Epidemiological alert
Risk to human health associated with infection with Western Equine Encephalitis Virus in equines

Corrigendum[1] – 26 December 2023

Given the recent detection of Western Equine Encephalitis (WEE) outbreaks in equines located in several provinces of Argentina and some departments of Uruguay, the risk to human health associated with the circulation of this virus and the high potential for spread to other countries of the Region of the Americas, the Pan American Health Organization / World Health Organization (PAHO/WHO) alerts Member States to the importance of strengthening epidemiological surveillance and diagnosis of equine encephalitis, intersectoral coordination, surveillance, and vector control in the Region.

Summary of the situation

Western Equine Encephalitis (WEE), ICD-11 1C83, is a disease caused by a virus of the same name, which belongs to the genus *Alphavirus* of the *Togaviridae* family. Like Eastern Equine Encephalitis and Venezuelan Equine Encephalitis, WEE is part of the group of encephalitis caused by arboviruses. Most of these encephalitis infections produce undifferentiated febrile illness. Neurologic manifestations include meningitis, encephalitis, or myelitis, with specific symptoms varying depending on the type of virus and clinical presentation. Meningitis is typically characterized by fever, headache, neck stiffness, and other meningeal signs. The encephalitis may present with fever, altered mental status, seizures, or focal neurologic signs including movement disorders (*1*). It has been documented that mortality in humans ranges from 3-4% *(2)*.

The WEE virus is transmitted primarily through the bite of infected mosquitoes that act as vectors. The main vector is *Culex tarsalis*, but there are multiple vectors, such as *Aedes melanimon*, *Aedes dorsalis* and *Aedes campestris*; these vectors maintain the circulation of the virus in wild enzootic cycles, where passerine birds act as the main reservoir of the virus (3). Humans and equines are final reservoirs of the virus, meaning they are not able to transmit the virus to mosquitoes *(2)*.

In arboviral encephalitis, the risk of infection is generally determined by exposure to infected vectors and depends on many factors, including environmental conditions, season, and human activities. People who live, work, or participate in outdoor activities in endemic areas or where there are reported active outbreaks of the disease in animals are at increased risk due to exposure to mosquitoes (*1*).

[1] This corrigendum is to modify the alert originally published on 19 December 2023 to incorporate laboratory guidelines for the detection and diagnosis of human infection with Western Equine Encephalitis virus, as well as to modify the name of the virus and the disease; **Encephalitis** instead of Encephalomyelitis. Additionally, corrections were made to the situation summary.

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Situation in South America

The World Animal Health Information System (WAHIS) shows that, from 2006 to 2023, Equine Encephalitis has been reported in animals in 17 countries of the Region of the Americas. During this period, WEE was reported in equines in Brazil (2006, 2007, 2017, 2018, 2019 and 2022), Mexico (2019), and, in 2023, cases were reported in Argentina and Uruguay *(4)*.

On 25 November 2023 in **Argentina,** the National Food Safety and Quality Service (SENASA per its acronym in Spanish) reported positive virological results for an *unspecified Alphavirus* in the provinces of Corrientes and Santa Fe (5). On 27 November 2023, the diagnosis of WEE was confirmed in three samples, and additional cases were confirmed in the following days within the provinces of Buenos Aires, Entre Ríos, Córdoba, Chaco, Formosa, and Santiago del Estero. On 30 November, SENASA declared a state of sanitary emergency throughout the national territory *(6)*. Per SENASA Resolution 1219/2023, the last official record of WEE in Argentina dates back to1988 *(7)*. According to SENASA's 18 December report, 920 outbreaks were confirmed (26 diagnosed by laboratory and 894 by clinical and epidemiology). The province of Buenos Aires reported the highest number of cases (n=501), followed by Santa Fe (n=147), Córdoba (n=127), Entre Ríos (n=69), Corrientes (n=41), Chaco (n=16), Formosa (n=6), Santiago del Estero (n=5), La Pampa (n=4) and Rio Negro (n=4) *(8)*.

In response to Argentina's first report, the animal health authorities of Paraguay and Uruguay, on 25 and 27 November respectively, issued statements urging the notification of neurological symptoms in equines *(9,10)*.

On 5 December 2023, the Ministry of Livestock, Agriculture and Fisheries (MGAP per its acronym in Spanish) of **Uruguay** confirmed a case of WEE in an equine in the municipality of Salto, on the coast of the Uruguay River (11). In the last weeks, visits were made to 219 establishments, across all departments of the country, with complaints of suspected WEE in equines. In the 13 December report, 20 cases of WEE were confirmed by laboratory in 9 municipalities: Río Negro (n=5), San José (n=4), Paysandú (3), Artigas (n=2), Soriano (n=2), Durazno (n=1), Flores (n=1), Salto (n=1), and Tacuarembó (n=1). In the same report, 252 cases with well-founded clinical suspicion of WEE were reported, for which the laboratory results are pending *(12)*.

Recommendations

Below is a summary of the main recommendations for surveillance, prevention measures, and risk communication.

Laboratory diagnosis of WEE in humans (13)

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 of 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 the detecting WEEV infections. In an outbreak context 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; therefore, in cases with clinical and epidemiological suspicion, a positive result

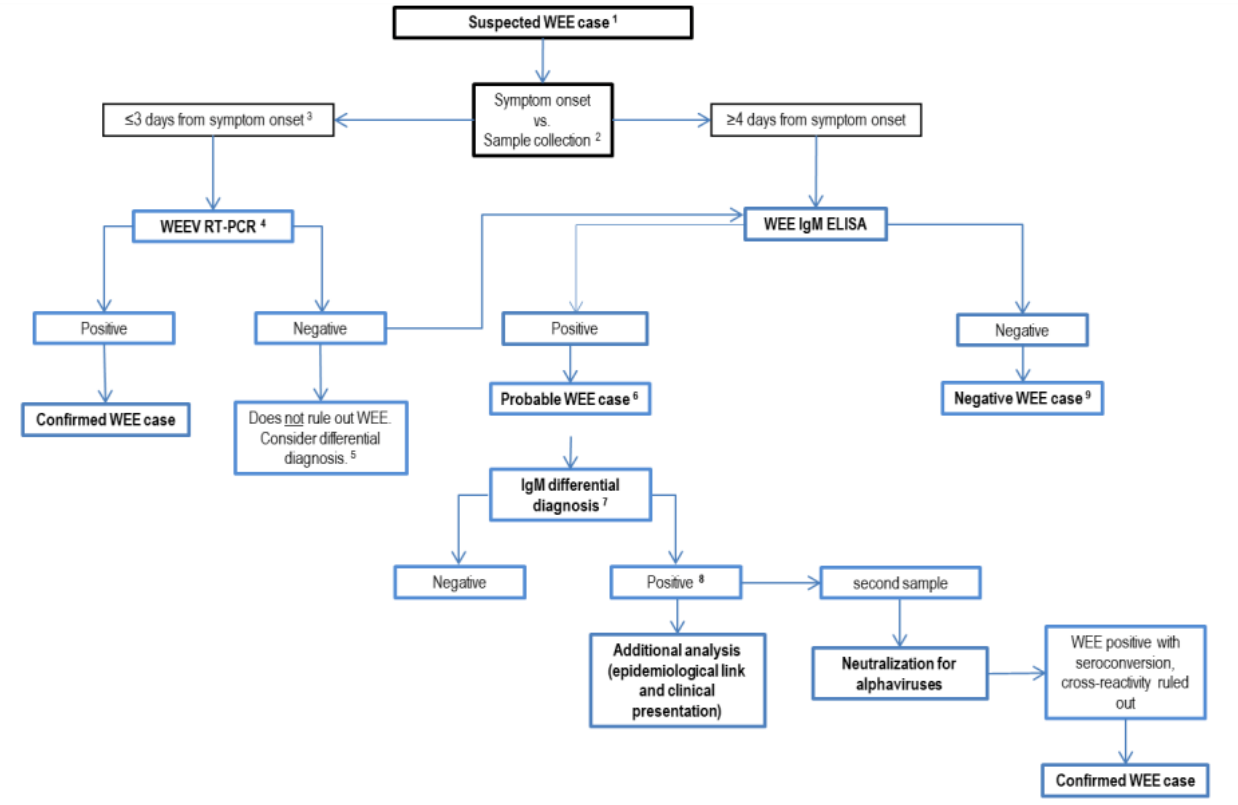
for IgM is considered a probable case of WEEV infection. Nevertheless, the specificity of IgM detection is estimated to be relatively high.

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 positivity 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 WEEV infection in a case with neurological manifestations.

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.

Figure 1. Algorithm for laboratory confirmation of Western Equine Encephalitis (WEE) virus infection.



[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 is longer. A positive result confirms the case; however, a negative result does not rule out WEEV infection and additional testing is recommended.
[4] Generic panalphavirus RT-PCR can also be used, followed by the identification of the etiological agent by sequencing.
[5] Consider other equine encephalitis viruses, West Nile virus, St. Louis encephalitis virus, and others, depending on the epidemiological situation of the area/country.
[6] A positive IgM result in a single sample is not confirmatory. Serological cross-reactivity with other alphaviruses might be observed.
[7] Consider the chikungunya virus and other alphaviruses, according to the epidemiological situation of the area/country.
[8] 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).
[9] IgM levels may be below the limit of detection if the sample was taken early in the acute phase (days 1-3). In these cases, consider collecting a second sample.

CSF: cerebrospinal fluid.

Human Case Surveillance

In areas at risk or with active outbreaks, it is recommended to implement or strengthen surveillance through the search for compatible neurological syndromes that do not have another defined diagnosis, taking into account the incubation period, geographical area and environmental conditions.

Patient Management and Infection Prevention Measures in Health Facilities

There is no specific antiviral treatment. Most infections are characterized by a mild clinical presentation in which treatment is symptomatic. Patients presenting with neurologic symptoms should be evaluated by a specialist and require close monitoring.

Prevention Measures

The preventive actions listed below must be organized within the framework of One Health, considering the inter-institutional and comprehensive action between animal health, human health, and the environment.

Managing the Environment

Considering the ecology and biology of the main vectors of the WEE virus, the main prevention measure is the modification of the environment and the environmental management of the environment, seeking to reduce the number of mosquitoes and their contact with equids and humans. These measures include:

- Filling or draining water collections, ponds, or temporary flooding sites that may serve as sites of female oviposition and breeding sites for mosquito larvae.
- Elimination of weeds around the premises to reduce mosquito resting and shelter sites.
- The equids can be protected by sheltering them in barns with mosquito nets, especially at times when mosquitoes are most active.
- Despite the fact that the main vectors do not have indoor habits, it is advisable to protect homes with mosquito nets on doors and windows; in this way other arboviruses are also prevented.

Vector Control

Vector control measures for WEE should be considered within the framework of Integrated Vector Management (IVM). It is important to consider that the decision to carry out vector control activities with insecticides depends on entomological surveillance data and the variables that may condition an increase in the risk of transmission, including insecticide resistance data.

- Insecticide spraying may be considered as an additional measure and, where technically feasible, in areas of transmission where high mosquito populations are detected. The methodology should be established based on the ecology and behavior of the local vectors.

Vaccination for equids

- Vaccines are available for equids. It is advisable to seek high vaccination coverage in susceptible equids in areas considered at risk and to carry out annual vaccination boosters in these equines.

Personal Protective Measures

- Use of clothing that covers the legs and arms, especially in homes where someone is sick.
- Use of repellents containing DEET, IR3535 or Icaridin, which may be applied to exposed skin or clothing; their use must be in strict accordance with the instructions on the product label.
- Use wire mesh/mosquito netting on doors and windows.

- Use of insecticide-treated or non-insecticide nets for daytime sleepers (e.g., pregnant women, infants, bedridden, elderly, and night shift workers).
- During outbreaks, outdoor activities should be avoided during the mosquitoes' peak feeding period (dawn and dusk).

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