead-end hosts without prominent contribution in natural maintanence. Different tick species favour distinct environments or biotopes, that define their geographical distribution and risk areas for vertebrate transmission. In addition, feeding behavior of the vector ticks, opportunities for contact with humans or livestock, seasonal variations in temperature, precipitation and similar climatic factors, presence of non-human hosts all play important, often inter-related roles in pathogen dispersion and disease epidemiology [10,11]. Moreover, human activities may profoundly influence the emergence or resurgence of tick-borne diseases. Globalisation has enormously increased the passage of people, animals and goods among countries, contributing to the the international spread of viruses, bacteria and parasites. Anthropomorphic factors may enable viruses to emerge in new locations, access to new hosts and cause outbreaks of unparalleled intensity [13]. Tick-borne infections can be promoted due to the exploitation of environmental resources and increase in human outdoor activities, allowing contact with tick vectors and pathogens normally present only in the field. Therefore, rapid and efficient detection of potential pathogens and timely development of appropriate preventive measures are imperative for reducing health and economic impacts of a future emergent infection.

Tick-associated viruses

Viruses transmitted by or detected in ticks are heterogenous and belong in a range of families with diverse characteristics. They comprise over 160 viruses and 50 are currently recognized or probable virus species [2,12]. The majority of the strains possesses an RNA genome and classified in *Flaviviridae*, *Nyamiviridae*, *Nairoviridae*, *Orthomyxoviridae*, *Peribunyaviridae Phenuiviridae*, *Reoviridae*, *Rhabdoviridae* and families, along with a single DNA family, the *Asfarviridae*. Viruses considered as significant human or animal pathogens, due to the severity of the clinical disease, distribution, potential to emerge and notable economic or social impact has been outlined in Table 1. Particular infections such as those caused by tick-borne encephalitis virus or louping ill virus could be controlled by vaccination efforts [2]. In the last decade, significant epidemiological changes and expanding activity zones for several tick-borne viruses documented, exemplified by the spread of Crimean-Congo Hemorrhagic fever virus (CCHFV) around the Mediterranean Sea, increased incidence and spread of Powassan/dear tick virus in north America, detection of Alkhurma hemorrhagic fever virus in the horn of Africa, expansion of Kyasanur Forest disease virus affected areas in India and the impact of African swine fever virus disease in multiple countries across Africa, Asia and Europe [2]. CCHFV is the top agent in the World Health Organization's blueprint list of priority diseases in 2018 [14]. The list also maintains a vacant spot for potential, yet unknown pathogens, to enable rapid

Table 1. Notable tick-borne pathogenic viruses.

Virus	Classification†	Genome	Vector‡	Distribution	Clinical disease‡
Crimean-Congo hemorrhagic fever virus	*Nairoviridae*, Orthonairovirus	Single strand RNA (– sense)	*Hyalomma* spp.	Africa, Asia, Europe	Hemorhaggic fever
Nairobi sheep disease virus	*Nairoviridae*, Orthonairovirus	Single strand RNA (– sense)	*Rhipicephalus* spp.	Africa, Asia	Febrile disease, abortus (sheep)
Severe fever with thrombocytopenia syndrome virus	*Phenuiviridae*, Banyangvirus	Single strand RNA (– sense)	*Haemaphysalis* spp.	Asia	Hemorhaggic fever
Heartland virus	*Phenuiviridae*, Banyangvirus	Single strand RNA (– sense)	*Amblyomma* spp.	North America	Febrile disease
Tick-borne encephalitis virus	*Flaviviridae*, Flavivirus	Single strand RNA (+ sense)	*Ixodes* spp.	Europe, Asia	Febrile disease, encephalitis
Omsk hemorrhagic fever virus	*Flaviviridae*, Flavivirus	Single strand RNA (+ sense)	*Dermacentor* spp.	Russia	Hemorhaggic fever
Kyasanur forest disease virus	*Flaviviridae*, Flavivirus	Single strand RNA (+ sense)	*Haemaphysalis* spp.	India	Hemorhaggic fever
Powassan virus	*Flaviviridae*, Flavivirus	Single strand RNA (+ sense)	*Ixodes* spp.	North America, Russia	Febrile disease, encephalitis
Alkhurma hemorrhagic fever virus	*Flaviviridae*, Flavivirus	Single strand RNA (+ strand)	*Ornithodoros* spp.	Middle East, Africa	Hemorhaggic fever
Colorado tick fever virus	*Reoviridae*, Coltivirus	Double strand RNA	*Dermacentor* spp.	North America	Febrile disease, encephalitis, hemorrhagic disease
African swine fever virus	*Asfarviridae*, Asfivirus	Double strand DNA	*Ornithodoros* spp.	Africa, Asia, Europe	Hemorhaggic fever (pigs)

† Given as family, genus.
‡ Frequent symptoms and well-established vectors are indicated.

response and preparedness in case of a serious international epidemic, a scenario which fits well to several of the newly emerged tick-borne viruses described below.

New tick-associated viruses of significance

Banyangviruses

Banyangvirus is a recently-established genus within the family *Phenuiviridae* (order *Bunyavirales*) [15]. The genus currently comprises three distinct species, namely *Huiayangshan banyangvirus*, *Heartland banyangvirus* and *Guertu banyangvirus*, represented by severe fever with thrombocytopenia syndrome virus (SFTSV), Heartland virus (HRTV) and Guertu virus (GTV), respectively. Originally reported in 2011 with cases dating back to 2009, SFTSV became the first well-characterized tick-borne human viral pathogen following a long gap of decades [16]. Within a 5-year period, China reported over 10,000 cases with an average mortality rate of 5.3% from 23 provinces [17]. The virus was subsequently detected in South Korea and Japan with higher mortality rates (up to 31.4%) and, recently emerged in Vietnam [18–20]. HRTV was identified in individuals with similar clinical presentation in the United States in 2009 and further cases were documented during the following decade, mostly from midwestern and southern states [21,22]. Exposure in several species of wild and domestic animals were documented for both viruses and clinical cases coincide with the distribution range of their predominant tick vectors, *Haemaphysalis longicornis* (the Asian long-horned tick) and *Amblyomma americanum* (the Lone Star tick) [22,23]. Both viruses are classified as category C priority pathogens by the National Institutes of Health (NIH) and SFTSV was also considered for inclusion in the list of blueprint priority diseases by the World Health Organization (WHO) [14,24]. The discovery of SFTSV and HRTV exemplifies that novel tick-borne viruses can emerge as causative agents of severe human diseases and may pose significant, previously unrecognized public health threats.

A new banyangvirus to be included in the genus was Guertu virus (GTV), which was initially reported from China in 2018 [25]. Closely related and sharing a recent common ancestor with SFTSV and HRTV, GTV appears as an intermediate species between these viruses. Although human clinical cases were yet to be reported, GTV shares many traits similar to SFTSV and HRTV, raising concerns about being a potential pathogen. The GTV genome was initially characterized using a metagenomic approach, in *Dermacentor nuttalli* ticks collected at the Guertu County of the Xinjiang province. Screening for viral nucleic acids with newly-designed primers resulted in identification of infected ticks in the region and subsequent virus isolation. Further investigations revealed the

ability of GTV to infect and replicate in several animal and human-derived cell lines including Vero, DH82, BHK-21, 293 and HepG2, producing high virus titers in most of the cells analysed [25]. GTV infection could interfere with the intracellular type I interferon signaling, as previously observed for SFTSV [24]. Seeming non-pathogenic to C57BL/6 mice, GTV produced multi-organ pathologies including neuronophagia, hemorrhage with infilrations in kidneys and lungs, as well as hepatocyte degeneration in challenge experiments using interferon receptor knockout mice. Serological screening revealed virus exposure in 19.8% of the local resident population. Viral nucleic acids were further detected in rodent tissues, suggesting transmission from ticks to various vertebrate hosts. Due to the pronounced amino acid similarities between GTV and SFTSV proteins, cross-neutralizing antibodies that can prevent Vero cell infection by the reciprocal virus were produced in mice [25]. This is likely to complicate serological assessments in populations and probable patients. GTV remains to be explored as a potential human pathogen, co-circulating with SFTSV in particular regions in China.

Other phenui & related viruses

The latest expansions in the *Bunyavirales* order also included novel tick-associated viruses other than Banyangviruses within the family *Phenuiviridae* [15]. A new genus named *Kabutovirus* was created to accomodate two virus species, *Huangpi kabutovirus* and *Kabuto mountain kabutovirus*, representing Huangpí tick virus 2 from *Haemaphysalis doenitzi* and Kabuto mountain virus from *Haemaphysalis flava* ticks, respectively [7,26]. Moreover, the *Phlebovirus* genus was expanded by one species, *Mukawa phlebovirus*, for Mukawa virus from *Ixodes persulcatus* ticks [27]. These viruses were replication-competent in particular arthropod and mammalian-derived cell lines and some could also adapt to, and induce pathological changes in suckling mice, suggesting the potential to infect human or animals.

In addition to those already described, there is a considerable number of unclassified viruses with genome sequence similarities to members of the family *Phenuiviridae*. These strains have been characterized during metagenome/virome studies on ticks or using generic detection techniques. The majority lacks a cell culture-adapted isolate, detailed information on biological features, host range or potential pathogenicity [7,27–38]. They form a phylogenetically separate cluster, distinct from well-characterized viruses (Figure 1) and exhibit a surprisingly wide range of distribution including several regions in Europe, Asia and the American continent, and several tick hosts. In particular locations, they co-circulate with pathogenic viruses and show considerable sequence diversity [33,35,36,38]. No evidence of vertebrate infections was reported so far and some strains require complete genome and biological characterization for a reliable assessment of pathogenicity [30–32,37,38]. Additional strains such as Malsoor virus from India, Hunter Island Group and Albatross Island viruses from Australia seem to have zoonotic potential and are genetically related to the currently-known pathogenic tick-borne phenuiviruses (Figure 1) [39–41].

Thogotoviruses

Thogotoviruses constitute a separate genus in the family *Orthomyxoviridae* and comprise a number of tick-borne viruses [42]. Thogoto virus (THOV) and Dhori virus (DHOV) are approved species within the genus and several additional viruses await classification. THOV and DHOV are zoonotic viruses distibuted in Africa, southern Europe, the Middle East, India and Russia, circulating among several tick species, vertebrate hosts and waterfowl. In East Asia, Thogotoviruses were not recognized until the detection of the THOV in Japan in 2015 [43]. THOV causes leucopenia in cattle and febrile disease and abortion in sheep, whereas DHOV is hepatotropic and causes diffuse neuronal necrosis in mice. Human exposure has been documented for both strains, with symptomatic cases presenting with systemic febrile disease, central nervous system and liver involvement and possible fatal outcomes [12]. New tentative members of the genus were identified from an even wider geographical range and from mosquitoes as well as ticks [44,45].

Thogotoviruses initially emerged in the western hemisphere in 2014, when a novel strain (Bourbon virus; BRBV) was isolated from a patient, residing in Bourbon County, KS, US (Table 2) [46]. The patient presented with a febrile condition with thrombocytopenia and leukopenia, that progressed to multi-organ failure, cardiopulmonary failure and death. Follow-up surveys revealed additional cases, viral nucleic acids in various stages of field-collected *Amblyomma americanum* ticks and neutralizing antibodies in wild animals, indicating ongoing virus circulation [47–49]. Robust replication of BRBV in tick and mammalian cells was also demonstrated [50]. Interestingly, ribavirin and favipiravir could block BRBN in cell culture, suggesting that ribavirin, a long-approved broad spectrum antiviral agent, could provide a potential therapeutic option in acute cases [47,51]. In experimental conditions, BRBV infected and induced severe pathology in mice with deficient type I–II interferon systems [51]. Furthermore, prophylactic or post exposure administration of favirapir prevented virus-induced mortality in immunocompromised mice,

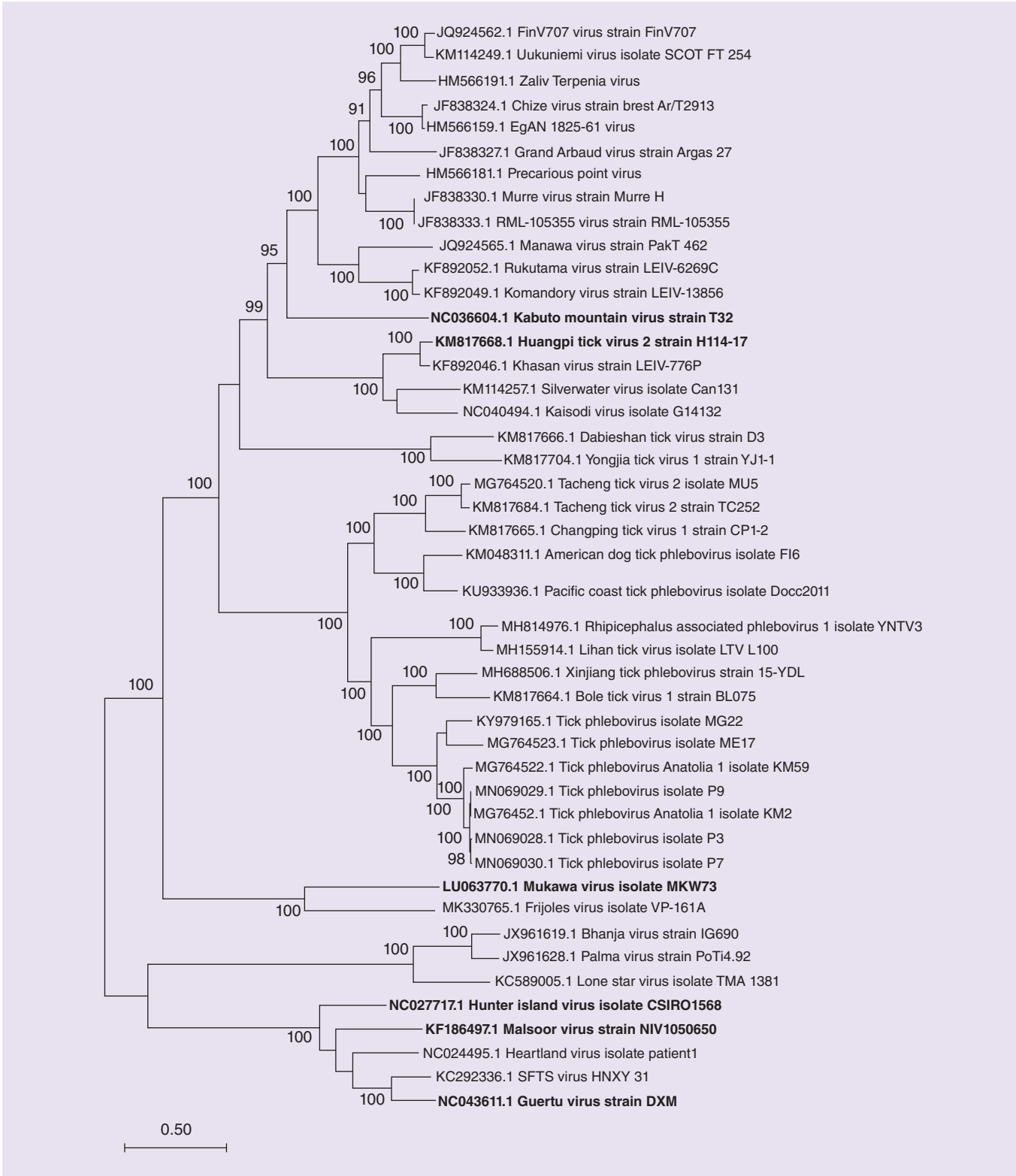

Figure 1. Phylogenetic tree of the tick phlebovirus near-complete L segment sequences (4692 nucleotides). The tree is inferred using the maximum likelihood method, General Time Reversible (GTR) substitution model, Gamma distributed with Invariant sites (G + I) for 500 replications. The GenBank accession number and strain/isolate name are used to indicate individual viruses included in the analysis. Viruses discussed in detail in the manuscript are in bold.

Table 2. New tick-borne viruses associated with symptomatic human infections.

Virus	Classification	Genome	Host ticks	Current Distribution	Nonhuman Exposure	Clinical disease	Ref.
Bourbon virus	*Orthomyxoviridae*, Thogotovirus	Double strand RNA	*Amblyomma americanum*	United States	Yes	Febrile disease, multiorgan failure	[46–51]
Jingmen tick virus	*Flaviviridae*, Jingmen virus group	Single strand RNA (+ sense)	Several species	China, Brasil, Kosovo, Turkey	Yes	Febrile disease	[53–61]
Alongshan virus	*Flaviviridae*, Jingmen virus group	Single strand RNA (+ sense)	*Ixodes persulcatus*	China, Finland	Yes	Febrile disease	[62–64]
Tacheng tick virus 1	*Nairoviridae*, Orthonairovirus	Single strand RNA (−/ambisense)	Several species	China	Yes	Febrile disease	[65]

providing a strong candidate therapeutic [47]. Recently, a novel thogotovirus, genetically-related to BRBV, was isolated from *Amblyomma testudinarium* ticks in Japan and provisionally named as Oz virus (OZV) [52]. OZV demonstrated a comparable host cell range and growth properties to BRBV and could efficiently replicate in several mammalian cell lines, including primate cells. Intracerebral inoculation into suckling mice yielded a high mortality rate. Rodents were suggested as potential amplifying or maintenance hosts for OZV [52]. It remains to be determined whether OZV is pathogenic and will be detected in human or animal infections.

Jingmenviruses

Among recently described tick-associated viruses, Jingmen tick virus (JMTV) and related strains are unique, due to their specific genome configuration and direct association with human disease (Table 2). JMTVs possess a multicomponent genome, comprising four positive-sense RNA segments [53]. Two genome segments encoding for the non-structural proteins of the virus are related to flavivirus proteins, whereas those encoding the structural proteins seem to have originated from yet uncharacterized ancestors [8,53]. In America, Africa and Asia, closely related viruses with four to five genome segments have been documented in insects and mosquitoes. These viruses are tentatively included in the *Flaviviridae* family as a distinct group [8,54,55]

The prototype JMTV isolates were detected in ticks from China, and subsequent reports documented JMTV and a closely-related virus, Mogiana tick virus (MGTV) in ticks and cattle from Brazil [53,55–58]. PCR-based screening identified the virus in several tick species, including those with vector competency for other tick-borne viruses. Further evidence for infections in vertebrates were revealed, with detectable viral RNA in primates and rodents [55,57–59]. Discovery of individuals co-infected with CCHFV and JMTV in Kosovo indicated probable human exposure via shared tick vectors [60]. JMTV was also detected in ticks from Turkey with endemic CCHFV circulation [61]. Initial documentation of human infections were reported from China, where JMTV was present in skin tissues and in circulation, as well as detached ticks from symptomatic individuals. The clinical presentation could be severe in particular cases [59]. Furthermore, Alongshan virus, another Jingmen group virus, was reported individuals with febrile disease, from another region in China, with evidence for natural infections in sheep and cattle (Table 2) [62,63]. It was also detected in ticks from southeastern Finland without human cases [64]. In each case, the clinical presentation ranged from mild illness to severe disease requiring hospitalization and could not be readily differentiated from infections by other tick-borne pathogens [59,62]. Initial serological screening findings from China and Finland suggest that human exposure is not highly prevalent for both viruses. Limited information on JMTVs is currently available, which appear as ubiquous viruses, capable of infecting humans and various animal hosts.

Nairoviruses

Current taxonomy defines *nairovirirdae* as a family within the order *Bunyavirales*, comprising 15 species and over 40 viruses [15]. Majority of the nairoviruses are tick-borne and infect multiple vertebrate host species during their life cycle in nature. The most notable pathogens within the family are CCHFV and Nairobi sheep disease virus, causing infections with significant human and animal health impact as well as economic concern (Table 1) [2,12,14]. These viruses are not covered in this review, as there are several sources available for updated epidemiological information and research. Other strains such as Dugbe, Erve, Issyk-kul, Kasokero and Tamdy viruses have been implicated in symptomatic infections in humans, mostly associated with febrile diseases. Several novel nairoviruses

have been described in the last decade, as well as expansion of particular strains into previously unaffected or low prevalence regions. One of the newly-described strains is Leopards Hill virus, which was isolated from bats in Africa and observed to produce severe gastrointestinal hemorrhagic disease with liver involvement in mice [66]. Soft tick bunyavirus, a novel strain related to Issyk-Kul virus, was detected in soft ticks (*Argas vespertilionis*) collected from bat feces in Japan [67]. Another novel strain, Tofla virus, was also characterized in Japan, in *Haemaphysalis* spp. ticks [68]. In interferon knockout mice, Tofla virus produced a lethal infection with prominent gastrointestinal damage and could replicate in several cell cultures of human and monkey origin. Grotenhout virus was isolated from *Ixodes ricinus* in Belgium [69]. Metagenome investigations have also described several sequences related to nairoviruses in various tick species [6,69,70]. Tamdy virus, originally described in Central Asia, have recently been detected in ticks from Turkey and China [35,71]. Moreover, Tacheng tick virus 1, a member of the *Tamdy orthonairovirus* species, was associated with human cases presenting with fever and rash in Northwestern China, as well as neutralizing antibodies detected in populations and viral RNA in various tick species and domestic animals (Table 2) [65]. Tamdy orthonairoviruses may exemplify the cryptic tick-borne viral agents, emerging in new regions.

Coltiviruses

The genus *Coltivirus* is among the genera in the subfamily *Spinareovirinae* of the *Reoviridae* family and includes two tick-borne viruses, Colorado tick fever virus and Eyach virus, as species [72]. The coltivirus genome comprises linear, double-stranded RNA in 12 segments. Colorado tick fever virus is endemic in North America, and detected in ticks, wild rodents and humans. It is the causative agent of Colorado tick fever, an acute, febrile disease, rarely complicated with central nervous system involvement or hemorrhagic manifestations [73]. Eyach virus was isolated from various *Ixodes* spp. ticks from Germany and France, and still reported to circulate in particular regions [69,73]. It was incriminated as a human pathogen, due to detectable antibodies in particular cases with neurological symptoms, with recent evidence for neurotropism in mice [69]. Coltiviruses have also been isolated from rodents and bats, in addition to ticks that serve as transmission vectors.

Recently, novel coltiviruses have been detected in new locations. Tarizumi tick virus was isolated and characterized from *Haemaphysalis flava* ticks in Japan, becoming the initial coltivirus documented in Asia [74]. Although the genome configuration is identical, it features distinct functional properties in particular segments, with evidence for reassortment. It produced variable cytotoxicity in mammalian cells and no clinical signs in intracerebrally inoculated suckling mice, thus, lacking clear evidence of pathogenic potential. Other novel viruses closely-related to coltiviruses have been reported in ticks from Australia (Shelly Headland virus), India (Kundal Virus) and from free-tailed bats in France [75–77].

Tick viromes in investigation

Despite variations in experimental methods and data processing, a considerable amount of data on tick-associated viruses produced by next generation sequencing has accumulated during the last decade [78]. Moreover, information on virus composition in many tick species from geographically-separated regions in the American continent, Europe, Asia and Australia is now available [8,9,34,35,78,79]. Overall, the tick virome mainly includes strains with RNA genomes in monopartite or segmented, single or double-strand, negative or positive sense configurations. DNA viruses genomes are scarce and can sometimes be considered as of environmental origin [78]. One of the frequent populators includes negative sense, single stranded RNA viruses belonging in *Bunyavirales* and *Monogenavirales*. Within these orders, *Rhabdoviridae* and *Phenuviridae*, *Nairoviridae* and *Orthomyxoviridae* represent the families with monopartite and segmented genomes, respectively. Members of the *Flaviviridae*, *Nodaviridae*, *Tetraviridae*, *Picornaviridae*, *Caulimoviridae*, *Virgaviridae*, *Narnaviridae*, *Luteoviridae* constitute the viruses with single-strand positive-sense RNA viruses commonly detected in the tick virome.

The deep sequencing approach applied for the study of tick virome also enabled identification many novel strains closely or distantly-related to pathogenic tick-borne viruses [8,9,75,78,79]. Some of the newly described strains seem extremely diversified, making predictions of biological behavior or virulence difficult, without a cell-culture based isolate. Moreover, presence of viral nucleic acids provides strong evidence but cannot confirm an active replication in or transmission by ticks, and the detected nucleic acids may have originated from various sources. Nevertheless, these techniques have enabled a broader understanding of the RNA virus biodiversity and helped to fill up major phylogeny gaps [8]. Among the recently-described viruses, members of the *Chuviridae* family constitute a monophyletic group of viruses with segmented, nonsegmented and circular genomes, forming an intermediate between with segmented and unsegmented negative strand RNA viruses [7,80]. They exhibit a variety of genome

organizations in circular (clade I) or segmented-linear (clade II) configuration [7]. Chuviruses infect a wide variety of invertebrates, including *Argasidae* and *Ixodidae* ticks, as well as in *Crustacea*, *Nematoda*, *Insecta*, *Myriapoda* and *Arachnida*. Tick-associated chuviruses (such as Bole tick virus 3, Changping Tick viruses 2–3, Wuhan tick virus 2–3, Tacheng tick virus 5, Lonestar tick chuvirus, Black-legged tick chuvirus, Suffolk virus, Genoa virus and Canne point virus) seem globally prevalent and can be detected in multiple tick species in the same geographic regions. So far, they are not associated with human or animal disease.

Tick-borne viruses: perils & opportunities

Newly emergent viruses are prone to abrupt genetic change

Majority of the tick-borne viruses identified so far possess RNA genomes in single or segmental configuration and therefore, likely to undergo genetic change via recombination or reassortment. In general, recombination describes the formation of a chimeric genome by polymerase template switching when two parent viruses replicate within the infected cell, while reassortment occurs when novel combinations of genomic components derived from the parental viruses are formed via co-encapsidation or cotransmission events [81]. In addition to the error-prone nature of the RNA-based replication, both mechanisms are pivotal in generating divergent genomes and significantly accelerate virus evolution, facilitating adaptation to new hosts, antigenic variations resulting in immune escape and increased virulence. Recombinations are frequent in single-stranded RNA viruses and reassortment is a notable mechanism involved in the emergence of novel strains and subtypes in segmented viruses, such as influenza virus and many bunya viruses [81,82].

The genomes of several tick-borne viruses exhibit evidence for past recombination and reassortment events. The phylogeographic analysis of CCHFV and SFTSV have revealed reassortment as a key mechanism responsible for spatial variations in viral genomes [83,84]. Interestingly, another factor likely to contribute to TBEV, CCHFV and SFTSV dispersal as well as genome divergence was identified as bird migration. Here, infected ticks remain attached to their avian hosts and can travel long distances, even overseas, to new habitats. This sort of dispersal might introduce the viruses in new geographic areas and accelerate virus evolution by enabling exchange among spatially-separated genetically-divergent virus strains. Recently, experimental evidence using virus-like particles proved that Uukuniemi virus and SFTSV S and L segment-based minigenomes can be successfully packaged using HRTV glycoproteins [85]. These findings demonstrate that viral components required for transcription, replication and packaging are compatible among tick-borne phleboviruses and coinfection with these viruses could lead to the generation of viable reassortant progeny. Despite the lack of well-characterized vector competency data for most of the newly-emerging agents, overlapping of tick vectors and vertebrate hosts suggest that co-infections with multiple, closely or distantly-related viruses occur in natural conditions. New reassortant viruses will surely have to withstand important restrictions due to fitness cost, host adaptation, transmission efficiency and superinfection exclusion [85]. Nevertheless, the potential of reassortment to produce novel pathogenic tick-borne viruses has already been well-documented, both *in vivo* and *in vitro*.

Infections with novel agents are difficult to identify

The clinical presentation and routine laboratory findings in most of the tick-borne viral infections, especially in the mild cases at the early stage, are undifferentiated and nonspecific [12,21,22,46]. Development of hemorrhagic manifestations or rarely, CNS involvement, has been documented for several viruses, but these are relatively less frequent and usually preceded by a febrile episode, that can be easily misdiagnosed as other endemic tick-associated infections. Therefore, novel viruses with human spillover from the natural tick-vertebrate reservoir cycle is hard to detect, without an accumulation of cases with unidentified etiology, such as in an outbreak setting. This has led to the descriptions of tick-associated rash illness or southern tick-associated rash illness, indicating infections following *Amblyomma testudinarium* and *Amblyomma americanum* exposure in Japan and the USA [2,86]. Despite the detection of particular *Borrelia* spp. in some cases, the etiologic agent remains obscure in most of the clinically compatible infections worldwide. The spillover is also likely to happen in environments of urban-rural interface, where access to specialized laboratory testing would normally be limited [11,79,86]. Lack of awareness or interest by the local physicians and veterinarians may also hamper the detection of infections due to novel agents. Here, information sharing on new and emerging tick-borne diseases among practicing medical and veterinary professionals is essential and may facilitate timely and critical interventions for containment and prevention [2–4]. Syndromic surveillance methods will further enable identification of case clusters by the tracking of symptoms, signs, behavioral patterns or laboratory findings, common in tick-borne infections through a variety of data sources.

New techniques will facilitate rapid detection of the next emergent virus

Metagenomic or targeted deep sequencing in ticks and clinical specimens have proved very efficient in producing critical information on well-characterized or novel tick-borne viruses. The technology has also been successfully applied for diagnostics, using various platforms and approaches [87,88]. However, issues on equipment requirement, trained personnel, cost and availability still restrict a broader application of these techniques for screening and diagnosis of tick-borne viruses. Miniaturization of the PCR-based diagnostics and sequencing have produced field-deployable devices, albeit with particular limitations [88]. Target amplification at fixed incubation temperatures (isothermal) and separation of the DNA duplex by non-thermal methods have been increasingly employed in recently developed assays for rapid molecular diagnostics [89,90]. These methods abolish the requirement for thermal cycling and can be performed using simple, portable devices that can run on low power. Therefore, they are ideal for point-of-care diagnosis and screening of ticks, enabling real-time detection of pathogens in the field. Several recombinase polymerase amplification and LAMP-based assays have been developed, including those targeting tick-borne viruses [91]. Recently, enzymes from CRISPR/Cas systems have been adapted for detecting viral nucleic acids, allowing multiplexed, portable and ultra-sensitive detection [92,93]. The next generation molecular assays will be those that can simplify or automatize all prepatory procedures, speed up the detection and analysis steps and run in a portable device, without compromising sensitivity or specificity. Adaptation of available technologies to detect tick-borne viruses will facilitate rapid identification and screening of potential emergents.

Despite their obvious advantages and strengths, assays based on nucleic acid identification can only produce results when there is ongoing or very recent infection, hence detectable nucleic acids in target hosts. On the contrary, serological evaluation does not require an active infection and can provide information about past infections, enabling temporal assessment of exposure. Therefore, serological testing may be a powerful tool for gaining insights into infection history, without infected specimen handling [94]. It can also be possible to identify viruses that cross the species barrier and cause asymptomatic infections [79]. Routine serology for diagnostic or screening purposes often employs enzyme-linked immunosorbent assays, which can be automated for rapid testing and incorporates one or few target antigens. Viruses grown in cell culture can be readily used to prepare crude, nascent antigen preparations for antibody detection via immunofluoresence assays. However, given the extended repertoire of the target viruses and potential hosts to be screened, these methods will be insufficient with the requirement of new approaches for robust testing [79]. Identification of protective or diagnostically-useful antigens in new virus strains can also be a daunting task using the standard methods [94].

Recent advances in antibody profiling have enabled a broad-scale antigen analysis and characterization [94]. Employing huge numbers of recombinant antigens, it is now possible to undertake an in-depth assessment of humoral responses in viral infections by various approaches. These methods are also likely to impact antigen discovery, facilitate development of new diagnostic assays and tools for vaccine monitoring, as well as providing new insights into humoral responses in the host. For tick-borne infections, vertebrate exposure due to virus spillover and diagnostic efficiency of novel multiplex detection method have been evaluated via in house-developed assays [79,94,95]. Overall, a comparable sensitivity to commercially available assays for antibody detection can be achieved using different approaches and discriminatory antigenic epitopes. Multispot immunoassays utilizing label-free optical detection methods also appear promising, enabling analysis with a simple device consisting of a smartphone with a portable cartridge and optical components, without costly electronics or microfluidic systems [96]. Used-designed, broad range assays for antibody detection will provide invaluable tools for screening as well as diagnosis in tick-borne viruses.

Understanding virus pathogenesis yields novel targets for therapy & prevention

Many tick-borne and hemorrhagic fever-associated viruses have evolved common strategies to surpass immune responses for efficient viral replication in the host. Particular mechanisms include type I interferon antagonism and impairment of dendritic cell maturation to disrupt innate-adaptive immunity cascades [97]. They may also trigger an aberrant, uncontrolled pro-inflammatory response upon infection, called the systemic inflammatory response syndrome, directly associated with clinical disease severity. Utilizing different mechanisms, the SFTSV and HRTV NSs proteins have been documented to block interferon signalling within the infected cells [24]. The NSs proteins of particular phenuiviruses also exhibit similar effects in host cells, where the mechanism and strength of inhibition seem to be related with virulence [98]. A notable example is the tick-borne Uukuniemi virus, possessing weak interferon antagonising activity and no well-documented human infections, in contrast to mosquito-borne and severely pathogenic Rift Valley Fever virus [99]. It appears that viruses causing severe disease counteracts the

innate immune induction and signaling pathways by targeting multiple components at several stages [98]. CCHFV also antagonizes innate immune signaling by encoding an OTU domain, reported to suppress innate immune responses by deubiquitinating proteins involved in signaling pathways [100]. Viral OTU domains of nairo viruses exhibit significant sequence variation, with distinct preferences for certain host proteins and diverse enzymatic activity profiles, likely to influence host adaptation and pathogenesis [101]. A better understanding of the virus-host interaction and commonly-targeted immune evasion strategies will help to elucidate infection pathogenesis and facilitate development of effective vaccines and new treatment strategies.

Future perspective

Our knowledge of the viruses circulating in ticks has been dramatically increased during the last decade, mostly owing to the availability of advanced sequencing technologies. The Pandora's box of viruses has been opened, revealing a wide spectrum of new viruses, some with new and surprising characteristics. These viruses co-circulate in the ecosystem with the previously-known agents and potentially influence the evolution of novel pathogens and subsequent disease emergence. Therefore, succesful prevention and control strategies must recognize the changing paradigms and respond to the current threats appropriately.

A realistic approach to understanding tick-borne viruses and their environmental dynamics should rely on a holistic view of the viruses, vectors and hosts, including humans, as described by the 'One Health' approach to diseases [3,4]. Identification of multiple, often interacting factors affecting introduction and establishment of tick-borne viruses requires interdiciplinary efforts to elucidate underlying mechanisms of dispersal, pathogenicity, evolution and emergence.

Tick-borne viral diseases usually exhibit complex epidemiological features due to the involvement of various tick vectors and animals host and therefore, difficult to control. Screening and surveillance efforts should include all potential vectors as well as domestic, feral and wild animals, potentially hosting the tick vectors. Transmission and adaptation in synanthropic animals may provide the viruses an interface to evolve and subsequently establish infection in humans. Therefore, the description of the host range and community ecology of the viruses will facilitate effective preventive measures to block transmission as well as development of appropriate animal models to study infection dynamics.

Various viruses seem to employ similar strategies to initiate and maintain infection in ticks and vertebrate hosts. Understanding molecular drivers that promote survival, spread and pathogen transmission will provide opportunities to interfere with these processes. Interactions among microorganisms within the realm of the tick vector may provide opportunities to disrupt vector competency and transmission to nonarthropod hosts. Given the paucity of vaccines and specific antivirals for tick-borne virus infections, novel targets and strategies for control and prevention will be of considerable impact. The immune recognition of tick-associated viruses in vertebrate hosts and the mechanisms used by the viruses to counteract the immune response appear as promising foci for further research in this regard.

Despite powerful tools currently available for genome characterization in single isolates or populations, new assays are needed for monitorization. The next big step will be the availability of field-deployable assays and portable devices that can be operated without expert knowledge or training, in screening or healthcare settings. Several promising assays are in progress for validation and widespread availability. The produced genome-wide data will continue to translate into research, focusing on functional interpretation of the information, such as identification of virulence-associated markers, antigenic epitopes for immune recognition or to be targeted for diagnosis. In addition to the portable testing, development of broad spectrum serological assays will improve our understanding of host tropism and will enable realistic interpretation of infection prevalences, by recognizing previous exposures.

Tick-borne diseases are on the rise with increasing global risks [4]. Impact of human activity on the environment has become a worldwide concern in many aspects, where tick-borne infections represent another, perhaps a minor threat. We have to adopt appropriate prevention and mitigation measures to counteract, at least in the case of tick-borne viruses, the effects of ongoing environmental degradation.

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Executive summary

Tick-associated viruses are on the rise
- There has been a proliferation of novel tick-associated viruses being detected in vertebrates and arthropods.
- Some of these viruses produce severe diseases and challenge public and veterinary health.
- Early recognition and characterization is critical for mitigation and control.
- Understanding the interactions between viruses, vectors and hosts provides new targets for future control strategies.

An abundance of novel viruses have been revealed
- Several new banyang, phenui, thogoto, jingmen, colti and nairoviruses have been isolated and characterized.
- Boubon virus, Alongshan virus, Jingmen tick virus and Tacheng tick virus 1 are currently documented to cause human infections.
- Indirect evidence for infection and pathogenicity in vertebrates is available for several viruses.

The tick virome exhibits a surprising diversity
- Most frequent components comprise RNA viruses with various genome configurations.
- Many novel strains closely or distantly related to pathogenic tick-borne viruses are present.

The viruses are prone to abrupt genetic change
- Majority of the tick-borne viruses possess RNA genomes in single or segmental configuration. They exhibit significant diversity and co-infect various ticks and hosts.
- Therefore, they are likely to undergo genetic change via recombination or reassortment.
- Impact of these mechanisms have already been documented for pathogenic viruses.

Infections with novel viruses are hard to detect
- The symptoms and laboratory findings in affected individuals are non-specific.
- The causative agent cannot be identified in many cases.
- Specific or broad range assays, usually surpassing the capabilities of local laboratories, are required for accurate diagnosis.
- Awareness by the primary health care personnel is required for optimal sampling and reporting.

New techniques will help detection of the next emergent virus
- Miniaturization of the currently used PCR-based assays and point-of-care tests with isothermal detection are being explored.
- Field-deployable assays and portable devices are required for rapid diagnosis and screening.
- Multiplex serology will be a powerful tool for insights into infection history, without infected specimen handling.

Tick-associated viruses share strategies to surpass host immune responses
- These include interferon antagonism and impairment of dendritic cell maturation.
- An aberrant, uncontrolled pro-inflammatory response may also occur upon infection, which is directly associated with disease severity.
- Banyang and phenuivirus NSs proteins and nairovirus ovarian tumor-like deubiquitinase domains are involved.
- Elucidation of pathogenesis will facilitate the development of effective vaccines and new treatment strategies.

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