



Clinical features and laboratory diagnosis of emerging arthropod-transmitted viruses

A Report from the Pan American Society for Clinical Virology Clinical Practice Committee

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ARTICLE INFO

Keywords:

Eastern Equine Encephalitis
Chikungunya
West Nile
Powassan
Arbovirus
Arthropod-borne

ABSTRACT

Arthropod-borne viruses (arboviruses) are an increasing global threat due to their ability to cause human disease and their expanding geographical distribution. They circulate in nature between arthropod vectors and vertebrate hosts. Infection of susceptible human hosts leads to harmful developmental and neurological manifestations. Arboviruses have caused recent outbreaks with significant public health implications, such as the Zika virus outbreak in the western hemisphere which caused fetal abnormalities in some infected pregnant women, or Eastern Equine Encephalitis which caused 15 deaths in 2019. This review discusses several arboviral infections and their clinical manifestations while highlighting the importance of laboratory diagnostics to detect infections and current attempts at vaccine development. The ability to accurately diagnose an arbovirus infection is critical for initiating a timely response to infections in order to improve patient outcomes.

1. Introduction

Arboviral diseases are spread through the bite of arthropods such as mosquitoes and ticks and are rising in incidence and expanding in geographical distribution [1]. Mosquito-borne diseases historically thought of as tropical or subtropical such as Dengue, Chikungunya, West Nile, and Zika virus, have expanded their territory [1]. Many tick species are also expanding their range and exhibiting high population growth including *Ixodes scapularis*, the tick species that carries the recently identified Powassan virus [2]. Many factors are involved in these epidemiological changes such as adaptation to new vector species, human migration and travel patterns, and climate change [1]. Diseases such as Chikungunya and Zika virus are now being identified in the Americas and in other temperate regions due to adaption to the widely distributed *Aedes albopictus* mosquito species [3,4]. A 2016 outbreak of Zika virus, an emerging arboviral pathogen which can cause fetal abnormalities like microcephaly when acquired during pregnancy, resulted in the publication of detailed testing guidance from the CDC [5,6]. Likewise, testing guidance for Dengue virus, a disease spread by *Aedes*

mosquitoes, has also been published by the CDC [7]. In this review, we will discuss four emerging arboviral threats for which diagnostic algorithms are still unclear including disease description, epidemiology, geography, and recommendations for laboratory diagnosis.

1.1. Eastern Equine Encephalitis

Eastern Equine Encephalitis Virus (EEEV) is an alphavirus in the family *Togaviridae* which has a single-stranded positive sense RNA genome [8,11]. The incubation period is 4–10 days and initial symptoms are non-specific and flu-like, including fever, chills, malaise, myalgia, and arthralgia [8]. This initial systemic illness can persist for 1–2 weeks, followed by complete recovery for most patients. Neuroinvasive disease may begin after several days of systemic illness in children and adults but occurs soon after symptom onset in infants [8,11]. Symptoms include fever, headache, nausea, vomiting, weakness, and neck stiffness [11]. In one study, up to 25 % of patients developed seizures, 89 % became comatose, and a median of 18 days of hospitalization was reported [11]. Eastern Equine Encephalitis is considered to be the most

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<https://doi.org/10.1016/j.jcv.2020.104651>

Received 17 June 2020; Received in revised form 11 September 2020; Accepted 27 September 2020

Available online 30 September 2020

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fatal arboviral disease, as approximately one-third of patients die, typically within 2–10 days of symptom onset. Many of those who survive have long-term neurological sequelae.

EEEV was first discovered in 1933, and the first large outbreak was in Massachusetts in 1938 and resulted in 34 cases of encephalitis and 25 deaths. Currently, cases of EEEV are most commonly reported from Florida, Massachusetts, New York, and North Carolina [8]. From 2009–2018, 3–15 cases of neuroinvasive EEEV were reported to the CDC annually [8]. There was a sharp increase in cases in 2019, with nine states reporting human cases of which 14 were fatal [10]. While neuroinvasive cases of EEEV are reportable, only 4–5 % of EEEV infections result in Eastern Equine Encephalitis, so transmission to humans occurs more frequently but remains limited [10]. This is due to the need for bridging vectors as there is no direct transmission from equine to human [8]. There is concern that if EEEV were to adapt to allow efficient transmission by *Aedes aegypti*, human cases and fatalities would increase [10]. EEEV is maintained in endemic areas through a cycle between *Culiseta melanura* mosquitoes and songbirds, predominantly in freshwater hardwood swamps. *C. melanura* rarely feed on humans, so transmission to humans predominantly occurs through bridging vectors like *Aedes*, *Coquillettidia*, or *Culex* mosquitoes with both horses and humans being dead-end hosts [8]. Transmission via organ donation has also been reported [9]. While anyone living in or visiting endemic areas are at risk of infection, people who work outdoors or do recreational activities around hardwood swamps are at greatest risk [8].

Diagnosis of EEEV can be very challenging. Analysis of cerebrospinal fluid shows pleocytosis in 97 % of patients, elevated protein in 94 % of patients, and elevated red blood cells in 77 % of patients [11]. While there are no FDA-approved tests for EEEV, serologic testing for serum and CSF is available at multiple reference labs, through state health departments, and the CDC [8]. IgG and IgM testing is typically performed via indirect immunofluorescence or ELISA, with positive results confirmed with a plaque reduction neutralization assay (PRNT) due to potential cross-reactivity with other alphaviruses. Serologic testing may be negative during the first week of infection [10]. Nucleic acid testing may be positive during this time frame but is not the preferred method of diagnosis as it is unusual to detect the virus (either by antigen or nucleic acid testing) in the blood or CSF, and is only performed through state public health departments and the CDC [12].

1.2. Powassan virus

Powassan virus is a member of genus *Flavivirus* and family *Flaviviridae* and has a positive sense RNA genome [13]. Powassan virus has an incubation period of 1–5 weeks and typically presents with a prodromal illness of approximately one week characterized by fever, headache, vomiting, and generalized weakness [13,14]. Neuroinvasive disease such as encephalitis, aseptic meningitis, or meningoencephalitis can develop and last for weeks to months with patients exhibiting vomiting, fever, seizures, and difficulty breathing, speaking, or moving [13,14]. Approximately 10 % of neuroinvasive Powassan virus disease is fatal [13]. Up to 50 % of survivors will have long-lasting neurological sequelae, which can range from headaches to cognitive disability [13, 14]. It is transmitted to humans via the bite of the *Ixodes scapularis* tick, which can also transmit *Borrelia burgdorferi*, *Anaplasma*, and *Babesia* [14]. From 2009–2015, the number of cases of Powassan virus reported to the CDC ranged from 6 to 12. This increased to 21–33 cases per year in 2016–2018, with a peak in 2017 [14]. The geographical foci of Powassan virus is similar to other diseases transmitted by *Ixodes scapularis*, with cases primarily in the Northeast Great Lakes regions of the United States. From 2009–2018, the states with the most reported cases of Powassan virus were Minnesota (34), Wisconsin (25), Massachusetts (22), and New York (19) [14]. No other state reported more than 8 cases in this time period [14].

Testing options remain limited as there are no FDA approved assays for Powassan virus and no targeted testing is done outside of public

health laboratories. Analysis of cerebrospinal fluid from patients with neuroinvasive disease typically shows pleocytosis with lymphocytic predominance, though neutrophils can predominate early in disease [14]. During the prodromal phase of illness, nucleic acid testing, which is offered at CDC and some state public health labs, is the test of choice, though a negative result does not rule out Powassan virus infection [13, 14]. PCR can be performed on serum, cerebrospinal fluid, or tissue [14]. Immunohistochemistry stains are also available for tissue specimens [14]. Once neurologic symptoms begin, serologic testing should be used which is also available at CDC and some state public health labs [13,14]. An IgM capture ELISA is performed initially, and, if positive, the result is confirmed with PRNT [14]. Negative serology results have been reported in a patient taking rituximab who was later confirmed to have Powassan virus, so results from immunosuppressed patients should be interpreted with caution [15].

1.3. Chikungunya virus

Chikungunya virus is a Togavirus in the Alphavirus genus with a single-stranded positive sense RNA genome [3,16]. Three genotypes, the West African, East/Central/South African, and Asian lineage, have been identified. Although the historical mortality rate is low and the infection self-limiting, it causes a crippling disease characterized by rapid onset of fever, musculoskeletal inflammation, polyarthralgia, myalgia, rash, and headache [4,17]. It is transmitted to humans through the bite of infected *Aedes* mosquitoes as the primary viral vector [17]. Maternal transmission also occurs with the highest risk to the infant when the mother is viremic at the time of delivery [4,18].

Although the earliest known case of Chikungunya was during a 1952 Tanzanian outbreak, it was likely present earlier but misdiagnosed as Dengue fever [4,16,19]. In the acute phase, the diseases very closely resemble one another, but Chikungunya can cause a long term reoccurring rheumatism (months to years after infection) [3,4]. The virus is endemic in tropical regions of sub-Saharan Africa and Southeast Asia with non-human primates serving as the primary reservoir in an enzootic transmission cycle. Since its identification, outbreaks of Chikungunya have occurred sporadically in Africa and Asia, but there have been recent outbreaks in the Caribbean and the Americas [4,16,19]. This is due to viral spread through *Aedes albopictus*, a widely distributed mosquito species found in temperate regions including the eastern and southern United States. Mutations in the viral genome (E1-A226 V mutation) have improved adaption to the *albopictus* mosquito species, which likely exacerbated a 2004 outbreak from Kenya to the Indian Ocean Islands [19]. *Aedes aegypti* and *albopictus* mosquitoes, which require low amounts of water for egg laying, have also been shown to cause urban transmission outbreaks in areas such as Southeast Asia with human-mosquito-human transmission cycle. There is no vaccine or specific antiviral regimen for Chikungunya, and treatment is limited to supportive care and pain relief with anti-inflammatory drugs.

Since its identification in 1952, the virus has been spreading rapidly and has now caused outbreaks in at least 40 countries [20]. In the United States, average case counts are low (in the low to mid one-hundreds) and are mostly attributed to importation from travelers, but globally, incidence is increasing [20,21]. The 2004 Kenya outbreak totaled almost a half million cases with seroprevalence between 70–75 % as the outbreak spread to surrounding areas into 2005 [21]. Another significant outbreak occurred in the islands of French Guiana and the Caribbean between 2013–2014 where case counts totaled 2.9 million with 296 deaths [21]. Although typically described as having a low mortality rate, deaths due to Chikungunya have been increasing since 2005 as more severe disease forms have been described [22].

Testing can be considered for patients experiencing polyarthralgia with acute fever onset who have traveled to or live in endemic regions or areas with current or recent outbreaks. Testing is available through CDC, several state health departments, and some reference labs and may include serological IgM/IgG testing, RT-PCR, and viral culture of serum

or plasma [22]. Viral culture is available through the CDC under BSL-3 conditions but is only appropriate in the first 3 days of illness [22]. BSL-3 conditions are required because although rare, laboratory transmission is possible via aerosol, exposure directly or indirectly through broken skin, or splash into mucous membranes [23]. RT-PCR can detect viral RNA in serum in approximately the first 8 days of illness. Virus-specific antibodies begin to form towards the end of week one of illness and serological testing is available for both IgM and IgG. If a patient initially tests negative for serologic testing, a convalescent sample should be obtained and tested to rule out chikungunya infection [22]. Serum samples collected less than 6 days after illness onset should be tested using RT-PCR, whereas samples collected greater than or equal to 6 days after illness onset should be tested using serological (ELISA) testing [22]. Positive results should be confirmed using PRNT before reporting. Positive results by ELISA that do not confirm by PRNT are considered negative for Chikungunya virus and have non-specific reactivity [24].

1.4. West Nile Virus

West Nile Virus (WNV) is a neurotropic mosquito-borne virus in the Flaviviridae family [25]. It has a positive sense single-stranded genome and is divided into 5 distinct phylogenetic lineages [32]. Lineage 1 is the most geographically widespread and associated with outbreaks of neurological disease. Lineage 2 strains have emerged in Europe and have been responsible for WNV outbreaks [33]. Less is known about lineages 3, 4, and 5 [34]. Human WNV infection incubation period ranges from 2 to 14 days [35]. However, approximately 80 % of human infections are asymptomatic [41]. Symptomatic patients present with self-limiting West Nile Fever characterized by acute onset of fever, headache, malaise, back pain, myalgia, and weakness. Eye pain, pharyngitis, and gastrointestinal symptoms can also occur. A transient rash that predominates the torso and extremities has also been reported. Some patients have experienced a more severe illness without neurological manifestations but sought medical attention due to difficulty concentrating and neck stiffness, along with fatigue and malaise that lasted for approximately one month [42]. Less than 1% of persons infected with WNV develop neuroinvasive disease [41,43]. Neuroinvasive disease manifests as meningitis, encephalitis or acute flaccid paralysis. Risk for encephalitis increases with age and for immunocompromised organ transplant patients. WNV encephalitis clinical severity ranges from mild to severe encephalopathy, coma and death.

WNV cycles between *Culex* spp. mosquitoes and birds in nature, with birds being the natural host [26]. It was first isolated from blood of a febrile patient in Uganda in 1937 and introduced into the United States in 1999 [27,28]. WNV spread rapidly throughout the western hemisphere and is now the leading cause of encephalitis in the United States [29]. Globally, WNV is found throughout Africa, the Middle East, Europe, Asia and North America. Infection incidence increases from early summer to September and in the United States, WNV incidence varies from state-to-state suggesting that ecological factors are important for transmission [30,31]. Successful spread of WNV depends on *Culex* spp. mosquitoes feeding on viremic birds, allowing WNV to replicate within the mosquito. Other routes of infection include blood transfusion [35], organ transplantation [36], breast milk, and conjunctival exposure to laboratory workers [37]. To date, no human-to-human transmission of WNV through casual contact has been documented. In the United States, *Culex* spp. have been responsible for WNV transmission to humans and other mammals such as horses [38,39]. However, humans and horses are considered dead-end hosts because the levels of viremia in these hosts do not meet the threshold required to infect mosquitoes [40].

The most common method for laboratory diagnosis of WNV infection in patients with symptom onset greater than eight days is the detection of WNV-specific IgM and/or IgG antibodies in serum or CSF. Testing using Enzyme Immunoassays (EIAs) or enzyme linked immunosorbent assays (ELISAs) using commercially available kits, submitting to a

reference laboratory, or through the state public health laboratories is available. IgM antibodies against WNV are typically detected in serum and CSF within 8–10 days after disease while IgG can be detected after 10 days [41]. Microsphere immunoassays (MIAs) have been developed to detect WNV antibodies with the ability to multiplex against other arboviruses [44]. A PRNT is used as a confirmatory test after testing positive with EIA, MIA or ELISAs. If the patient is less than eight days from symptom onset, other methods such as RT-PCR and viral culture can be used for CSF, serum or tissue [45]. These methods are only appropriate for acute infection and testing is available through state public health laboratories, CDC, and certain reference laboratories [46]. A negative result would not rule out infection due to timing considerations.

2. Future directions

2.1. Diagnosis through metagenomic sequencing

Next generation metagenomic sequencing is being increasingly utilized as a diagnostic tool, particularly for patients who test negative for common pathogens using conventional methods. Arboviruses are ideal candidates for detection by metagenomic sequencing as clinical presentation can be non-specific. Various examples of the use of this powerful technology have been recently published. Metagenomic sequencing of serum samples was used to identify Zika/Chikungunya virus co-infections during the 2015–2016 Zika outbreak in Brazil [47] and neuroinvasive Chikungunya virus during a Chikungunya outbreak in Bangladesh [48]. West Nile Virus in a 14 year old renal transplant patient was diagnosed using metagenomic sequencing of cerebrospinal fluid which was later confirmed by serology testing of convalescent samples [49]. Powassan virus RNA has also been detected by metagenomic sequencing of cerebrospinal fluid in a 61 year old man who had recently reported multiple tick bites [50]. As reports of using metagenomic sequencing to detect arboviral infections are limited, more data is necessary to determine sensitivity of sequencing as compared to traditional PCR assays. Additionally, as PCR is typically only able to detect viral nucleic acids during early disease, metagenomic sequencing will likely be most useful for specimens collected during similar time periods in acute illness. The Food and Drug Administration has not approved any next generation metagenomics platform for arbovirus identification. Due to the high cost and general unavailability of metagenomic sequencing assays, this technology would most likely be useful when conventional methods have ruled out organisms that cause the clinical manifestations in a patient with ongoing symptoms. However, if the technology becomes readily available and cost improves, this could prove a useful front-line tool due to the diversity of organisms that can cause similar clinical presentations.

2.2. Vaccine development

Many candidate vaccines for the arboviruses discussed in this review have reached human testing, though none have entered phase 3 clinical trials. A total of twelve vaccines against Chikungunya virus have reached human trials [51]. Early designs were inactivated vaccines and were primarily developed by the United States Military. More recent vaccines have been virus-like particles, Chikungunya/Measles virus chimeric vaccines, or live attenuated vaccines. Subunit vaccines and DNA vaccines have also been developed, though these have not yet entered clinical trials. At least six West Nile Virus vaccines have entered clinical trials and have successfully resulted in the production of neutralizing antibodies [52]. Despite this promising data, there is concern that immunity derived through West Nile Virus vaccination may hinder diagnosis of other flaviviruses such as Dengue or Zika viruses, as antibodies typically cross-react in serological assays. Additionally, cross-reactive antibodies may exacerbate diseases caused by other flaviviruses through antibody dependent enhancement, which

means that any vaccine candidate for West Nile Virus epitopes must not be conserved among flaviviruses. Although an effective equine vaccine is available for Eastern Equine Encephalitis, a human vaccine is not yet available. The United States Army began development of an inactivated vaccine against EEEV in 2008. Phase 2 clinical trials began in 2016 and were completed in 2018, though results are not yet available. No vaccine for Powassan virus has reached clinical trials.

3. Conclusion

As the geographical range of arboviral vectors continues to expand, the geographic ranges of the diseases they carry will expand as well. Up-to-date knowledge of which arboviruses are circulating in a specific region will be critical to ensure they are properly included in differential diagnoses. As choice of test can be critical in different disease stages, laboratorians are advised to work closely with clinical care providers to guide appropriate test selection and result interpretation. This review provides the most current diagnostic recommendations as of publication, but as new technologies such as metagenomic sequencing or multiplex RT-PCR become available, it is critical that algorithms for diagnosis be revised accordingly. This will aide laboratorians to rapidly and accurately identify these viral pathogens to help lessen the severe diseases associated with arboviruses.

4. Disclosures/disclaimers

This Report is a product of the PASCV Clinical Practice Committee (CPC) developed to provide guidance and recommendations to health-care professionals considering implementation of CLIA-waived molecular tests. It is not clinical advice and does not represent all possible approaches to implementation. The 2018–2020 PASCV CPC included N. Esther Babady (Chair), James J. Dunn, Roberta Madej, Omar Perez, Eleanor Powell and Meghan Starolis.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgments

The authors thanks Dr. James J. Dunn, Dr. Jane Hata, Dr. Roberta Madej and Dr. Esther Babady for thoughtful critique and review of this document.

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