AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews®

REVIEW

# Tick-Borne Flaviviruses, with a Focus on Powassan Virus

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**SUMMARY** The tick-borne pathogen Powassan virus is a rare cause of encephalitis in North America and the Russian Far East. The number of documented cases described since the discovery of Powassan virus in 1958 may be <150, although detection of cases has increased over the past decade. In the United States, the incidence of Powassan virus infections expanded from the estimated 1 case per year prior to 2005 to 10 cases per year during the subsequent decade. The increased detection rate may be associated with several factors, including enhanced surveillance, the availability of improved laboratory diagnostic methods, the expansion of the vector population, and, perhaps, altered human activities that lead to more exposure. Nonetheless, it remains unclear whether Powassan virus is indeed an emerging threat or if enzootic cycles in nature remain more-or-less stable with periodic fluctuations of host and vector population sizes. Despite the low disease incidence, the approximately 10% to 15% case fatality rate of neuroinvasive Powassan virus infection and the temporary or prolonged sequelae in >50% of survivors make Powassan virus a medical concern requiring the attention of public health authorities and clinicians. The medical importance of Powassan virus justifies more research on developing specific and effective treatments and prevention and control measures.

**KEYWORDS** Powassan virus, arbovirus, viral encephalitis

## INTRODUCTION

Ticks are known to host and transmit a variety of viruses, bacteria, and protozoa pathogenic to animals and human beings (1). The medical and veterinary importance of numerous tick-borne viral infections has been recognized for decades. Tick-borne viruses belong to different virus families, including *Asfarviridae*, *Bunyaviridae*, *Flaviviridae*, *Ortho-*

**Citation** Kemenesi G, Bányai K. 2019. Tick-borne flaviviruses, with a focus on Powassan virus. Clin Microbiol Rev 32:e00106-17. https://doi.org/10.1128/CMR.00106-17.



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**Published** 12 December 2018

*myxoviridae*, *Reoviridae*, and *Rhabdoviridae* (2). The most severe disease manifestations associated with tick-borne viruses include central nervous system (CNS) infections and hemorrhagic symptoms, which may be lethal in a high proportion (up to 20% to 30%) of patients. Tick-borne flaviviruses (TBFVs) comprise a variety of viruses worldwide that are thought to occur predominantly in the temperate zone and subtropical areas of the Northern Hemisphere and less prominently in (sub)tropical areas of Africa and Australia (3).

Powassan virus (POWV) is a neglected tick-borne virus, the only known member of the TBFVs that occurs naturally in North America. Although the number of associated human diseases can be considered low, the reporting system for notifiable diseases in the United States has shown a tendency toward an increase in the annual number of cases over the past decade. The case fatality rate of POWV encephalitis exceeds 30% in some reports, and long-term sequelae are very common (4, 5). The aim of this paper is to give an overview of POWV. The introductory sections focus on the general features of TBFVs, whereas the second part gives a comprehensive view on Powassan virus, with particular attention being given to epidemiology, genetic diversity, ecology, clinical disease, and diagnosis.

## TICK-BORNE FLAVIVIRUSES

### Taxonomy and Classification

At present, the International Committee on Taxonomy of Viruses lists four genera within the family *Flaviviridae*: *Hepacivirus*, *Pegivirus*, *Pestivirus*, and *Flavivirus*. The genus *Flavivirus* includes over 50 virus species (6); mosquitoes and ticks serve as primary vectors for numerous members of the genus, while mammals and birds serve as common primary hosts. Viruses without vertebrate hosts (e.g., Culex flavivirus and Aedes flavivirus, etc.) and viruses without arthropod vectors (e.g., Tamana bat virus) are also known (6–8).

Dual-host flaviviruses can be divided into mosquito/vertebrate and tick/vertebrate viruses (9). Tick-transmitted flaviviruses include the following species and viruses (listed in alphabetical order): *Gadgets Gully virus*, *Kadam virus*, Karshi virus, *Kyasanur Forest disease virus* (KFDV), *Langat virus*, *Louping ill virus* (LIV), *Meaban virus*, *Omsk hemorrhagic fever virus* (OHFV), *Powassan virus* (POWV), *Royal Farm virus*, *Saumarez Reef virus*, *Tick-borne encephalitis virus* (TBEV), and *Tyuleniy virus*.

Antigenic classification of flaviviruses is based on serological cross-reactivity. In this system, TBEV (including Russian spring-summer encephalitis and Central European encephalitis), *Langat virus*, KFDV, *Royal Farm virus*, Karshi virus, Negishi virus (a representative strain of LIV), and POWV were found to share considerable antigenic cross-reactivity, a finding based upon which these viruses were classified into the tick-borne encephalitis (TBE) serocomplex (10). Genetic classification, based on genome sequence data and phylogenetic relationships, provided further insights into the relationship among flaviviruses, including TBFVs. TBFVs were classified into two genetic clades, named mammalian and seabird TBFVs, to indicate a difference in the primary vertebrate host (11). Currently, mammalian TBFVs include seven species of known or putative medical importance (KFDV, Karshi virus, *Langat virus*, LIV, OHFV, POWV, and TBEV) (Table 1), which are distributed over the Northern Hemisphere: TBEV and LIV occur in Europe and Asia, and OHFV, *Langat virus*, KFDV, and Karshi virus are endemic in Asia, while POWV is endemic across North America and occurs in the Russian Far East (12). In humans, OHFV and KFDV cause hemorrhagic fever, whereas TBEV, LIV, *Langat virus*, Karshi virus, and POWV cause meningitis and encephalitis. Lesser-known members are those not associated with causing diseases in humans or animals; these include *Gadgets Gully virus*, *Kadam virus*, and *Royal Farm virus* (11).

### Structure and Biology

Infectious flavivirus particles are enveloped, smooth, spherical structures about 50 nm in diameter. The virion contains an electron-dense, 30-nm nucleocapsid which encapsulates the genomic RNA. The nucleocapsid protein (C protein) interacts with viral RNA and forms a less-ordered structure beneath the lipid membrane. This structure is

**TABLE 1** General features of human-pathogenic TBFVs

| Virus[a] | Yr of discovery | Region(s) affected | Important vector (tick) species | Common intermediate hosts | Disease(s) in humans (incidence [cases/yr]) |
|---|---|---|---|---|---|
| TBEV | 1937 | Europe, Asia | *Ixodes ricinus*, *Ixodes persulcatus*, *Dermacentor reticulatus* | Rodents (e.g. *Myodes* spp., *Apodemus* spp.) | Encephalitis, meningitis (0–15) |
| KFDV | 1957 | India, China, Saudi Arabia | *Haemaphysalis* spp., *Argas* spp., *Dermacentor* spp., *Hyalomma* spp., *Ixodes* spp., *Ornithodoros* spp., *Rhipicephalus* spp. | Monkeys (*Semnopithecus entellus*, *Macaca radiate*), camel, sheep | Hemorrhagic fever (400–500) |
| OHFV | 1947 | Russia | *Dermacentor reticulatus* | Rodents (e.g., *Microtus gregalis*, *Ondatra zibethica*, *Arvicola terrestris*) | Hemorrhagic fever (7–70) |
| POWV | 1958 | North America, Russia | *Ixodes cookei*, *Ixodes marxi*, *Ixodes scapularis* | Small- and medium-sized mammals (e.g., *Marmota monax*, *Mephitis mephitis*, *Peromyscus leucopus*) | Encephalitis (9–10) |
| LIV | 1929 | Europe, Russian Far East, Japan | *Ixodes ricinus* | *Ovis aries*, *Lepus timidus*, *Lagopus lagopus scotica* | Influenza-like illness, encephalitis (<1) |
| LGTV | 1956 | Malaysia, Thailand | *Ixodes granulatus*, *Haemaphysalis papuana* | Unknown | Fever, encephalitis (seen in clinical studies) (incidence unknown) |
| KSIV | 1972 | Uzbekistan, Kazakhstan | *Hyalomma asiaticum*, *Dermacentor daghestanicus*, *Ornithodoros parkeri*, *Ornithodoros sonrai*, *Ornithodoros tartakovskyi* | Unknown | Fever, encephalitis (incidence unknown) |

[a]Abbreviations: TBEV, tick-borne encephalitis virus; KFDV, Kyasanur Forest disease virus; OHFV, Omsk hemorrhagic fever virus; POWV, Powassan virus; LIV, Louping ill virus; LGTV, Langat virus; KSIV, Karshi virus.

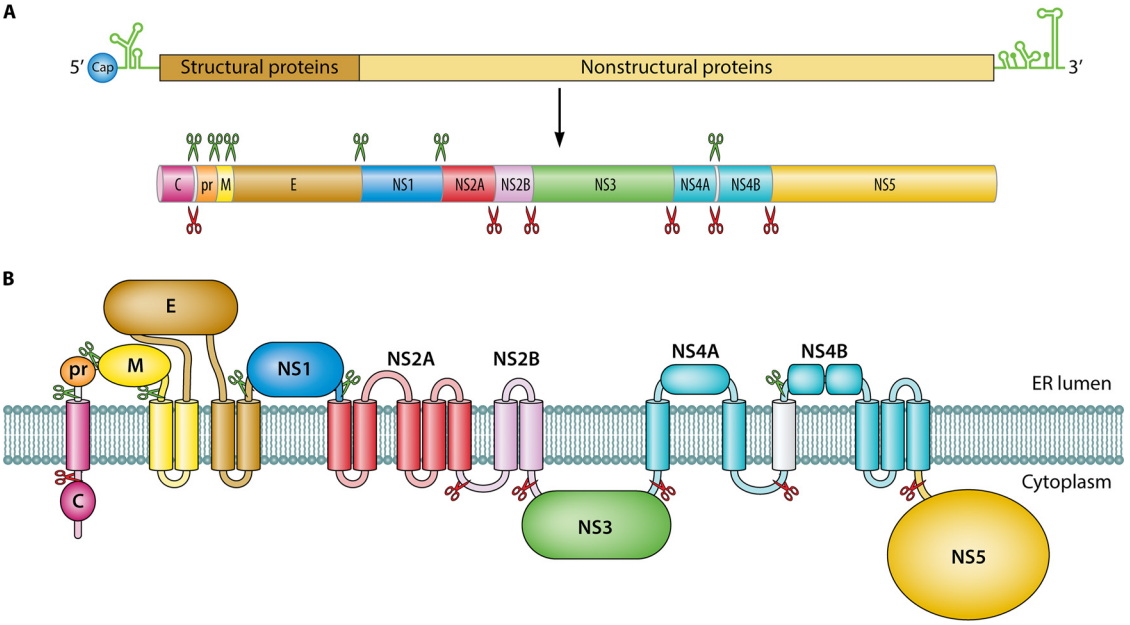


**FIG 1** (A) Schematic representation of the flavivirus genome and translation of the viral polyprotein. (B) Proteolytic cleavage sites, individual proteins, and localization of nascent proteins in the endoplasmic reticulum. Scissors indicate viral (red) and cellular (green) proteases that are responsible for cleavage.

surrounded by a cell-derived lipid membrane and viral E and M proteins, which are embedded via their transmembrane domains into the external lipid layer (13, 14). In the mature virion, the E proteins form homodimers in a head-to-tail orientation, displaying a herringbone pattern typical for all flaviviruses (13). The M protein protrudes at 5-fold symmetry axes between the dimerization domains of E proteins (9).

Members of the *Flavivirus* genus possess a single-stranded plus-sense RNA genome with an average length of 11 kb (Fig. 1). The GC content is around 52% to 55% (15). The 5′ end of genomic RNA has a type I cap structure (m7GpppAmpN$_2$), whereas the 3′ end lacks a poly(A) tail and terminates in a stable stem-loop structure (16). The cap directs the process of translation and also protects the viral RNA from degradation by cellular exonucleases (16). The genomic RNA is infectious upon transfection. The coding region of flavivirus genomic RNA encodes a single large polyprotein, composed of roughly 3,300 to 3,400 amino acids (aa) (17, 18). The coding region is flanked by 5′ and 3′ noncoding regions (NCRs). The lengths of the 5′ NCR and 3′ NCR are ~100 nucleotides (nt) and ~400 to 700 nt, respectively; the 3′ NCR may show significant length variation even within closely related TBFV strains (19). The NCR structures contain both sequential and structural motifs and are essential for flavivirus life cycle processes such as translation, replication, and virion assembly (20).

The structural proteins are encoded at the 5′ end of the genomic RNA (Fig. 1). The C protein forms the nucleocapsid; its C-terminal helices have a net positively charged interface and interact with the negatively charged genomic RNA (9). The M protein has two functional forms. In the immature virion, the longer, glycosylated precursor M (prM) protein stabilizes the E protein, preventing the virion from undergoing maturation into the structure that displays fusion activity (21, 22). During virion maturation, furin removes the N-terminal region of prM, leaving the C-terminal region to be anchored into the virion membrane (23). The E protein has multiple roles; it mediates virus attachment to cellular receptors and, consequently, is a major determinant of host and tissue tropism, and it also mediates membrane fusion and specifies immunological properties responsible for eliciting neutralizing antibodies *in vivo*. It contains three structural domains. Domain I and domain II are responsible for dimerization and contain a highly conserved hydrophobic peptide region that mediates membrane

fusion following internalization of the virion. Domain III contains receptor binding sites and the major antigenic sites with predominantly linear epitopes that are the main targets of neutralizing antibodies (24).

The nonstructural proteins are encoded by the central region and at the 3′ end of the genomic RNA (Fig. 1). Flavivirus NS proteins are components of the viral replication complex embedded in the endoplasmic reticulum (ER), play a role in flaviviral polyprotein processing, and/or serve as effectors against cellular antiviral defense mechanisms. The glycosylated NS1 protein has multiple forms. Of interest, antibodies raised against NS1 protect mice from virus challenge (25, 26). The multidomain NS3 protein with its N-terminal part functions as a serine protease, playing a role in flaviviral polyprotein processing, whereas the C-terminal part of the protein contains RNA triphosphatase and RNA helicase activities involved in capping and synthesis of viral RNA (27). NS5 consists of an N-terminal methyltransferase domain and the C-terminal RNA-dependent RNA polymerase domain containing both viral RNA capping and RNA synthesis activities. NS5 also plays a role in the evasion of the innate immune response in both mosquito- and tick-borne flaviviruses (28, 29).

The flavivirus replication cycle begins with the attachment of mature virus particles to the surface of the host cell. For TBEV, heparan sulfate has been shown to be involved in cellular binding (30). Additional cellular factors for attachment may be laminin binding protein and dendritic cell-specific intracellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) (31, 32). After budding into the cytoplasm, vesicles containing the virus particles fuse with endosomes, where the acidic pH triggers irreversible structural rearrangement in the E protein layer of the virion and E protein binding to the endosomal membrane. Following the fusion of the virion lipid layer with the endosome membrane, the nucleocapsid is released into the cytoplasm. Subsequently, the nucleocapsid disassembles, releasing the genomic RNA. Virus proteins are translated from the viral RNA on the cytoplasmic surface of the ER membrane as a single large polyprotein (33). Specific signals mediate the translocation of the nascent protein chain between the cytosolic and the ER interior spaces. Co- and posttranslational processing of the flaviviral polyprotein involves proteolysis by viral and cellular proteases (Fig. 1). The membrane-bound nonstructural and structural proteins accumulate on the cytoplasmic surface and the ER lumen (33). The replication complex on the ER forms specific membrane-bound compartments and provides a protective milieu where replication intermediates are hidden from cellular factors responsible for activating the double-stranded RNA (dsRNA)-dependent pathways of the innate immune system (34). Assembly of virions of the nascent genomic RNA and the three structural protein components (C, prM, and E) occurs in close proximity to the viral replication complex on the endoplasmic reticulum. The virions mature during the journey through the secretory pathway involving the Golgi network (27). The mature virions are released from the cell through exocytosis (33).

## Epidemiology and Ecology

Adaptation of tick-borne flaviviruses (TBFVs) to specific invertebrate and vertebrate hosts directly influences the dispersal of the pathogens and shapes the epizootiology of these viruses. Therefore, the emergence and reemergence of TBFVs are strongly correlated with population dynamics of the vector and the amplifying host species. It should be noted that similarly to other vector-borne infections, a broad range of factors affect the dynamics of TBFVs, such as climatic, ecological, or human-related changes (i.e., mobility and agricultural patterns) (11, 35). Since the occurrence and distribution of vector tick species are highly influenced by climate, global warming and its side effects may contribute to the future expansion of the geographic distribution of some tick species (36, 37). Such scenarios are well discussed in the cases of *Dermacentor reticulatus* and *Ixodes ricinus*, two tick species that seem to be establishing new foci in Europe (38, 39). Migratory birds are also thought to play a role in the natural dispersal of some TBFVs at long distances (40). Human activities were also reported as factors in the long-distance spread of TBEV, and at least two major events were described: the

building of a Siberian highway and the construction of the Trans-Siberian Railway (41). Human activities not only affect virus dispersal events but also have been demonstrated to influence the incidence of TBFV diseases. For instance, DDT (dichlorodiphenyl-trichloroethane) usage in the former Soviet Union greatly reduced the tick vector populations, which in turn resulted in a significant decrease in TBE incidence (42). Discontinuing the routine use of DDT worldwide may have led to an increase in the tick population, which played a role in the increase in disease incidence over the past 20 years (43). Based on these findings, it is anticipated that utilization of pesticides that are environmentally safe may successfully reduce the risk of TBFV infections by controlling the tick vector populations.

Ticks can serve as both reservoirs and vectors, while the vertebrate host provides a transport route between cofeeding ticks with so-called nonviremic transmission (Milan Labuda's enduring paradigm) (44–49). This unique transmission route seems to be facilitated mainly by leukocytes migrating between collaterally present tick feeding sites and, interestingly, does not depend on the viremic stage of the vertebrate host (49, 50). The major role in the maintenance of these viruses can therefore be attributed to horizontal means of transmission with leukocyte-mediated cofeeding transmission, which might be more important than transovarial transmission from the adult female tick to the eggs (51, 52). Although this transmission route has been fully characterized for TBEV, it was also stated that transovarial transmission is not the main route of transmission between tick vectors (53, 54). While the viremic stage seems to be less important for the natural transmission of TBFVs, viral RNA can be detected in various rodent organ samples (55–57). Replication in the vertebrate host might play an important role in virus overwintering (58). The significance of different tick species in overwintering of TBEV has also been reported (59, 60).

Numerous rodent, ungulate, lagomorph, and bird species have been recognized as intermediate hosts for TBFVs (Table 1). Despite the fact that TBFV infection induces viremia in these hosts, the question of whether these species are able to participate in virus maintenance in nature remains open. These vertebrates are thought to serve as intermediates for horizontal transmission events, which directly influence the transmission cycle by providing hosts for reproducing ticks (61, 62). Probably due to the very short and low viral titer during the viremic period, humans are considered dead-end hosts for TBFVs (36).

TBEV is the most significant TBFV, with an estimated 10,000 to 15,000 annual cases in Europe and Asia. The incidence greatly depends on vaccination coverage among residents at the greatest risk of infection in affected areas (63). The case fatality rate largely depends on the infectious subtype of TBEV and is known to range between 1% (for the Western subtype) and 20% (for the Far Eastern subtype) (36, 64–66). Although TBEV is the most abundant of the TBFVs, other viruses should be noted as characteristic tick-borne pathogens worldwide. OHFV is represented in Siberia (67), while KFDV is a commonly identified pathogen in some regions of India (68, 69). LIV causes infections in the United Kingdom, Ireland, and southwestern Norway, with an introduction to Spain (70), and POWV is the causative agent of severe illnesses in North America (71). Alkhurma virus (AHFV), a subtype of KFDV, is present in Saudi Arabia and causes infections associated with hemorrhagic fever manifestations (69). Langat virus and Karshi virus are not considered medically important TBFVs (Table 1).

## Pathogenesis

TBFVs can be divided into two groups based on their different tissue tropisms. Due to the high affinity of the virus for neural tissue, neurological disease manifestation is a characteristic pathogenetic scheme in TBEV, LIV, and POWV infections (70, 72–76). In contrast, KFDV, AHFV, and OHFV infections are typically associated with hemorrhagic manifestations, although neurotropism has been described for KFDV (68, 77–80). A strict hematopoietic and vascular tissue tropism has been linked to OHFV infections (81). Details about the pathomechanism of TBFVs are only partially understood, and most data are available from TBEV infections. Similarly to the majority of arthropod-

borne viruses, the initial replication site of TBFVs is usually proximal to the tick bite. Macrophages and dendritic cells (DCs) are believed to transport the replicating virus to draining lymph nodes, where replication continues. This event results in a transient viremic stage of the host, which may lead to multiorgan dissemination of the virus. In POWV-based infection experiments, macrophages and fibroblasts have been identified as early cellular targets of infection close to the tick feeding site (82).

During the viremic stage, neurotropic flaviviruses can invade the central nervous system (CNS) by crossing the blood-brain barrier (BBB). Studies suggest that tumor necrosis factor alpha (TNF-$\alpha$) may serve as a key factor in modulating the permeability of the BBB for some neurotropic flaviviruses (83). Although the exact molecular background of flaviviral BBB crossing is largely unknown, the brain capillary endothelium may play a key role in this mechanism (84). The main target cells for TBEV in the CNS are neurons, and virus-induced neuronal injury has been characterized in detail (74, 85–87). In addition, glial cells may also be involved in viral pathogenesis, particularly in the neurological stage of infection. Interestingly, rat astrocytes resist TBEV-mediated cell death, which might explain dormant TBEV infection in rodents (88). This capacity of astrocytes might contribute to maintaining the enzootic cycle in nature via long-term infections (58). In this process, a rapid type I interferon (IFN) response is thought to protect astrocytes from flavivirus-induced cell death (89). For TBFVs causing hemorrhagic disease, a characteristic liver and spleen tropism is seen, resulting in viscerotropic disease manifestations (11, 67, 90).

Genetic factors of the host and virus may be important factors in clinical disease severity. Recent data indicate that a lower expression level of the chemokine receptor CCR5 in peripheral blood lymphocytes is associated with greater susceptibility to TBEV infection (91). Toll-like receptor 3 gene mutations were also suggested to be an important genetic marker for the severity of TBEV infection; however, this observation needs further confirmatory studies (92). Mutations in the promoter region of the CD209 gene (encoding DC-SIGN, a C-type lectin pathogen recognition receptor [PRR]) may be associated with a predisposition for severe forms of tick-borne encephalitis (31). Evidence that viral genetic factors may also contribute to the severity of infection was supported by the genetic characterization of different TBEV strains from symptomatic and asymptomatic infections. Mutations affecting the virion proteins, NS3, NS5 proteins, and even the 3′ NCR were linked to altered pathogenic properties (93, 94). Despite the progress in investigating viral pathogenesis, the background of the TBFV pathomechanism remains poorly understood, especially for less-common TBFVs.

## Immunity

Tick-borne flaviviral infections affect the immune system of vertebrate hosts in a unique manner, in which the proximity of the tick bite site is the first step. This special environment, along with its distinctive molecular mechanisms, can highly affect the outcome of infection. Saliva of the tick *Ixodes ricinus* is able to alter dendritic cell-induced responses by decreasing TNF-$\alpha$ and interleukin-6 (IL-6) production and reducing TBEV-induced apoptosis (95). This proinflammatory environment is the first barrier against infection, and it has been reported that the bite of a POWV-infected tick induces more-rapid immune cell recruitment at this site than a bite from an uninfected tick (82, 96).

The mammalian immune response against flaviviral infection includes both innate and adaptive immune processes. As an early response, innate immunity activates multifaceted pathways rapidly after virus introduction with the primary aim of reducing the replication efficiency at the site of infection. The virus is primarily detected by PRRs of the host cell. PRRs detect pathogen-related single- and double-stranded RNA molecules and initiate multiple signaling pathways that lead to the production of IFNs (97, 98). Type I IFNs play a central role in the immune response. Processes involved in this pathway have been reviewed in detail by Kawai and Akira (98). IFNs stimulate the JAK-STAT signal transduction pathway and amplify further IFN responses, resulting in the expression of numerous interferon-stimulated genes.

DCs are the early targets of invading flaviviruses (47, 99), along with macrophages and fibroblasts, as was suggested in a recent study (82). Additional important functions of DCs are PRR-based virus detection, which leads to the production of type I IFNs, the transportation of the virus into draining lymph nodes, and antigen presentation that induces the adaptive immune response (100). The latter mechanism is what makes DCs the bridge between innate and adaptive immunity. TBFVs developed multiple countermeasures against the DC-dependent immune response, as exemplified by the antagonism of IFN regulatory factor 1 (101) or the suppression of JAK-STAT signaling via virus-encoded IFN antagonists (28, 102, 103).

DCs undergo a maturation program which leads to the activation of naive T cells, resulting in the production of T helper type 1/type 2 and cytotoxic T lymphocytes. DC-related PRR-based virus recognition controls the activation of multiple signaling pathways, such as higher expression levels of major histocompatibility complex (MHC) class II molecules, T-cell-costimulatory molecules, and proinflammatory cytokines (104). DCs are also responsible for generating protective $CD8^+$ T-cell immunity using molecular signaling (104). The $CD8^+$ T-cell response was recently examined in depth in acute human TBEV infections, including temporal dynamics, specificity, and functional and phenotypical characteristics of the response (105). Another recent study suggested some major differences in the natural killer (NK) T-cell response against human TBEV infection compared to other human viral infections (106).

In primary TBFV infections, the antibody response appears mostly at the end of the viremic phase, when IgM antibody titers increase rapidly. Normally, within 1 to 2 days following infection, specific IgG levels also start to increase. In instances of secondary infections, IgM levels show only a slight increase, whereas the IgG response is rapid and robust and efficiently terminates viremia (107).

## Prevention, Control, and Treatment

With the exception of TBEV, specific prophylactic treatment against TBFVs is not available. For prevention of TBEV infection, four commercially available inactivated vaccines have been marketed to date. Two of them are licensed in Russia and produced from the Far Eastern subtype of the virus (TBE-Moscow; EnceVir), while the other two vaccines available in Europe contain the inactivated European subtype (FSME-Immun; Encepur) (108). Vaccination in areas of endemicity significantly reduced the incidence of TBE, but it remained high in nonvaccinated populations (109). The annual incidence of TBEV infections has slightly increased during the past decades in several European countries (110, 111). Therefore, some authors argue for the need for mass immunization programs in affected areas and recommend the extension of vaccination to possible new regions of endemicity (112).

Immune therapy using hyperimmune IgG is not recommended for TBEV infections because of concerns of possible antibody-enhanced infection (113, 114).

Along with vaccination programs, tick bite prevention actions are the most efficient way to avoid infections. The risk of viral infection must be highlighted for those visiting areas of endemicity for professional or sporting activities (e.g., forestry, farming, hunting, and military activities) and even for leisure (e.g., hiking and orienteering). Also, control of small mammals (mainly rodents) should be implemented around home environments to minimize contact with putative primary vertebrate hosts, and our pets (dogs and cats) should be protected against tick bites and examined regularly to prevent tick introduction into the living space. Preventive arrangements and basic risk factors were recently reviewed (115).

Novel approaches based on targeted microRNA (miRNA) control of TBFV infections have recently been successfully tested using cell culture and mouse models. This novel therapeutic method could theoretically inhibit virus replication in both arthropod and vertebrate hosts. The available data on abundant host microRNAs (116, 117) enable the design of target sites along with various virus genomes specific for host microRNAs (118, 119). One major disadvantage of virus attenuation by this strategy is the instability of the target miRNA sequence in the modified genome, which can result in a reversion

of the virus to the virulent phenotype, although several results on counterstrategies were reported (120–122). Other approaches offer replication-defective, chimeric vaccines, in which parts of the TBFV genome are replaced by homologous genomic regions derived from a heterologous (e.g., mosquito-borne) flavivirus. In animal models, these vaccine candidates have been shown to be highly immunogenic even after a single dose (123).

Dual-action vaccines have been extensively examined during the past years in order to control tick-borne pathogens. The principle of dual-action vaccination lies in the inhibition of blood-feeding mechanisms and rupture of the tick midgut, causing death of engorged ticks. Promising results have been obtained in controlling parasitic and bacterial tick-borne pathogens with such experiments. In laboratory mice, TBEV infection was successfully prevented with recombinant tick cement protein (a protein derived from the cement cone that secures the tick's mouthparts during feeding) (124, 125). Results of subsequent studies were recently reviewed (126).

Concerning virus-specific therapy, although etiological treatment has not been approved to date, several studies dealing with possible antiviral agents for treatment of TBFV infections were reported. For example, 6-azauridine, 2′-*C*-methylcytidine, and IFN-$\alpha$2a successfully inhibited AHFV, KFDV, OHFV, and POWV replication in cell culture (127). Further studies were performed with the adenosine analogue NITD008, which was considered a potentially efficient panflaviviral inhibitor (128). Additionally, high antiviral activity along with low cytotoxicity were observed *ex vivo* with a 7-deaza-2′-*C*-methyladenosine nucleoside analogue, which might be another antiviral candidate in the future (129).

## POWASSAN VIRUS

### History

Powassan virus (POWV) was named after the town Powassan (Ontario, Canada), where it caused the death of a 5-year-old child diagnosed with encephalitis (130). In the United States, the first human POWV case was described in 1970 in New Jersey (131). Russia was the third country that identified human POWV infection in 1978 (132). Interestingly, the first POWV isolates originated from tick vectors and had been collected before human infections were documented; this is exemplified by the earliest U.S. isolate of POWV from a *Dermacentor andersoni* tick collected during 1952 in Colorado (133) and the first POWV isolates from Russia, including those detected in ixodid ticks collected in 1970 (GenBank accession number KT224351).

### Epidemiology

As a result of detections across North America and the Russian Far East, POWV is now considered the only known member of the TBFVs that naturally occurs in both the Old and New World (Fig. 2). Human illnesses associated with POWV infection have been reported in Canada, the United States, and Russia (130–132, 134). Humans most likely contract POWV from biting ticks carrying the virus. However, most patients with POWV disease (or their caregivers) cannot recall a preceding tick encounter, very likely because tick bites are easily overlooked (135–139). Alternative infection routes cannot be excluded either; however, the hypothesis that individuals who consume unpasteurized goat milk in areas where POWV is endemic are, theoretically, at risk of POWV infection awaits formal demonstration (140, 141). From the perspective of disease prevention, understanding the time required for the transmission of the pathogen from tick to vertebrate is a key factor. In infection experiments that aimed at determining the dynamics of transmission of POWV from ticks to laboratory mice, the majority of experimental animals exposed to feeding by *Ixodes scapularis* ticks carrying POWV became infected 15 min following attachment, and all animals became infected after 30 min (142). Although the length of attachment time required for the development of human disease is unknown, relatively short tick attachment times (less than 3 h) associated with subsequent clinical illness progression have been reported (143). The short time for effective tick-to-vertebrate host transmission of POWV in mice, and likely

**FIG 2** Geographic distribution of POWV. Dots indicate the locations where POWV was identified and/or serological assays provided indirect evidence for local circulation.

in humans, sharply contrasts with the longer (up to 72 h) skin contact time needed for effective transmission of nonviral tick-borne pathogens, including *Borrelia burgdorferi* (142, 144). Thus, while the removal of ticks several hours after attachment to skin may prevent infection with *Borrelia burgdorferi*, it may not prevent infection with POWV.

From the late 1950s to the mid-2000s, around 40 to 45 human POWV illnesses were documented in North America, with the majority of cases being reported in the United States, and at least 16 cases were reported in the Russian literature, encompassing a 10-year study period in the 1980s (132, 137, 145–147). The low POWV detection rate in this period (~1 to 1.5 cases per year) was followed by a marked increase in reporting of POWV illnesses during the subsequent decade in the United States (4). Between 2007 and 2016, a total of 98 cases were documented by the U.S. Centers for Disease Control and Prevention (CDC), with an average of ~10 cases (range, 2 to 22 cases) per year (Fig. 3) (see https://diseasemaps.usgs.gov/mapviewer/ and https://www.cdc.gov/powassan/). POWV illness occurs in all age groups; however, while early reports showed a greater incidence in children under 15 years of age (~70% [reviewed by Gholam and coworkers for 1958 and 1998 {139}]), more-recent data reported by the U.S. CDC showed that only 8% of patients with POWV illness were younger than 18 years of age, whereas ~50% of patients were older than 60 years of age (139, 148–153) (Fig. 3). Similarly to other tick-borne infections in North America, POWV infections tend to show a peak incidence from May to November (Fig. 3). In the United States, the most heavily affected states are Massachusetts, Minnesota, New York, and Wisconsin, but human illnesses were also reported in New Hampshire, New Jersey, Maine, North Dakota, Pennsylvania, Tennessee, Vermont, Virginia, and Connecticut (135–137, 143, 154–158). The highest incidence of POWV neuroinvasive disease (≥0.5 cases per 100,000 residents) was documented in some counties in the states of Minnesota and Wisconsin (see https://diseasemaps.usgs.gov/mapviewer/ and https://www.cdc.gov/powassan/). POWVs were identified in a few tick species (including *Ixodes* and *Dermacentor* spp.), whereas POWV or POWV-specific antibodies were detected in at least 38 wild and domestic mammals and a few bird

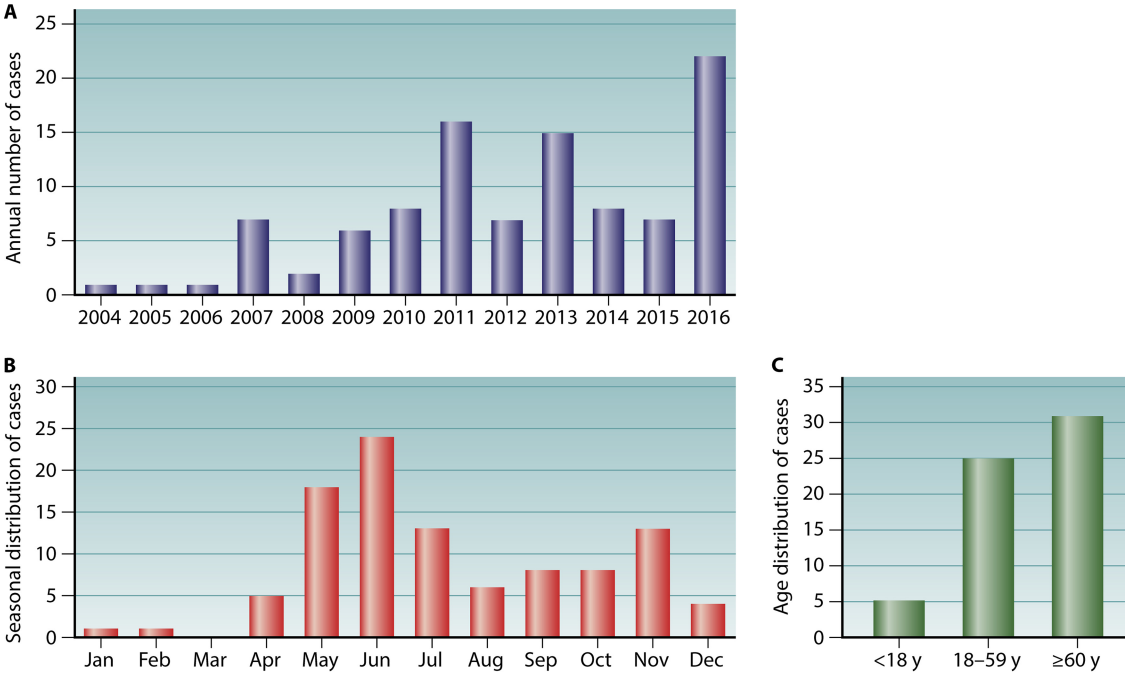


**FIG 3** Epidemiological features of POWV infections between 2004 and 2016 in the United States. The panels show the annual number (A), the seasonal distribution (B), and the age distribution (C) of cases reported to the Centers for Disease Control and Prevention.

species from well-known areas of endemicity and from other regions across the United States, including Alaska (147, 159). Virus serology data suggest that POWV may occur in northwest Mexico (Sonora state) (160). In Canada, human infections have been identified primarily in New Brunswick, Quebec, and Ontario; however, epidemiological and ecological surveys also detected POWV in Nova Scotia, Prince Edward Island, Alberta, and British Columbia (139, 161–163). In Russia, POWV disease has been reported in the Russian province of Primorsky Krai (Maritime Territory), whereas seropositive rodents whose sera reacted with POWV antigen were also detected in other parts of Siberia (134, 146, 159). It is important to note that while data based on serology assays indicate a wide distribution of POWV across North America, cautious interpretation is needed for results obtained by some of the available laboratory tests that tend to exhibit cross-reactivity due to shared epitopes on POWV-derived antigens and those expressed by other flaviviruses (147, 159).

Because of the low incidence of human illness in areas of endemicity, seroprevalence of POWV in the general population is thought be a good proxy to better describe the overall exposure to and geographical distribution of human infections with POWV. However, data reported so far are scant and not spatiotemporally representative. Seroprevalence rates ranged between 0 and 5.8% among residents of Ontario in the late 1950s and 1960s, showing variation among communities, a finding that suggested a focal occurrence of both tick vectors and POWVs (141, 164, 165). In seroepidemiological studies conducted later among residents in British Columbia, New York, Minnesota, and Wisconsin, 6.1%, 0.7%, 4%, and 4% of individuals, respectively, had POWV antibodies in their sera (147, 163, 166, 167). These data from areas of endemicity are consistent with the hypothesis that many infections remain undetected due to absent or mild symptoms. In addition, a portion of symptomatic POWV infections could be masked by the clinical manifestations associated with other human-pathogenic tick-borne microorganisms cotransmitted with POWV by tick bite. So far, no longitudinal serological studies have been conducted to determine the dynamics of POWV seroprevalence in residents of areas of endemicity, and only limited data are available for animals. Notably, a recent seroepidemiological study that used sera collected in Connecticut from white-tailed deer (*Odocoileus virginianus*) demonstrated a marked tem-

poral variation in seroprevalence but overall an increasing trend of seropositivity for POWV, rising from <25% before 1996 to 80% to 91% between 2005 and 2009 (168).

The increased reporting of POWV encephalitis in humans may be, in part, explained as a result of enhanced surveillance activity for arboviruses in North America established following the incursion of West Nile virus (WNV) in the United States in the late 1990s (4, 169, 170). However, the increasing seroprevalence in white-tailed deer cannot be explained by the enhanced monitoring of arbovirus-related human diseases. Thus, other contributing factors must also be taken into account. The dramatic territory expansion of an important competent tick species, *Ixodes scapularis*, seen over the past decade is probably the most important contributing factor (171).

Risk factors for human POWV infection include outdoor activities in areas of endemicity, contact with wild animals thought to serve as natural hosts of POWV, as well as family pets infested by ticks. To reduce the risk of being infected with POWV, it is recommended that individuals use tick repellents, wear adequate clothes (long sleeves and pants), avoid dense underbrush and wooded habitats, minimize the presence of wild mammals around homes, and, very importantly, perform thorough tick checks after spending time outdoors (136, 147) (https://www.cdc.gov/powassan/index.html).

## Diversity and Evolution

POWV is not genetically uniform. Evidence of genetic diversity was reported in 1997 when a novel POWV, called deer tick virus (DTV), was detected in *Ixodes scapularis* (172). Comparison of the genome sequences of representative POWV-like (genome size, 10,839 bases; encoded polyprotein, 3,415 aa) and DTV-like (genome size, 10,834 to 10,837 bases; encoded polyprotein, 3,415 aa) isolates showed 84% to 85% nucleotide and 92% to 95% amino acid similarities between the two related groups of isolates. Minor length differences are localized to the 3′ untranslated region (UTR) (134, 173–176). Analysis of the coding region revealed that POWV-like isolates (unlike DTV-like isolates) have two consecutive initiation codons for the C protein. One or two amino acid differences were described at the cleavage sites of the polyproteins of POWV and DTV at junctions of C/PrM, PrM/M, NS1/NS2A, NS2B/NS3, NS3/NS4A, and NS4A/NS4B (174). The functional consequences of these changes, if any, remain to be elucidated. Despite the genetic differences, the phenotypic implications are currently unclear, although Ebel (71) anticipated that phenotypic differences may exist in the respective primary tick vectors. Experimental data indicate that DTV and POWV are antigenically closely related, as demonstrated by hemagglutination inhibition assays and cross-neutralization tests, and also display similarities in neurovirulence in laboratory mice (174, 175). Both major variants are pathogenic for humans (130, 131, 177, 178).

Currently, the publicly available sequences are derived from around 60 separate virus isolates or clinical specimens. Several sequencing studies were initiated to better understand associations between the major variants of POWV and their tick vectors and to characterize strains identified from human disease (134, 177, 179–184). Thus, the majority (>90%) of POWV sequences originate from ticks and humans, and only a few sequences are available from wild animals (e.g., black-faced bunting, fox, woodchuck, and red squirrel) or other possible vectors (*Aedes* sp.). Although POWV-specific sequence data are accumulating slowly, researchers have used the available genetic information to clarify the evolutionary history and genetic relationships among North American isolates and between North American and Russian POWVs.

Molecular epidemiology and phylogenetics studies showed that POWV and DTV form two major genetic clusters (Fig. 4), designated lineage I for POWV-like strains and lineage II for DTV-like strains (71, 185). POWV isolate LB that originated from the first recorded human case in 1958 (Powassan, Ontario, Canada) is the prototype isolate of lineage I, while the first isolate from *Dermacentor andersoni* collected in 1952 (Colorado) is the prototype DTV isolate representing lineage II (174). Epidemiological surveillance conducted by combining various laboratory methods (including virus neutralization tests, reverse transcription-PCR [RT-PCR], and sequencing) indicated that lineage I POWVs occur in the United States, Canada, and Russia, while lineage II POWVs are

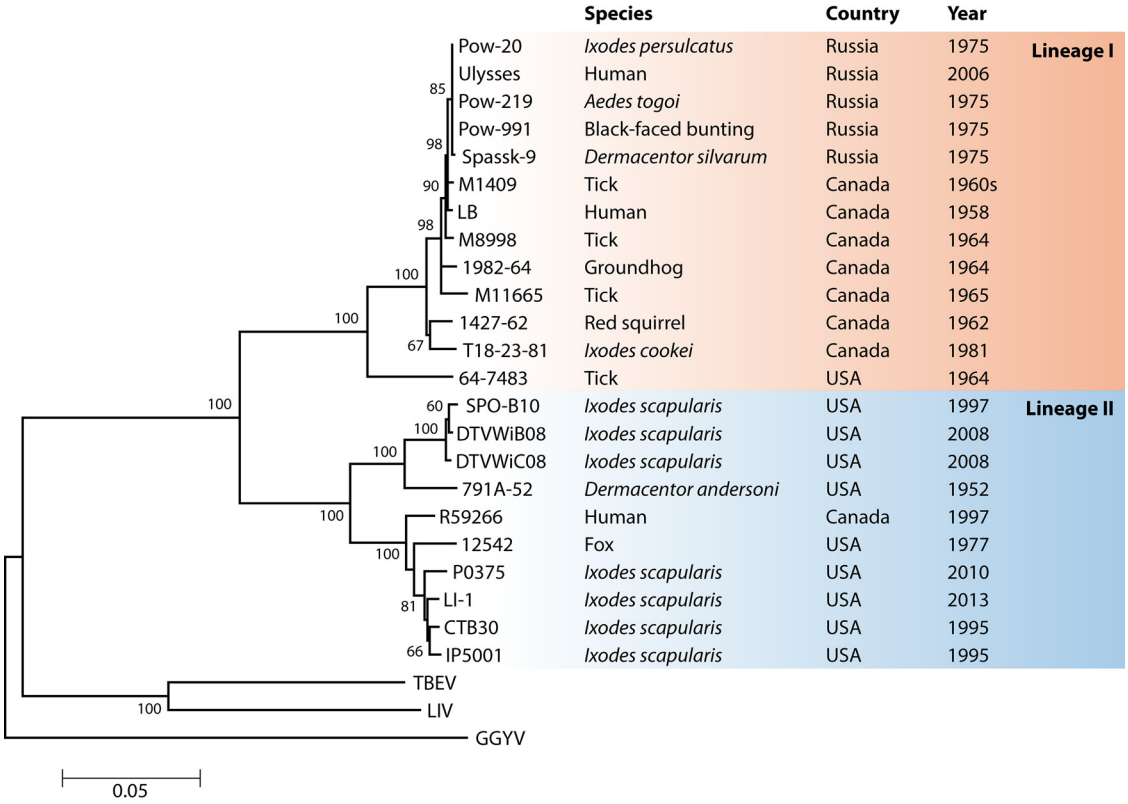


**FIG 4** Phylogenetic tree showing the geographic distribution and species association of lineage I and II POWVs.

known to occur in the United States and Canada (134, 176). Although the geographic dispersals of lineage I and II POWVs seem to overlap in at least Ontario, Canada, and in the northeastern regions of the United States, collecting additional sequence data from different geographic locations is needed to construct a more detailed distribution map for the two lineages.

POWV is a distant relative of other, Eurasian TBFVs, which is believed to have diverged ca. 12,000 to 13,000 years ago as a likely consequence of animal migration across the Bering land bridge during the latest ice age (186). Further sequence analyses offered intriguing explanations of the genetic diversity and the geographic distribution of POWV. The level of genetic divergence between and the molecular evolutionary analysis of lineage I and II POWVs suggested that they diverged around 500 to 2,000 years ago (this uncertainty comes principally from differences in data sets and analytical methods used to estimate the time when the most recent common ancestors existed) (176, 187, 188). Additional analyses identified molecular traces on the E protein being under selective pressure, providing evidence for the adaptation of POWV lineages to different enzootic cycles (176). Phylogenetic analysis also demonstrated further divergence to sublineages within both major POWV lineages; however, the evolutionary driving forces behind this diversification have not yet been clarified (176). Of interest, all currently known Russian isolates of POWV cluster with the prototype LB strain identified in Ontario, Canada, and share very high genetic similarity (up to 99.8% nucleotide identity) with all North American lineage I POWV strains, suggesting a single introduction of POWV into Russia during recent human history. Russian authors hypothesize that although natural expansion of POWV to Russia, for example, by long-distance-migrating birds, cannot be excluded, a more likely scenario links this introduction to human activity, such as importation of muskrat or mink to supply fur farms in Russia or shipment of commercial or military cargo (134).

Apparently, further studies are needed to clarify the evolutionary history of POWVs, and these estimates will require larger data sets that include sequences from neglected

tick and vertebrate species. Nonetheless, POWVs continue to evolve in their vectors and vertebrate hosts. Overall, the rate of evolution was estimated to be higher in North American POWVs than in Russian POWVs ($5.7 \times 10^{-4}$ versus $2.4 \times 10^{-5}$ substitutions per site per year for the E protein, respectively) (134, 176). This phenomenon is not clearly understood; however, differences in the vector-host relationships that may act as a limiting factor for POWV evolution leading to lower virus diversity in the Old World territory of POWV have been implicated (134). It was observed that the population complexity of lineage II POWV in ticks is very low and that POWV resists the pressure to diversify in response to RNA interference (RNAi) within ticks; these features contrast with the RNAi-mediated diversification of mosquito-borne flaviviruses in mosquitoes (189, 190). Viral population genetic analyses demonstrated that the majority of genetic changes occur during the few days of horizontal transmission from tick to small mammal. However, seeing the low rate of evolution in nature, which corresponds to $\leq 2$ mutations per genome per year, it is conceivable that horizontal transmission is infrequent and may occur only once a year (190).

**Ecology, Vectors, and Vertebrate Hosts**

Efforts to describe the natural history of POWV began after the first human case in Powassan (Ontario, Canada) was identified. Initial virus isolation attempts and serological surveys performed in the vicinity of Powassan revealed that the natural cycle of POWV includes some small mammals and ticks and that peak transmission from ticks to mammals occurs during spring and summer (191–194). Virus activity was found to vary year by year, which may be affected by the tick population density, the rate of tick infestation of mammals, and the availability of various vertebrate host species. Antibody prevalence was detected in a number of small- to medium-sized mammalian species and also some large animals but not universally in all study sites, showing geographic differences in the possible role for these host species in the natural cycle of POWV infection (71). The study design, with a small sample size tested, or the antibody detection methods chosen may have an impact when determining the reservoir role for a particular host species in a geographic region. Unlike serological evidence that showed numerous possible reservoirs and tangential host species, POWV itself was relatively infrequently isolated in vertebrate hosts.

Regardless of the serological methods used to measure circulating serum antibody, the mammalian hosts incriminated in the ecology of POWV infections included a variety of rodents and lagomorphs, such as red squirrel (*Tamiasciurus hudsonicus*), gray squirrel (*Sciurus carolinensis*), chipmunk (*Tamias striatus*), yellow-pine chipmunk (*Tamias amoenus*), Columbian ground squirrel (*Citellus columbianus*), golden-mantled ground squirrel (*Callospermophilus lateralis*), groundhog (*Marmota monax*), yellow-bellied marmot (*Marmota flaviventris*), deer mouse (*Peromyscus maniculatus*), white-footed mouse (*Peromyscus leucopus*), Pinon mouse (*Peromyscus truei*), northern red-backed vole (*Myodes rutilus*), southern red-backed vole (*Myodes gapperi*), porcupine (*Erethizon dorsatum*), and snowshoe hare (*Lepus americanus*). Virus isolation or serological studies also found evidence of natural infection in mustelids, such as spotted skunk (*Spilogale putorius*), striped skunk (*Mephitis mephitis*), long-tailed weasel (*Mustela frenata*), and short-tailed weasel (*Mustela erminea*), and some other mammals, including Virginia opossum (*Didelphis virginiana*), coyote (*Canis latrans*), fox (*Vulpes vulpes*), gray fox (*Urocyon cinereoargenteus*), raccoon (*Procyon lotor*), and white-tailed deer (*Odocoileus virginianus*) (184, 191–209). Birds are not thought to play a role in POWV maintenance in nature; however, a recent survey analyzing virus prevalence and serological reactivity to POWV in New York state birds detected serum antibody in veery (*Catharus fuscescens*), gray catbird (*Dumetella carolinensis*), northern cardinal (*Cardinalis cardinalis*), and Eastern towhee (*Pipilo erythrophthalmus*) (184). In addition, POWV was isolated from common teal (*Anas crecca*), mallard (*Anas platyrhynchos*), and black-faced bunting (*Emberiza spodocephala*) in Russia (209) (GenBank accession number KU297222). Collectively, while the host species list of wild animals that are susceptible to POWV infection is

becoming more complete, the role of most species in POWV perpetuation remains unknown (71).

Seroepidemiological studies conducted in some regions showed that domestic animals display significant variation in susceptibility to POWV infection and, in general, are not thought to be involved in the persistence of POWV in nature. However, some species might have a role in the transmission of POWV to humans, primarily by increasing the likelihood of human exposure to infected ticks. Experimental infection resulted in severe neuroinvasive disease in horses inoculated with POWV, and seroepidemiological studies conducted in Ontario found that 13% of examined horses had circulating serum antibodies against POWV (210). Cows, sheep, and goats occasionally sampled in serosurveys were not found to be naturally infected with POWV; however, goats infected experimentally developed transient viremia without clinical signs of infection and also released infectious virus in milk (140). In infection experiments, rabbits have developed viremia (210). Experimentally infected dogs and cats did not show symptoms of neuroinvasive disease; however, antibody was detected in their sera. Family cats and dogs were also shown to possess POWV-specific antibodies in a case report of a patient with POWV encephalitis, and around 1% of dogs surveyed in Ontario and British Columbia had antibodies to POWV (138, 211–213). Collectively, some lactating ruminant species may pose a theoretical risk for the alimentary route of infection with POWV in cases when unpasteurized milk is consumed, although this route of infection for POWV has never been documented. In addition, pets may serve as incidental hosts for ticks involved in the natural life cycle of POWV; thus, given that they may bring ticks into the home, pets may pose a risk for family members to become bitten by ticks carrying POWV.

The host preference of ticks has been studied extensively (214). These studies provide information about different vectors that may pose a major public health risk for disease transmission due to their host selection. For POWV, tick species that show high host specificity may be effective in POWV maintenance in the enzootic cycle but are thought to be inefficient in POWV transmission to humans. On the contrary, ticks that are more opportunistic in host feeding may pose a greater public health risk because they may more frequently transmit POWV from the common enzootic cycle to incidental hosts, including humans (71). Although the first isolation of POWV was made from a *Dermacentor andersoni* tick, the most important vectors in North America are thought to be *Ixodes* species, including *Ixodes cookei*, *Ixodes marxi*, *Ixodes spinipalpis*, and *Ixodes scapularis*. Detections of POWVs have been made from *Ixodes cookei*, *Ixodes marxi*, and *Ixodes scapularis* at a fairly constant rate over study sites and time periods that rarely exceeds 5% (184, 192, 193, 198, 199, 203). Other North American tick species are also thought to play a role in POWV maintenance; however, these other tick species are rarely studied for POWV carriage (71). Thus, for example, *Dermacentor* spp. have been only occasionally assayed for POWV, and no evidence so far indicates that *Dermacentor variabilis* or *Dermacentor andersoni* would play a major role in the infection cycle of POWV. In addition, laboratory investigations demonstrated that *Dermacentor andersoni* does not support the vertical transmission of POWV, thus confirming previous ecological observations (215). Nonetheless, data reported from Russia are consistent with the possibility that a number of other tick species may serve as vectors for POWV transmission, given that prototype POWVs in the Russian Far East were isolated from *Ixodes persulcatus*, *Dermacentor silvarum*, *Haemaphysalis japonica*, and *Haemaphysalis longicornis* ticks (216) (GenBank accession numbers KU297218, KU297219, KU297221, KU160627, and MG652438).

When trying to put epidemiological surveillance data in context with results from ecological studies, it is important to recall that POWVs exist in at least two genetic lineages that are associated with different vectors (71). Considering the feeding behavior and host preference of the implicated tick species, the natural cycles of lineage I POWV maintenance include *Ixodes cookei* and groundhog/skunk, and *Ixodes marxi* and red squirrel, whereas the hitherto-recognized natural cycle for lineage II POWV consists of the more opportunistic tick *Ixodes scapularis* and a variety of vertebrate hosts,

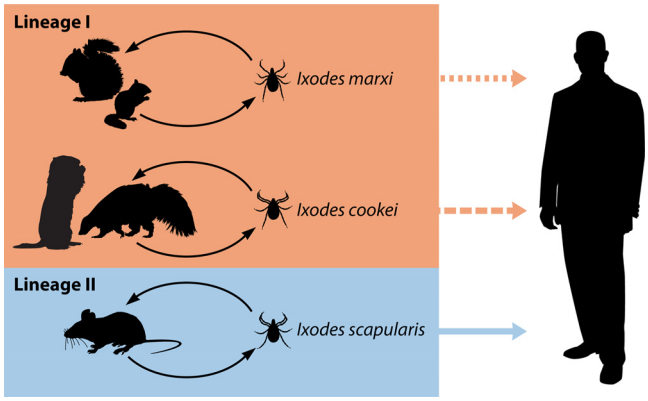


**FIG 5** Enzootic cycles of lineage I and II POWVs linked to the main tick vector and some important vertebrate hosts. Humans are considered tangential hosts; arrows pointing to the silhouette on the right are indicative of the relative public health risk posed by different tick species (i.e., low risk for *Ixodes marxi* and *Ixodes cookei* and greater risk for *Ixodes scapularis*).

including white-footed mouse. All three tick species may transmit POWV to humans, although *Ixodes cookei* and *Ixodes marxi* are generally considered more host specific, whereas *Ixodes scapularis* is more opportunistic and aggressively attacks humans, thus representing a major role in disease transmission to humans (71). Of interest, some host-specific tick vectors may display an extended host preference, as exemplified by *Ixodes cookei*, which shows a greater-than-usual preference for feeding on humans in Maine and thus may be a more common source of POWV infection in humans in this area than in other geographic regions (71) (Fig. 5). Although up-to-date information on POWV ecology in far-east Asia is not available, early reports from Russia suggest that ixodid ticks and murine rodents play a role in the perpetuation of lineage I POWV in enzootic foci (217, 218). Interestingly, virus monitoring conducted in the Russian Far East documented the isolation of POWV from mosquitoes (*Aedes togoi*); however, due to a lack of convincing evidence, the role for mosquitoes in POWV maintenance in enzootic areas was questioned (71, 219, 220).

## Clinical Disease and Diagnosis

The majority of POWV infections are thought to remain unseen by health professionals due to the presumably high rate of subclinical infections (164). When POWV infection is symptomatic, patients may develop flu-like illness or encephalitis/meningoencephalitis; these are the most commonly seen manifestations and are the major indications for the need for laboratory testing. Specimens collected from patients with CNS involvement are more likely to be tested for POWV. For example, the U.S. CDC reported that between 2010 and 2015, 85% (annual range, 75% to 100%) of tested patients had encephalitis or meningitis, while 15% (annual range, 0% to 25%) were reported to present with nonneuroinvasive disease (148–153). These findings suggest that the majority of patients without CNS manifestations are not routinely tested for the virus, and thus, published reports might underestimate the incidence of nonneuroinvasive POWV disease.

Clinical characteristics (Table 2) of POWV infection have been comprehensively overviewed in previous publications (147, 221, 222). Typical POWV infections have a 7- to 34-day incubation period followed by a prodrome phase with nonspecific flu-like symptoms, such as fever, chills, malaise, generalized weakness, sore throat, headache, myalgia, lethargy, somnolence, dizziness, and gastrointestinal symptoms (nausea and vomiting), in the majority of patients. Headache and fever up to a temperature of 38.5°C to 41°C are reported by all patients (4, 145, 157). A fine erythematous morbilliform rash was observed in some cases (138, 223–225). The prodrome phase lasts approximately 1 to 3 days and precedes CNS manifestations. The proportion of patients who develop CNS infection and manifest CNS symptoms is unknown.

**TABLE 2** Clinical presentation of POWV infections[a]

| Phase of disease | Symptom(s) | Reference(s) |
|---|---|---|
| Prodrome | Fever | 4, 130, 137–139, 143, 147, 157, 161, 162, 221, 224, 226 |
| | Chills | 4, 137, 224 |
| | Headache | 4, 137, 139, 147, 157, 161, 162, 178, 221, 225, 226 |
| | Malaise | 137, 147, 161, 162 |
| | Sore throat | 147, 226 |
| | Lethargy | 138, 147, 157, 178, 221 |
| | Somnolence | 4, 147, 157, 161, 226 |
| | Dizziness | 130, 139, 147, 157, 221 |
| | Confusion | 4, 157, 178, 221, 225, 226 |
| | Slurred speech | 4, 139 |
| | Muscle/generalized weakness, fatigue | 4, 137, 157, 178, 225 |
| | Myalgia | 4, 147, 157, 224 |
| | Tremor | 130, 226 |
| | Anorexia | 4, 138, 161 |
| | Nausea | 137, 147, 157, 221 |
| | Vomiting | 4, 143, 147, 157, 161, 162, 178, 221 |
| | Diarrhea | 157, 221 |
| | Visual blurring, photophobia, diplopia | 4, 137, 157 |
| | Erythematous rash | 137, 138, 147, 157, 178, 221, 223–225 |
| Acute illness | Fever | 130, 137–139, 157, 161, 178, 221, 226 |
| | Headache | 130, 137, 178, 221, 224 |
| | Dizziness | 130, 178 |
| | Drowsiness | 130, 139, 157 |
| | Altered sensorium (lethargy, confusion, stupor, coma) | 4, 130, 138, 139, 147, 157, 161, 162, 178, 221, 224, 226 |
| | Generalized muscle weakness, myalgia, ataxia | 4, 137, 225, 226 |
| | Meningism/meningeal irritation (in children) | 130, 138, 147, 161, 162, 226, 227 |
| | Neck stiffness | 4, 130, 137, 138, 161, 162, 178 |
| | Seizure, convulsion (in children; rare in adults) | 138, 143, 147, 157, 161, 221, 226, 228, 229 |
| | Hemiplegia, quadriplegia, paralysis, facial palsy | 4, 130, 139, 147, 157, 161, 162, 178, 221, 224–227 |
| | Pyramidal tract signs | 130, 138, 139, 147, 157, 161 |
| | Tremor | 4, 130, 147, 157, 161, 178 |
| | Twitching | 4, 130, 139, 143, 147, 161 |
| | Spasticity, rigidity | 139, 157 |
| | Myelitis, radiculitis | 147, 221, 227, 232 |
| | Visual blurring, photophobia, diplopia | 4, 137, 226 |
| | Nystagmus | 130, 157, 221 |
| | Anorexia | 137 |
| | Dysarthria, dysphasia, aphasia | 139, 157, 178, 221 |
| | Ophthalmoplegia | 4, 221, 230 |
| | Irregular respiration, apnea, respiratory failure | 4, 130, 157, 161, 226, 231 |
| | Hallucination | 162, 224, 228 |
| | Acute flaccid paralysis | 232 |
| Sequelae in survivors | | |
| Short term | Apnea | 178 |
| | Psychosis | 147, 178 |
| | Drowsiness | 161 |
| | Altered sensorium | 137, 162 |
| | Dysarthria, mutism | 161, 178 |
| | Spasticity, myoclonus | 138, 161, 178 |
| Long term | Spasticity, rigidity | 137, 161, 226 |
| | Spastic hemiplegia, quadriplegia | 4, 138, 162, 178 |
| | Prolonged headache | 157 |
| | Cognitive deficit, mental retardation | 138, 157, 161, 178 |
| | Depression | 137, 226 |
| | Dysarthria, anarthria, aphasia | 157, 161, 178 |
| | Muscle weakness | 137, 138, 161, 162 |
| | Imbalance | 137, 157, 178 |
| | Ophthalmoplegia | 4, 178 |
| | Memory dysfunction | 4, 137, 161, 162 |
| | Limitation in activities of daily living | 4, 157, 178 |
| | Bed bound | 178 |
| | Ventilator dependence | 178 |

[a]As documented in case reports, case series analyses, and review articles. Data were derived from 44 patients (10 children and 34 adults).

Altered sensorium ranging from confusion and stupor to coma is a universal finding in patients. Many patients (mainly children) exhibit evidence of meningismus or meningeal irritation upon physical examination (130, 138, 161, 162, 226, 227). Although children are commonly observed to have seizures, which are rarely seen in adults, febrile seizures are more common in children and thus may not be due simply to POWV infection (130, 138, 143, 161, 162, 226–229). Severe neuroinvasive symptoms, such as hemiplegia or even quadriplegia, are commonly observed in patients of all ages. Also, paresis, tremors, twitching, focal palsies, and pyramidal tract signs may occur. Moreover, but less frequently, nystagmus, ophthalmoplegia, facial palsy, myelitis, hallucinations, and respiratory failure were reported. A case of acute flaccid paralysis with residual consequence was also recorded (130, 139, 161, 162, 227, 228, 230–232).

POWV infection is associated with a high case fatality rate in patients with CNS disease and a high incidence of severe long-term sequelae (4, 130, 139, 178, 233). In one report, 12 of 14 patients (85%) with neuroinvasive POWV infection were admitted to the intensive care unit, and 5 patients (35%; all >60 years of age) died (178). Combining data from multiple case reports, for patients with POWV neuroinvasive illness, an ~10 to 15% case fatality rate is estimated; however, this might be a conservative estimate, as extended follow-up is performed for only a portion of patients (136, 139, 178). Where data on outcomes have been described, either short-term or long-term significant neurological deficits were observed in about 75% of cases. Apnea, dysarthria, psychosis, and spasticity were recorded as short-term sequelae. Long-term sequelae include prolonged headache, cognitive deficit, significant limitation in activities of daily living, bed-bound state, spastic hemiplegia and quadriplegia, hemiparesis, mental retardation, aphasia, hearing impairment, muscle weakness, imbalance, ventilator dependence, ophthalmoplegia, and memory dysfunction (Table 3) (4, 145, 147, 178).

General laboratory findings showed mild thrombocytopenia, lymphocytic pleocytosis (with fewer than 500 white blood cells per $\mu$l cerebrospinal fluid [CSF]), and elevated protein levels in the CSF in a majority of patients with CNS involvement. Neuroimaging findings and histopathological examinations helped to understand the background of clinical symptoms. In patients with POWV meningoencephalitis and encephalitis, magnetic resonance imaging (MRI) demonstrated deep foci of increased T2/FLAIR (T2-weighted fluid-attenuated inversion recovery) signal intensity (157). T2-hyperintense foci predominantly affected the gray matter, and microvascular ischemic changes were predominantly found throughout the brain. In 13 hospitalized patients with POWV encephalitis, cerebral cortex, basal ganglia, brain stem, cerebellum, thalamus, and meninges, all dominantly on the left side, were the affected brain areas (Table 3) (178, 221). Histological examination of brain samples obtained at autopsy revealed changes characteristic of acute meningoencephalitis. Histopathological analysis showed reactive gliosis, increased numbers of microglial cells, and necrotizing inflammation with a lymphocytic infiltrate, predominantly affecting the gray matter (139, 178).

Because the geographic distribution of POWV overlaps those of a variety of encephalitogenic viruses and bacteria in the Americas and Russia, and due to the wide range of clinical manifestations, a broad differential diagnosis is warranted. Depending on the geographic location of exposure, differential diagnosis should take into account TBEV, WNV, Saint Louis encephalitis virus (SLEV), or herpes simplex virus (HSV) encephalitis; bacterial meningitis; Lyme disease; anaplasmosis; ehrlichiosis; and tick-borne relapsing fever. Coinfections further complicate the situation and may contribute significantly to patient morbidity and mortality from tick-borne infections due to diagnostic difficulties and inadequate treatment (167). Despite the dramatic consequences for disease management, little is known about the prevalence of tick-borne pathogen coinfections in patients with POWV disease. One study reported the occurrence of *Anaplasma phagocytophilum* coinfection in one out of three patients with POWV encephalitis (137). In another study, among 41 patients with evidence of *Borrelia burgdorferi* infection, 7 (17.1%) showed serological evidence of acute POWV infection, and 3 (7.3%) had laboratory-confirmed POWV infection (167). In a case series analysis, which processed data from 14 patients hospitalized with POWV encephalitis, 2 patients (14.2%) with

**TABLE 3** Prevalence of clinical symptoms, radiography findings, and neurological deficits among patients hospitalized with POWV neuroinvasive illness between 2004 and 2012 in New York[a]

| Finding | No. (%) of cases |
|---|---|
| Sign or symptom | |
| Neurological | |
| Fever | 14 (100) |
| Generalized weakness | 12 (86) |
| Lethargy | 10 (72) |
| Confusion | 8 (57) |
| Seizure | 6 (43) |
| Vomiting | 5 (36) |
| Focal deficit | 5 (36) |
| Neck stiffness | 5 (36) |
| Aphasia | 3 (21) |
| Tremor | 2 (14) |
| Dizziness | 2 (14) |
| Dysarthria | 1 (7) |
| Balance disturbances | 1 (7) |
| Myoclonus | 1 (7) |
| Nonneurological | |
| Rash | 6 (43) |
| Dyspnea | 2 (14) |
| Abdominal pain | 3 (21) |
| Body aches | 3 (21) |
| Diarrhea | 1 (7) |
| Dysuria | 1 (7) |
| Rhinorrhea | 1 (7) |
| Brain area affected | |
| Region | |
| Cerebral cortex | 7 (54) |
| Basal ganglia | 7 (54) |
| Brain stem | 4 (31) |
| Cerebellum | 3 (23) |
| Thalamus | 3 (23) |
| Meninges | 2 (15) |
| Side | |
| Left | 9 (69) |
| Right | 2 (15) |
| Bilateral | 2 (15) |
| Deficits at hospital discharge | |
| Significant limitation in ADL ($n = 13$) | 11 (85) |
| Cognitive deficit ($n = 11$) | 6 (55) |
| Bed bound ($n = 13$) | 7 (54) |
| Focal deficit ($n = 10$) | 4 (40) |
| Quadriplegia ($n = 9$) | 3 (33) |
| Ventilator dependence ($n = 11$) | 3 (27) |
| Aphasia ($n = 11$) | 3 (27) |
| Imbalance ($n = 11$) | 2 (18) |
| Headache ($n = 11$) | 2 (18) |
| Ophthalmoplegia ($n = 9$) | 1 (11) |

[a]Data are from reference 178. ADL, activities of daily living.

erythema migrans had detectable antibodies to *Borrelia burgdorferi* (178). These findings are in agreement with vector surveillance data that showed that ticks infected with POWV may carry other tick-borne pathogens simultaneously. Rates of detection of such coinfections in *Ixodes scapularis* ticks ranged from 1.1% to 3.4% in Connecticut and New York, respectively; in these studies, the ticks, which carried lineage II POWV, carried either *Borrelia burgdorferi*, *Ehrlichia chaffeensis*, *Anaplasma phagocytophilum*, or a combination thereof (234, 235).

The case definition for POWV-associated neuroinvasive infection includes clinical criteria (fever of >38°C with any peripheral or central nervous system dysfunction) and at least one of the following laboratory findings: (i) direct detection of POWV in tissue,

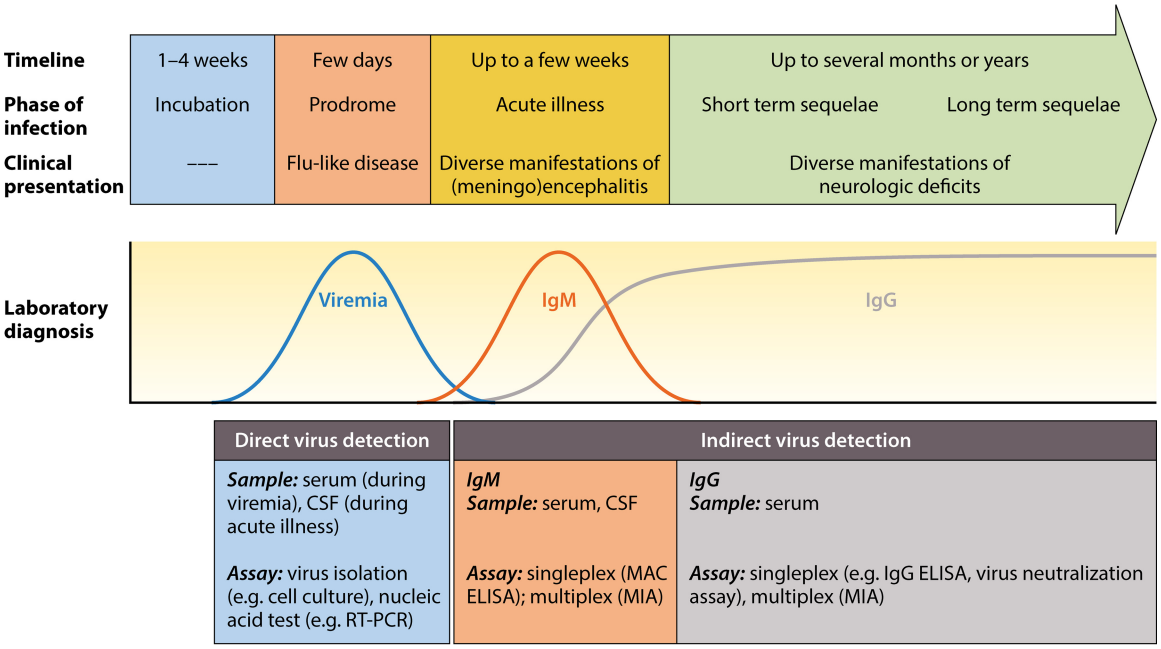


**FIG 6** Clinical course and laboratory diagnosis of POWV infections. The timeline aligned with the phase of infection and typical clinical manifestations is shown in the top panel, whereas laboratory diagnostic opportunities relative to the timeline of clinical disease are shown in the middle and bottom panels.

blood, or CSF by virus culture or a molecular detection method; (ii) a 4-fold rise of POWV-specific antibody titers in paired serum samples; (iii) the presence of POWV-specific IgM in serum and the presence of POWV-neutralizing antibodies in the same specimen or a specimen collected during convalescence; and (iv) the presence of POWV-specific IgM in CSF and the simultaneous absence of IgM for other arboviruses endemic to the region where exposure occurred (https://wwwn.cdc.gov/nndss/conditions/powassan-encephalitis-meningitis/case-definition/2001/) (Fig. 6). Commercial diagnostic tests for POWV serology are not available for use in hospital laboratories, and although POWV-specific molecular detection methods have been marketed, these tests are recommended currently for research use only. In the United States, until recently, laboratory diagnosis of POWV has been carried out exclusively in specialized diagnostic laboratories, including state health laboratories and the CDC, where multiple diagnostic assays (including serology, virus isolation, and RT-PCR) were developed and validated (236–238). More recently, a POWV serology-based diagnostic panel has been developed at a private commercial laboratory (239).

The gold standard for laboratory diagnosis of POWV infection remains serology despite the fact that it may take several weeks to obtain adequate results by this approach due to the need for paired clinical specimens. A positive IgM test via an immunofluorescence assay (IFA), IgM antibody capture enzyme-linked immunosorbent assay (MAC-ELISA), or a microsphere-based immunoassay (MIA), with confirmation using plaque reduction neutralization testing (PRNT), is the primary choice for laboratory diagnosis. However, PRNT requires infectious viruses and an adequate biosafety level for handling infectious POWV, and the sensitivity of PRNT may be inferior to the sensitivity of other serological assays. Commercial serology tests specific for another flavivirus (e.g., ELISAs that are available for WNV and SLEV diagnoses) may give a positive test result with sera collected from POWV-infected patients due to cross-reactivity among flavivirus antigen epitopes (240, 241); importantly, IgM cross-reactivity can occur for other non-flavivirus-associated conditions, such as lupus or Epstein-Barr virus infection. In recent years, ELISAs and IFAs have been replaced by more-convenient medium- to high-throughput, multiplexed microsphere-based immunoassays (MIAs)

that may target numerous North American arboviruses (including POWV) in a single test run to detect either IgM or IgG antibodies in CSF or sera (238).

Immunohistochemistry and virus culture from autopsy tissues can be useful and are indicative of active virus replication in the examined tissue sample. Keeping in mind that short-duration viremia may limit the sensitivity of direct virus detection, POWV can be isolated from the blood during the prodrome phase or later from the CSF when CNS manifestations develop. For the purpose of virus isolation, several cell lines are available, but suckling laboratory mice can also be used (130). Permissive cell lines include, but are not limited to, pig (PKE)-, monkey (Vero; LLC-MK2)-, and baby hamster (BHK21)-origin kidney cells and the human lung epithelial cell line A549 (127, 134, 180, 242). Although a cytopathogenic effect upon tissue culture may not be evident in some cultured cells, *ex vivo* culturing may be chosen to increase the virus titer for a subsequent *in vitro* detection method (134, 180, 242).

Nucleic acid amplification methods are increasingly becoming routine in virological diagnosis. Similarly to the virus isolation method, POWV RNA can be detected in brain tissue samples upon postmortem examination and, at various success rates, in CSF and blood samples collected during the prodrome phase and in CSF during the acute phase of illness (177, 224). The most widely used assay format is RT-PCR or RT combined with heminested PCR that preferentially targets either the E or the NS5 protein-coding genomic region; assays targeting the genomic NCRs have also been developed. Pan-flavivirus PCR-based detection has been reported to detect a variety of viruses in the *Flavivirus* genus, and some of these broad-range RT-PCR assays have been used to detect POWV from CSF and autopsy tissue samples (172, 243–248). Because POWV is sympatric with other flaviviruses in both North America and the Russian Far East, RT-PCR results obtained by a broad-range assay need to be confirmed. Confirmation of PCR-based amplification can be accomplished by sequencing or mass spectrometry of the amplified fragment or inclusion of virus-specific probes in a quantitative assay (142, 224, 249, 250). Multiplexing of molecular detection methods to potentially identify multiple targets simultaneously is an approach to reduce the assay cost while trying to keep the clinical sensitivity for each target; therefore, molecular diagnostic methods with increased multiplexity are being continuously developed. These assays may rely on expensive laboratory infrastructure and may require some special skills (including staff bioinformaticians).

In microsphere-based molecular detection assays, virus-specific probes are captured onto the surface of beads, and the number of targets may be flexibly chosen based on diagnostic needs, although the number of targets to be detected simultaneously is usually no more than a few dozen. For example, a commercial molecular diagnostic panel can identify 21 different tick-borne protozoal, bacterial, and viral pathogens, including POWV (Luminex). High-density panviral and panmicrobial DNA probe-based solid-phase microarrays are known to target hundreds to thousands of viral pathogens or a mixture of viral, bacterial, and fungal pathogens and parasites of vertebrates, respectively. Some of these assays were reported to include POWV-specific capture DNA probes (251–254). Also, a more recent advancement in panvirus detection methods is the combination of viral metagenomics with high-throughput next-generation sequencing (NGS). This approach has led to the discovery of numerous novel viruses but has limited value in clinical settings due to the low sensitivity. This low sensitivity was recently demonstrated for a patient with encephalitis of an unknown etiology, whose CSF sample, collected during the acute phase, was found to contain only 10 POWV (lineage II)-specific sequence reads out of 2.4 million total sequence reads generated for the sample (225). Targeted enrichment of possible viral targets by using a mixture of virus-specific capture oligonucleotides has been demonstrated to increase the sensitivity of NGS-based detection of numerous medically important viruses from clinical specimens (255). This approach is, theoretically, able to detect all currently known viruses; however, it lacks the unbiased feature of viral metagenomics and may be specific for viruses targeted based on known genetic sequence information and some others that are very closely related to the targeted viruses. Despite the availability

of these emerging new technologies, the clinical sensitivity of the most promising high-throughput methods awaits formal demonstration for a majority of targets, including the two lineages of POWV.

## CONCLUSIONS AND PERSPECTIVES

POWV was discovered nearly 60 years ago, and research efforts continue to focus on understanding clinical disease and pathology, ecology, epidemiology, and viral genetic diversity. The development of sensitive and highly specific laboratory detection methods was beneficial to achieve progress in these research areas. However, serological assays suitable for routine laboratory use in hospitals are not commercially available, which hampers diagnosis in settings where these tests are most needed. Given that POWV can be cotransmitted with various human-pathogenic microbes and viruses, utilization of diagnostic panels that target multiple tick-borne pathogens most typical in an area of endemicity could be useful, with the added benefits to better understand disease outcomes during mixed infections and to consider choosing the most adequate treatment options.

The number of case reports of POWV illness sharply increased over the past decade in the United States, from $<1$ case per year pre-2000s to 10 cases per year since the mid-2000s. Based on these findings, some researchers consider POWV an emerging threat in North America, and research investigations of this once neglected virus infection gained new momentum in the past few years. From a medical perspective, new research data that indicate an expanding range for *Ixodes scapularis*, a major arthropod vector that aggressively attacks humans and transmits lineage II POWV, seem to be relevant, a finding based upon which an increased POWV prevalence in the United States is anticipated. Continued virus monitoring and disease surveillance will certainly help to establish if this expectation holds true over an extended period. Epidemiological surveillance will require support from enhanced field and laboratory studies to explore other possible hosts and vectors whose distributions overlap those of known POWV hosts and vectors and to assess the public health risk potentially posed by these additional species.

Despite the low public health burden of POWV infections currently, the considerable case fatality ratio and the high risk for short- or long-term sequelae characterized by various motor, sensory, and cognitive disfunctions in affected persons justify implementation of more research efforts to help understand disease pathogenesis and immunity and to explore new options in prevention and therapy. Prevention focuses on the avoidance of tick infestations by using personal protective measures and tick control. Vaccines against POWV are not available for use, and in light of the low disease incidence, it is questionable if vaccines would be cost-effective. Therapy for patients with POWV illness is palliative. Some patients with POWV neuroinvasive illness who were administered intravenous immunoglobulin or high-dose corticosteroids survived; however, whether these treatments have any beneficial effect on favorable disease outcomes remains unclear and needs additional confirmatory data. Antiviral treatment is not available, and data on the susceptibility of POWV to various antiviral drugs are scant. The lack of comprehensive information on antiviral approaches against POWV is somewhat inexplicable despite the growing database of antiviral drugs that act directly on flaviviral gene products and may be effective against these viruses. As an alternative, the emerging field of antiviral treatment using drugs that target host proteins required for various processes of the flavivirus cellular life cycle may also be better explored. Contrary to conventional antiviral drugs, whose routine use could readily select drug-resistant viral clones, an approach that targets cellular components required for the flavivirus life cycle may have the added advantage that resistance is less likely to develop given that host cell targets tend to evolve very slowly.

## ACKNOWLEDGMENTS

We thank Kornélia Kurucz and György Szűcs for their assistance in preparation of illustrations. We are indebted to György Szűcs and Ferenc Jakab for the fruitful scientific

discussions in relation to selected sections of the manuscript, and we appreciate the help of Ádám Tóth and Jon Gentsch for critical reading and editing of the text.

G.K. was supported by the University of Pécs in the frame of the Viral Pathogenesis Talent Centre program and also by the European Union, co-financed by European Social Fund grant no. EFOP-3.6.1.-16-2016-00004, entitled "Comprehensive Development for Implementing Smart Specialization Strategies at the University of Pécs." K.B. was supported by the Momentum program. The funders had no role in collection or interpretation of data or the decision to submit the work for publication. Furthermore, there are no financial or other relationships that might lead to a conflict of interest.

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