Recommendations for the Detection and Surveillance of Oropouche in possible cases of vertical infection, congenital malformation, or fetal death

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Background

Since the first detection of Oropouche virus (OROV) in 1955 in Trinidad and Tobago, numerous outbreaks of Oropouche fever have been described in rural and urban communities in Brazil, Ecuador, French Guiana, Panama, Peru, and Trinidad and Tobago. In recent months, an increase in the detection of Oropouche fever cases has been observed, particularly in the Amazon region of Brazil where more than 6,000 cases have been reported, followed by Bolivia, Peru and Colombia reporting around 1,500 additional cases. In addition, recently, Cuba has demonstrated the autochthonous circulation and expansion in a large part of its territory, in an ecosystem different from the Amazon basin, accounting for a marked capacity for viral adaptation and dispersion. On the other hand, and although so far the clinical presentation of the disease has been relatively mild, biases and limitations in surveillance may be preventing the detection of more severe cases and symptoms. Likewise, Brazil has recently reported a case of spontaneous abortion where OROV was detected as a possible causative agent, that added to four (1) possible cases of microcephaly reviewed in retrospect, increase the possibility of vertical transmission that would have a great impact on public health.

In this context and in view of the need to strengthen surveillance and preparedness actions, including the active search and investigation of cases associated with congenital abnormalities or fetal death, laboratory diagnosis and interpretation constitute an essential component that must be fully articulated with clinical and epidemiological surveillance.

Oropouche Laboratory Diagnosis and Surveillance

Guidance on the diagnosis and laboratory surveillance of emerging arboviruses, including OROV, is detailed in the "Guidelines for the Detection and Surveillance of Emerging Arboviruses in the Context of the Circulation of Other Arboviruses" (2).

Virologic diagnosis of Oropouche infection

Sample types: serum and/or cerebrospinal fluid (CSF)[1]

During the acute phase of the disease (between 2 and 7 days), it is possible to detect the virus's genetic material (RNA) by molecular methods (real-time RT-PCR or qRT-PCR). RNA in cerebrospinal fluid (CSF) can also be detected in cases presenting with aseptic meningitis (a rare complication of Oropouche fever) or suspected neurological disease; however, a CSF sample should only be collected by medical indication and for the study of other causes. Most molecular methods for OROV detection are based on

[1] The use of a cerebrospinal fluid sample is recommended only when there is a medical indication for sample collection.

conserved S genetic segment detection (2 - 4).

Serological diagnosis of Oropouche infection

Sample types: serum and/or cerebrospinal fluid (CSF)[1]

OROV antibodies can usually be detected in serum as early as the fifth day after symptom onset. So far, there are no commercial assays for the serological diagnosis of OROV, so it can only be performed using in-house techniques such as plaque reduction neutralization (PRNT) and IgM/IgG ELISA (2).

Diagnosis from a single acute-phase serum sample is presumptive, so it is recommended to collect a second sample, one to two weeks after the first sample to demonstrate seroconversion (negative to positive) or up to four-fold increase in the antibody titer (with a quantitative test) (2).

However, it is worth noting that the availability of reagents for serological methods is extremely limited. Therefore, it is recommended to prioritize and use molecular methods (RT-PCR), if the appropriate samples are available.

Evidence of Oropouche vertical transmission and laboratory diagnosis

Currently, the possible vertical transmission of OROV is being investigated considering several laboratory findings (1). In a retrospective study conducted in Brazil, it was possible to detect the presence of IgM antibodies against OROV in serum and CSF in four neonates. These neonates presented microcephaly; however, it was not possible to establish a causal relationship between OROV infection and the neurological abnormalities observed. On the other hand, OROV genetic material was identified in umbilical cord blood, placenta and various organs from a fetal death that occurred at 30 weeks of gestation, which supports a possible vertical transmission of OROV. As reported by the Secretariat of Health and Environmental Surveillance of the Ministry of Public Health of Brazil, joint laboratory, epidemiology and clinical analyses are being carried out to conclude the final classification of this case (1).

Since the sudden increase in the number of OROV cases in the Amazon region, PAHO has provided technical support to countries in the region to improve their capacity to detect and characterize Oropouche and Mayaro viruses in a timely manner (5). The evidence emphasizes the need to strengthen surveillance of OROV in cases where vertical transmission is suspected. In this regard, some recommendations for laboratory surveillance of OROV in pregnant women, stillbirth's indicative of congenital infection, and healthy neonates and/or neonates with evidence of neurological complications or abnormalities are listed below.

Oropouche laboratory diagnosis in pregnant women

The diagnosis of OROV infection in pregnant women can be made according to the criteria described above: serum sample for detection of viral genetic material (RT-PCR) or detection of IgM, depending

on the stage of infection. However, since vertical transmission is being studied for OROV, it is important to closely monitor both the mother and the newborn.
Thus, a sample of amniotic fluid collected under a medical indication can be used for:

1. Molecular detection of viral genetic material (RT-PCR). A positive result indicates possible OROV transplacental transmission.
2. Antibody detection. A positive result for IgM means intrauterine infection of the fetus.

*See tables 1 and 2 for recommended samples.

Laboratory diagnosis of OROV associated with stillbirth's indicative of congenital infection

In cases of spontaneous abortions and stillbirth, a serum sample (if possible) for both, RT-PCR and IgM antibody detection (ELISA) should be guaranteed and in any case guaranteed a tissue sample (preferably from the nervous system). It is also recommended to analyze serum samples from the mother in parallel for determination of IgM antibodies.
On the other hand, and if an amniotic fluid sample is available (collected under a medical indication) it can be used for molecular detection by RT-PCR. In addition, depending on the gestational stage, CSF is recommended for molecular detection of viral genetic material by RT-PCR and for serology (IgM).

*See tables 1 and 2 for recommended samples.

Oropouche laboratory diagnosis in neonates

Healthy neonates of infected mothers: It is recommended to try to detect OROV (molecular and serological) in placenta samples collected at the time of delivery, umbilical cord fluid, and serum from both the newborn and the mother.

Neonates with evidence of neurological complications or abnormalities: In addition to attempting to detect OROV (molecular and serological) in placenta samples collected at the time of delivery, umbilical cord fluid, and serum from both the newborn and the mother, detection of the virus in CSF is also recommended[1].

*See tables 1 and 2 for recommended samples.

Table 1. Recommended samples, days after onset of symptoms and laboratory assays indicated for OROV detection.

Sample	Days after symptom onset	Quantity	Sample collection and transport	Handling and transport temperature	Storage >1 week	Laboratory Assay
Serum	1 to 5	5-7 mL	No additives	4 / 8 ºC	−20 /−70 ºC	PCR
Serum	5 to 7	5-7 mL	No additives	4 / 8 ºC	−20 /−70 ºC	PCR, ELISA IgM
Serum	7 onwards	0,5-1mL	No additives	4 / 8 ºC	−20 /−70 ºC	ELISA IgM
Urine*	5 to 15	5-7 mL	No additives	4 / 8 ºC	−20 /−70 ºC	PCR
CSF**		0.5 mL	No additives	4 / 8 ºC	−20 /−70 ºC	PCR, ELISA IgM

* There is insufficient evidence to determine the dynamics of the presence of OROV in urine, however, to facilitate investigation and better understand the infection process, it is recommended, if possible, to obtain a urine sample when no other samples are available

** Under medical indication for the diagnosis of neurological disease

Table 2. Other recommended samples, storage and transport conditions, and laboratory assays indicated for OROV detection.

Sample	Quantity	Sample collection and transport	Handling and transport temperature	Storage >1 week	Laboratory Assay
Mother's serum	5-7 mL	No additives	4 / 8 ºC	−20 /−70 ºC	PCR, ELISA IgM, PRNT, other
Cord blood	0,5-1mL	No additives	4 / 8 ºC	−20 /−70 ºC	PCR, ELISA IgM, PRNT, other
Placenta	3 x 3 cm	Buffered formalin	4 ºC - RT*	4 ºC - RT*	Immunohistochemistry
Placenta	3 x 3 cm	Sterile saline solution or dry tube	4 / 8 ºC	−20 /−70 ºC	PCR
Umbilical cord (tissue)		Buffered formalin	4 ºC - RT*	4 ºC - RT*	Immunohistochemistry
Umbilical cord (tissue)		Sterile saline solution or dry tube	4 / 8 ºC	−20 /−70 ºC	PCR
Newborn serum	0,5-1mL	No additives	4 / 8 ºC	−20 /−70 ºC	PCR, ELISA IgM, PRNT, other
Amniotic fluid**	0,5-1mL	No additives	4 / 8 ºC	−20 /−70 ºC	PCR
Newborn CSF**	0,5 mL	No additives	4 / 8 ºC	−20 /−70 ºC	PCR, ELISA IgM, PRNT, other
Mother's whole blood	5-7 mL	EDTA, other	4 / 8 ºC	4 ºC	Biochemistry, others
Newborn whole blood	2-5 mL	EDTA, other	4 / 8 ºC	4 ºC	Biochemistry, others
Tissue***	3 x 3 cm	Buffered formalin	4 ºC - RT*	4 ºC - RT*	Immunohistochemistry
Tissue***	3 x 3 cm	Sterile saline solution or dry tube	4 / 8 ºC		PCR

* Room temperature

** Under medical indication for suspected neurological syndrome

*** Fatal cases: Brain, liver, kidney, abortion product, other

Sample storage

- Keep samples refrigerated (4 ºC – 8 ºC) if processed (or sent to a reference laboratory) within 48 hours.
- Keep samples frozen (-10 ºC to -20 ºC) if processed after 48 hours.
- Keep samples frozen (-20 to -70 ºC) if processed after one week.
- For long term storage, samples for serological methods might be kept frozen at –10 to –20 °C (or less) while samples for virological methods should be kept at –70 °C or less, if possible.

Sending samples by air to the reference laboratory

- The cold chain of the samples must be guaranteed. Ship (if possible) with dry ice or at least with cooling gels. Always use triple packaging.
- Samples should be shipped, preferably, within the first 48 hours of collection.
- Original samples must be packaged, marked, labeled (if dry ice is used), and registered as category B.
- Shipments must be accompanied by complete clinical and epidemiological records.

Additional Comments and Recommendations

- Given the clinical presentation of Oropouche fever, for detection and follow-up it is suggested to process acute samples (up to 7 days after the onset of symptoms) from dengue surveillance, which meet a definition of a suspected case of dengue, but which are negative for the molecular detection of the dengue virus. Depending on laboratory capacity and epidemiological context, a percentage of dengue acute negative samples processed by molecular detection can be processed (which can range from 10% to 30%) or a limited number of representative samples (2).
- There are different protocols (primers and probes) for the detection of OROV by RT-PCR (both conventional and real-time). However, several countries in the region have been using the protocol developed by Naveca et al. (4) that allows the simultaneous molecular detection of the OROV and Mayaro viruses (MAYV), strengthening the laboratory diagnostic capacity for both viruses.
- Viral isolation is not considered a diagnostic technique and is recommended only for research trials complementary to public health surveillance.
- Laboratories that do not have the capacity for virological confirmation (RT-PCR, viral isolation, sequencing) or serological confirmation (PRNT), should send the samples to a reference laboratory or Collaborating Center of the World Health Organization. Prior to any shipment, please contact the contact persons at each center and the Pan American Health Organization office in Washington DC.

References:

1- Nota Técnica nº 15/2024-SVSA/MS — Ministério da Saúde (www.gov.br)

2 - Pan American Health Organization / World Health Organization. Guidelines for the Detection and Surveillance of Emerging Arboviruses in the Context of the Circulation of Other Arboviruses. April 18, Washington, D.C.: PAHO/WHO; 2024. Available in: Guidelines for the Detection and Surveillance of Emerging Arboviruses in the Context of the Circulation of Other Arboviruses - PAHO/WHO | Pan American Health Organization

3 - Pan American Health Organization. Recommendations for Laboratory Detection and Diagnosis of Arbovirus Infections in the Region of the Americas. Washington, D.C.: PAHO; 2022. Available in: Recommendations for Laboratory Detection and Diagnosis of Arbovirus Infections in the Region of the Americas (paho.org)

4 - Naveca FG, Nascimento VAD, Souza VC, Nunes BTD, Rodrigues DSG, Vasconcelos P. Multiplexed reverse transcription real-time polymerase chain reaction for simultaneous detection of Mayaro, Oropouche, and Oropouche-like viruses. Mem Inst Oswaldo Cruz. 2017;112(7):510-3. Available in: https://doi.org/10.1590/0074-02760160062

5 – Pan American Health Organization / World Health Organization. Countries of the Americas Strengthen Preparedness for Oropouche Virus. July 9, 2024. Available in: Countries of the Americas Strengthen Preparedness for Oropouche Virus - PAHO/WHO | Pan American Health Organization