Laboratory Guidelines for the Detection and Diagnosis of Western Equine Encephalitis Virus Human Infection

Updated, February 5, 2024 [1]

Context and general considerations

Western Equine Encephalitis is caused by the virus of the same name (WEEV), a member of the *Togaviridae* family, *Alphavirus* genus. Alphaviruses are widely distributed across all continents and are classified into New and Old World alphaviruses according to the first described endemic areas. The two groups are generally associated with notable differences in human infection pathogenesis. Old World alphaviruses such as chikungunya (CHIKV), O'Nyong-Nyong (ONNV), Ross River (RRV), Semliki Forest (SFV) and Sindbis (SINV) viruses primarily cause febrile illnesses with arthritic syndromes. On the other hand, New World alphaviruses like WEEV, Eastern Equine Encephalitis virus (EEEV) and Venezuelan Equine Encephalitis virus (VEEV) generally cause encephalitis in equines, humans and other mammals. Mayaro virus (MAYV), a New World arthralgic alphavirus, is an exception.

WEEV mainly circulates in the western regions of Canada and the United States and in the southern cone. In Argentina, outbreaks in equines, associated with human cases were identified in 1972/73 and 1982/1983. Since late November 2023, an intense circulation of WEEV has been observed with a significant number of outbreaks in horses in Argentina and Uruguay, and the detection of human cases in these two countries (*1, 2*). In addition, a case has also recently been reported in a horse in Brazil (*3*).

Host, vector and life cycle

WEEV is maintained in a primary enzootic cycle among its natural vertebrate hosts, birds, and mosquitoes, particularly *Culex tarsalis.* A secondary cycle has also been described involving lagomorph mammals and *Aedes melanimon* mosquitoes. Other reservoir (rodents, bats, reptiles) and mosquito species (*Aedes albifasciatus*, as well as *Culex ocossa*, *Psorophora pallescens* and *Anopheles albitarsis*) could potentially contribute to the life cycle, particularly in the southern cone. The involved vectors can also infect equines and humans which are dead-end hosts that do not develop sufficient viremia to infect mosquitoes and maintain the cycle.

Clinical presentation

The incubation period of the disease is 2 to 10 days. WEEV infection in equines and humans can be asymptomatic. Symptomatic infections are rare, but can be severe, causing conditions such as aseptic meningitis and encephalitis, and leave sequelae. Mortality is estimated at approximately 15-20% and 3-4% in equines and humans, respectively.

[1] This document is an update of the first version published on December 20, 2023. The recommendations presented in this document may be subject to subsequent modifications based on advances in knowledge about the disease and the etiological agent.

In humans, the disease has an acute onset with headache followed by fatigue, chills, fever, myalgia, and general discomfort. These symptoms may worsen the following days, with vomiting, drowsiness, confusion and prostration. The most frequent neurological symptoms include weakness and widespread tremors, especially in the hands, lips and tongue. Improvement generally begins several days after the fevers subsides, typically around 7 to 10 days. There is no human vaccine nor specific antiviral treatment. Management of cases includes rest, adequate hydration, and symptomatic therapy.

International notification

Disease in equines must be reported to the World Organization for Animal Health. An event involving human infection should be assessed using the "Decision instrument for the assessment and notification of events that may constitute a public health emergency of international concern", Annex 2 of the International Health Regulations (2005) (*4*) for reporting through the mechanisms of the Regulations.

Case definitions

Suspected case

Patient who:

1) presents or has presented **acute onset of fever,** accompanied by a headache; and

2) presents **mental confusion** or other **acute neurological manifestations** (including prostration, tremors, vomiting and somnolence), **meningitis or encephalitis** without another apparent etiology.

Depending on the epidemiological situation, the history of residence in or travel to a place or geographic area with confirmed cases of WEE in animals and/or humans during the 10-15 days prior to the onset of symptoms should be considered.

Confirmed case

Any suspected case with laboratory confirmation, using any of the following criteria:

1) detection of viral RNA by RT-PCR in any sample type; or

2) detection of anti-WEEV IgM antibodies by ELISA in a cerebrospinal fluid sample; or

3) seroconversion of anti-WEEV IgM antibodies by ELISA in paired acute and convalescent samples collected more than 7-10 days apart; or

4) seroconversion or increase in the titer of neutralizing antibodies by PRNT (or microneutralization) in paired acute and convalescent samples collected more than 7-10 days apart.

Probable case

Any suspected case with detection of anti-WEEV IgM antibodies by ELISA in a single serum sample (without a paired sample), and who, therefore, does not meet the definition of a confirmed case.

Negative/discarded case

Any suspected case with no detectable anti-WEEV IgM antibodies by ELISA in a single serum sample (without a paired sample) collected more than 10 days after the onset of symptoms.

Note: In cases for whom no sample was collected, or who only have a single sample collected within the first 10 days from the onset of symptoms with a negative result, and where it is not possible to obtain a paired sample, it will not be possible to confirm or rule out the suspicion. Clinical and epidemiological information should be carefully considered for the final classification of the case.

Laboratory diagnosis

The diagnosis of WEEV infection requires confirmation through laboratory techniques since the clinical presentation is not specific. These laboratory methods include virological (direct) diagnostic methods by nucleic acid amplification or potentially cell culture and serological (indirect) methods, which aim at detecting antibodies produced against the virus. Generally, samples for diagnosis include serum and cerebrospinal fluid (CSF). CSF should only be collected in cases with neurological symptoms and by clinical indication.

Biosafety

Fresh biological samples, of any type, should be considered potentially infectious. Samples should be processed and handled exclusively by trained professionals after a local risk assessment, considering all biosafety indications and appropriate personal protective equipment. Any procedure involving sample manipulation should be conducted in certified Class II biosafety cabinets. The manipulation of extracted RNA does not require biosafety cabinets. Additionally, all necessary precautions should be taken to prevent percutaneous exposure. The manipulation of materials or cultures with high viral load and/or high volume should be considered only after a local risk assessment considering the necessary containment measures is conducted.

Virological methods

The detection of viral RNA can be performed on serum and CSF samples using real-time or endpoint **RT-PCR** with specific primers (and probes) for WEEV. Generic protocols (pan-alphavirus) can also be used, followed by specific RT-PCR or nucleotide sequencing.

Viral isolation is carried out using the same types of samples as RT-PCR. Mammalian cell lines (e.g., Vero cells) and mosquito cells (e.g., C6/36 cells) are used. In general, viral isolation is not routinely applied nor is it a requirement for diagnostic confirmation. Technical complexity, containment requirements, costs, as well as the need to identify isolated viruses by RT-PCR or immunofluorescence, limit the use and timeliness of the diagnosis by viral isolation.

In fatal cases, RT-PCR (or viral isolation) can also be performed on tissue samples (in particular, nervous system tissue).

A positive result by RT-PCR (or viral isolation) **confirms** the infection. However, viremia in WEEV infections is low and of short duration. Furthermore, if the case is detected in the neurological phase, the virus is likely no longer present in the blood. Therefore, a negative result **does not rule out** infection and, in cases with clinical and epidemiological suspicion, serological methods should be used. Differential diagnosis by molecular methods, particularly for other arboviruses that can cause neurological syndromes, should also be considered. Depending on the epidemiological situation, other equine encephalitis viruses (EEEV and VEEV) as well as neurotropic flaviviruses (e.g. West Nile virus, St. Louis encephalitis virus) could be considered (Figure 1).

While RT-PCR generally has a low sensitivity due to the level and duration of viremia (it may be possible to detect the viral RNA up to 3 days after the onset of symptoms, at most 5 days), its high specificity and fast turnaround make it an important tool in detecting WEEV infections. In the context of an outbreak with compatible symptoms, detection by RT-PCR in at least one case allows for the identification of the etiological agent.

Serological methods

IgM antibody detection is performed by **ELISA** using *in-house* methodologies. Detection can be performed in both serum and CSF. The kinetics of antibody production have not been fully described. However, it is likely that antibody detection can be performed early after the onset of symptoms, particularly neurological ones (Figure 1).

Antibody detection may be limited by potential cross-reactivity between WEEV and other alphaviruses and by the persistence of antibodies after an acute infection; therefore, in cases with clinical and epidemiological suspicion, a positive result for IgM in a single sample is considered a **probable case** of WEEV infection. Nevertheless, the specificity of IgM detection is estimated to be relatively high. Cases with seroconversion by IgM ELISA in paired samples (acute and convalescent samples collected more than 7-10 days apart) are considered **confirmed cases**.

The potential cross-reactivity can be assessed by conducting differential IgM serological tests for other alphaviruses, particularly CHIKV, always taking into account the epidemiological context. In cases of positive results to more than one alphavirus, additional clinical and epidemiological criteria should be used for the final interpretation of the case. Cases of cross-reactivity can also be evaluated by neutralization assays such as the **plaque reduction neutralization test (PRNT)** or **microneutralization**, ideally using paired samples (acute and convalescent samples collected with more than 7-10 days of difference, convalescent sample collected more than 14 days after the onset of symptoms). Depending on the epidemiological situation in the area where the infection likely happened, it is recommended to detect in parallel neutralizing antibodies against WEEV, EEEV, VEEV, CHIKV and MAYV (Figure 1). Finally, the detection of specific antibodies in CSF **confirms a** WEEV infection in a case with neurological manifestations.

On the other hand, a negative IgM ELISA result in a single sample only rules out infection if the sample was collected more than 10 days after the onset of symptoms. In cases with a negative result in a singe sample collected within the first 10 days from the onset of symptoms and where it is not possible to obtain a paired sample, it will not be possible to confirm or rule out the suspicion. Clinical and epidemiological information should be carefully considered for the final classification of the case.

Laboratory reagents

There are no validated commercial kits for molecular or serological detection of WEEV infection. The use of protocols validated by reference laboratories is recommended. For more information, contact the PAHO Regional Office (e-mail: laboratoryresponse@paho.org, ricoj@paho.org).

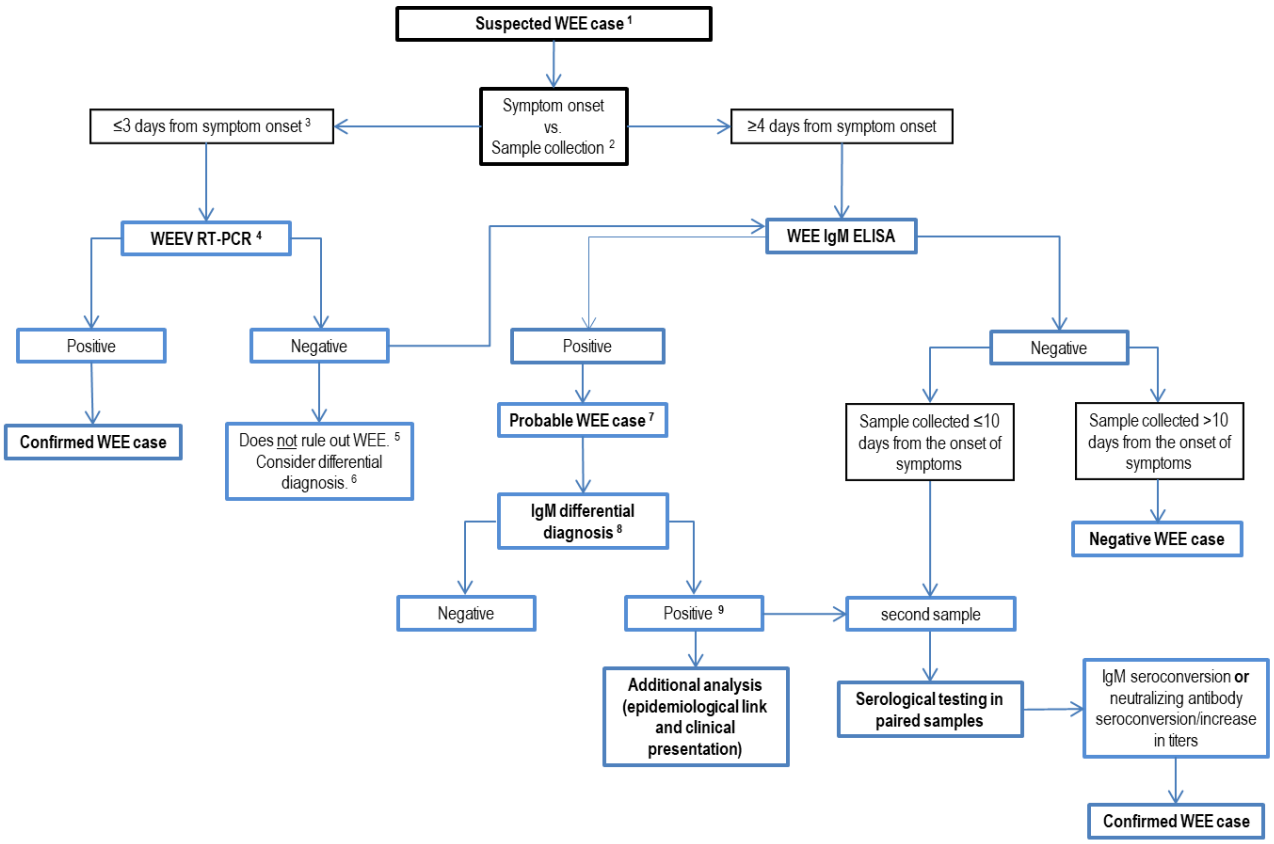
Sample storage

- Serum and CSF samples:
  - Keep refrigerated (2 - 8 ° C) if processed (or sent to a reference laboratory) within 48 hours.
  - Keep frozen (-10 to -20° C) if processed after 48 hours or within 7 days.
  - Keep frozen (-70° C or less) if processed more than one week after collection. The sample is adequately preserved at -70° C for extended periods of time.
- Tissue samples: freeze and ship on dry ice.
- Avoid multiple freeze-thaw cycles.

Shipping of samples to the reference laboratory

- Ensure the cold chain preferably with dry ice (tissues), or with refrigerant gels. Always use triple packaging.
- Ship samples preferably within the first 48 hours.
- The original samples should be packaged, marked, appropriately labeled, and documented as **category B.**
- The shipment must be accompanied by a complete clinical and epidemiological record, correctly identifying the sample type, the date of symptom onset, and the date of sample collection.

Laboratory algorithm



[1] See case definition.
[2] Laboratories that only have the capacity to perform RT-PCR or IgM ELISA should test samples with the available technique. Results should be interpreted according to the algorithm.
[3] In the first 3 days (or up to 5) from the onset of symptoms, it is recommended to use RT-PCR although it may have low sensitivity. The presence of viral RNA in the CSF may be longer.
[4] Generic panalphavirus RT-PCR can also be used, followed by the identification of the etiological agent by sequencing.
[5] A positive result confirms the case; however, a negative result does not rule out WEEV infection and additional serological testing is recommended.
[6] Consider other equine encephalitis viruses, West Nile virus, St. Louis encephalitis virus, and others, depending on the epidemiological situation of the area/country.
[7] A positive IgM result in a single serum sample is not confirmatory. Serological cross-reactivity with other alphaviruses or persistence of antibodies from a recent infection might be observed. A positive IgM result in a CSF sample confirms WEEV infection.
[8] Consider chikungunya virus and other alphaviruses, according to the epidemiological situation of the area/country.
[9] In cases of cross-reactivity, the IgM ELISA results do not allow confirmation of the etiological agent. However, this result does not rule out WEEV infection. Additional clinical and epidemiological criteria should be used for the final interpretation of the case. Neutralization can also be performed in a reference laboratory to analyze cross-reactive samples (ideally, in paired acute and convalescent samples).

Figure 1. Algorithm for laboratory confirmation of Western Equine Encephalitis Virus (WEEV) Infection.
CSF: cerebrospinal fluid.

References

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3. Secretaria da Agricultura, Pecuária, Produção Sustentável e Irrigação, Rio Grande do Sul. Diagnóstico confirma Encefalite Equina do Oeste no Estado. 26 January 2024. Available in Portuguese at: https://www.agricultura.rs.gov.br/diagnostico-confirma-encefalite-equina-do-oeste-no-estado

5. World Health Organization. International Health Regulations (2005), 3rd ed. January 1, 2016. Available at: https://www.who.int/publications/i/item/9789241580496