

Situation summary in the Americas

Since epidemiological week (EW) 1 to EW 7 of 2017, **Brazil, Colombia, Peru, and the Plurinational State of Bolivia**, have reported suspected and confirmed yellow fever cases.

The following is a situation summary in Brazil.

In **Brazil**, since the beginning of the outbreak in December 2016 to EW 7 of 2017, there were 1,368 cases of yellow fever reported (326 confirmed, 125 discarded, and 916 suspected cases remain under investigation), including 220 deaths (109 confirmed, 6 discarded, and 105 under investigation). The case fatality rate (CFR) is 33% among confirmed cases and 11% among suspected cases.

According to the probable site of infection, 83% of the suspected and confirmed cases were reported in the state of Minas Gerais (1,209), followed by Espírito Santo (185), São Paulo (10), Bahia (9), Tocantins (2), Goiás (1) and Rio Grande do Norte (1).¹ The confirmed cases are distributed in three states: Minas Gerais (269), Espírito Santo (53), and São Paulo (4). **Figure 1** illustrates the municipalities with confirmed cases and cases under investigation, as well as confirmed epizootics, and epizootics under investigation.

In Minas Gerais, the downward trend in cases in the four administrative regions of the state continues to be observed. Nonetheless, the decreasing trend will continue to be monitored to see if it continues in all the regions in the coming weeks (**Figure 2**).

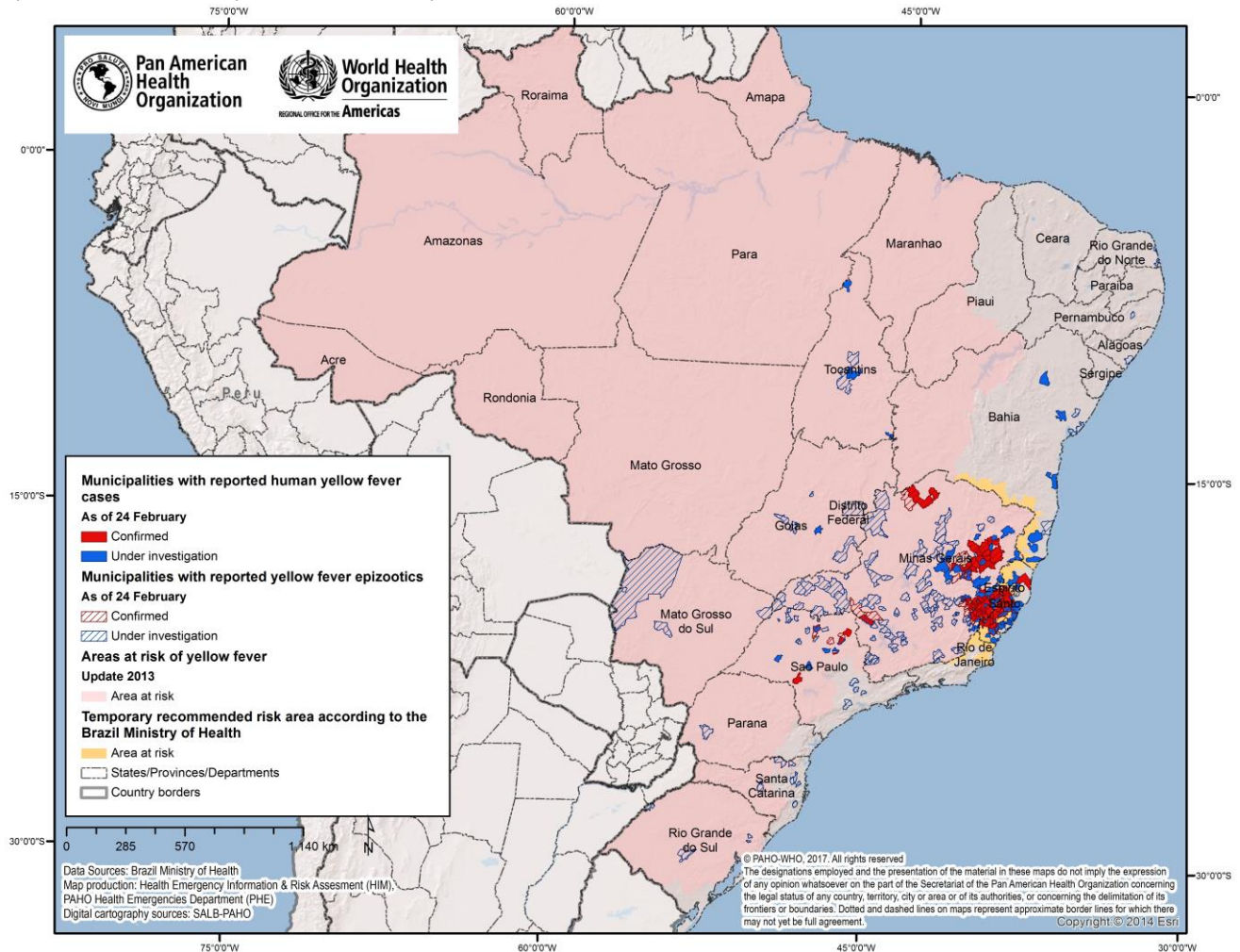
In Espírito Santo, there were 120 new cases (confirmed and under investigation) reported from 2 February to 24 February, bringing the total from 65 to 185 cases over a period of four weeks. In Minas Gerais, during the same period there were 256 new cases reported, bringing the total from 773 to 1,029 cases.

There is possibility of a change in the yellow fever transmission cycle in this current outbreak, however, to date *Aedes aegypti* has not been reported to have a role in transmission.

¹ There are also five suspected case for which the probable site of infection remains under investigation.

Suspected yellow fever cases in the municipalities close to large urban areas (such as Serra, Aracruz, and Vitoria in the state of Espirito Santo²) are under investigation.

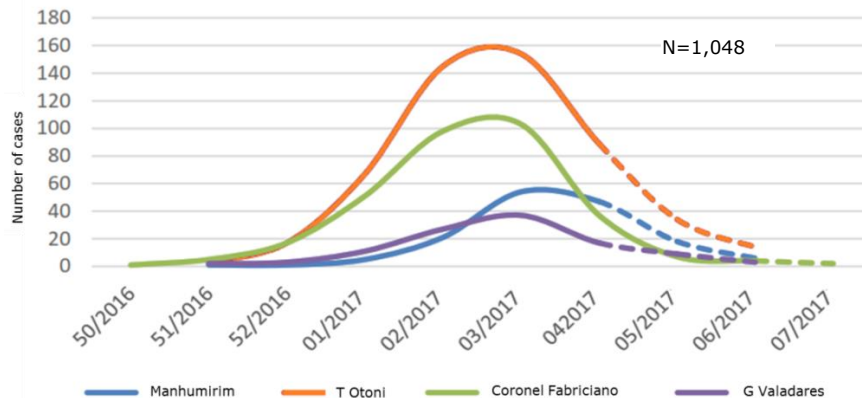
Figure 1. Geographic distribution of reported human yellow fever cases and yellow fever epizootics, Brazil, up to 24 February 2017.



Source: Data published by Brazil Ministry of Health (Monitoring of yellow fever cases and deaths) compiled and reproduced by PAHO/WHO

² Information published by the Brazil Ministry of Health and available at: http://portal.arquivos.saude.gov.br/images/2017/SVS/Municipios_casos_suspeitos_e_confirmados_febre_amar_ela_.pdf

Figure 2. Suspected and confirmed cases of yellow fever according to EW of onset of symptoms and administrative regions. Minas Gerais, EW 50 of 2016 to EW 7 of 2017



Source: Data published by the Minas Gerais Secretariat of Health and reproduced by PAHO/WHO

With regard to the confirmed deaths, 92 occurred in the state of Minas Gerais, 3 in the state of São Paulo and 14 in the state of Espírito Santo. In decreasing order, the CFR among suspected and confirmed cases by state is 75% in São Paulo, 34% in Minas Gerais, and 26% in Espírito Santo.

Since the last yellow fever Epidemiological Update³ up to 24 February 2017, a total of 76 new epizootics were reported in nonhuman primates (NHP) and are under investigation. Since the beginning of the outbreak, a total of 959 NHP epizootics were reported, of which 386 were yellow fever confirmed and 8 were discarded.

Epizootics in NHP were reported in the Federal District and in the states of Alagoas, Bahia, Goiás, Espírito Santo, Mato Grosso do Sul, Minas Gerais, Paraná, Pernambuco, Rio Grande do Norte, Rio Grande do Sul, Santa Catarina, São Paulo, Sergipe, and Tocantins.

To date, there have been no reports of yellow fever cases linked to the current outbreak in Brazil in other countries and/or territories in the Americas. However, the Pan American Health Organization, Regional Office of the World Health Organization (PAHO/WHO) reiterates that reports of epizootics, currently under investigation, in states of Brazil bordering other countries—Mato Grosso do Sul (bordering Bolivia and Paraguay), Santa Catarina (bordering Argentina), Rio Grande do Sul (bordering Uruguay and Argentina), and Paraná (bordering Argentina and Paraguay)—represent a risk of spread of the virus to the bordering countries, especially in areas with similar ecosystems.

The Brazil situation report on the yellow fever outbreak is published daily by the Brazil Ministry of Health and is available at:

<http://portalsaude.saude.gov.br/index.php/o-ministerio/principal/leia-mais-o-ministerio/619-secretaria-svs/11-svs/27300-febre-amarela-informacao-e-orientacao>.

The updated requirements for the **International Certificate of Vaccination or Prophylaxis (ICVP)** with proof of vaccination against yellow fever are available at:

http://www.paho.org/hq/index.php?option=com_topics&view=article&id=69&Itemid=40784&lang=en

³ PAHO/WHO Epidemiological Update: Yellow Fever. 23 February 2017. Available at:

http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=38226&lang=en

Recommendations

The PAHO / WHO guidance on Laboratory Diagnosis of Yellow Fever Virus Infection, February 2017, is included below and the complete document is available at: http://www.paho.org/hq/index.php?option=com_topics&view=rdmore&cid=5514&Itemid=40784&lang=en.

Laboratory diagnosis of yellow fever virus infection

The yellow fever virus belongs to the genus *Flavivirus* and is related to other viruses of the same genus such as dengue, Zika, Japanese encephalitis and West Nile viruses. The virus is transmitted to humans mainly by sylvatic mosquito vectors of the genera *Haemagogus* and *Sabethes* as well as by the *Aedes aegypti* mosquito. The clinical spectrum of yellow fever ranges from asymptomatic or mild infection to potentially fatal severe conditions with hemorrhage and jaundice. Suspicion of yellow fever is based on the patient's clinical features, places and dates of travel (if the patient is from a non-endemic country or area), activities, and epidemiologic history of the location where the presumed infection occurred. Thus, confirmation by laboratory techniques should be addressed for characterization of cases and of the outbreak.

The most important measure of prevention of yellow fever is vaccination which provides protective immunity against the disease to 80-100% of those vaccinated after 10 days and 99% immunity after 30 days. Although the yellow fever vaccine is safe and adverse events are uncommon, contraindications and safe immunization practices must be respected.

Sample types and laboratory procedures

The diagnosis of yellow fever is made by virological methods (detection of the virus or of its genetic material in serum or tissue) using virus isolation or Reverse transcription polymerase chain reaction (RT-PCR), or by means of serological testing for the detection of antibodies.

Biosafety considerations

All biological samples (whole blood, serum or fresh tissue) should be considered as potentially infectious. All laboratory personnel handling these samples must be vaccinated against yellow fever and use appropriate personal protective equipment. Likewise, it is recommended to carry out all procedures in certified class II biosafety cabinets and to take all necessary precautions to avoid percutaneous exposure. Procedures for handling of non-human samples should be carefully assessed according to the biosafety manual of the laboratory, and use of Class III biosafety cabinets should be considered.

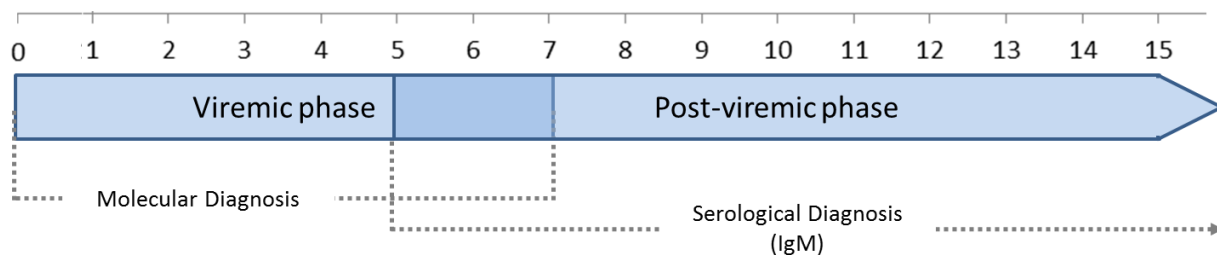
Virological diagnosis

- **Molecular diagnostics:** Viral RNA can be detected during the first 5 days from symptom onset (viremic phase) if using molecular techniques such as end-point of real-time RT-PCR. Occasionally, viral RNA can be detected up to 7 days from symptom onset. Thus, it is recommended to perform both RT-PCR and IgM ELISA for samples collected

between days 5 and 7 from the onset of symptom (**Figure 3**). A positive result (when using the appropriate controls) confirms the diagnosis.

- **Viral isolation:** Viral isolation can be performed through intracerebral inoculation in mice or in cell culture (using Vero or C6/36 cells; may be performed under BSL2 containment). However, because of its complexity, this methodology is rarely used as a diagnostic tool and is recommended mainly for research studies complementary to public health surveillance.
- **Postmortem diagnosis:** Histopathological analysis with immunohistochemistry performed on liver sections is considered the "gold standard" for the diagnosis of yellow fever in fatal cases. Additionally, molecular detection can also be performed in fresh or paraffin-embedded tissue samples to confirm the cases. The procedure may be carried out under BSL2 containment (See above the section *Biosafety considerations* for non-human samples).

Figure 3. Indications for yellow fever diagnosis according to the number of days since the onset of symptoms



Serological diagnosis

Serology (the detection of specific antibodies) is useful for diagnosing yellow fever during the post-viremic phase of the disease (i.e., from the 5th day since the onset of symptoms).

A positive IgM reaction by enzyme-linked immunosorbent assay (ELISA) (mainly IgM antibody-capture, MAC-ELISA) or any other immunoassay (indirect immunofluorescence) in a sample collected from the 5th day of symptom onset is presumptive of recent yellow fever virus infection. Currently, there are not commercially available, validated IgM ELISA kits. Therefore, in-house protocols using whole purified antigen may be standardized.

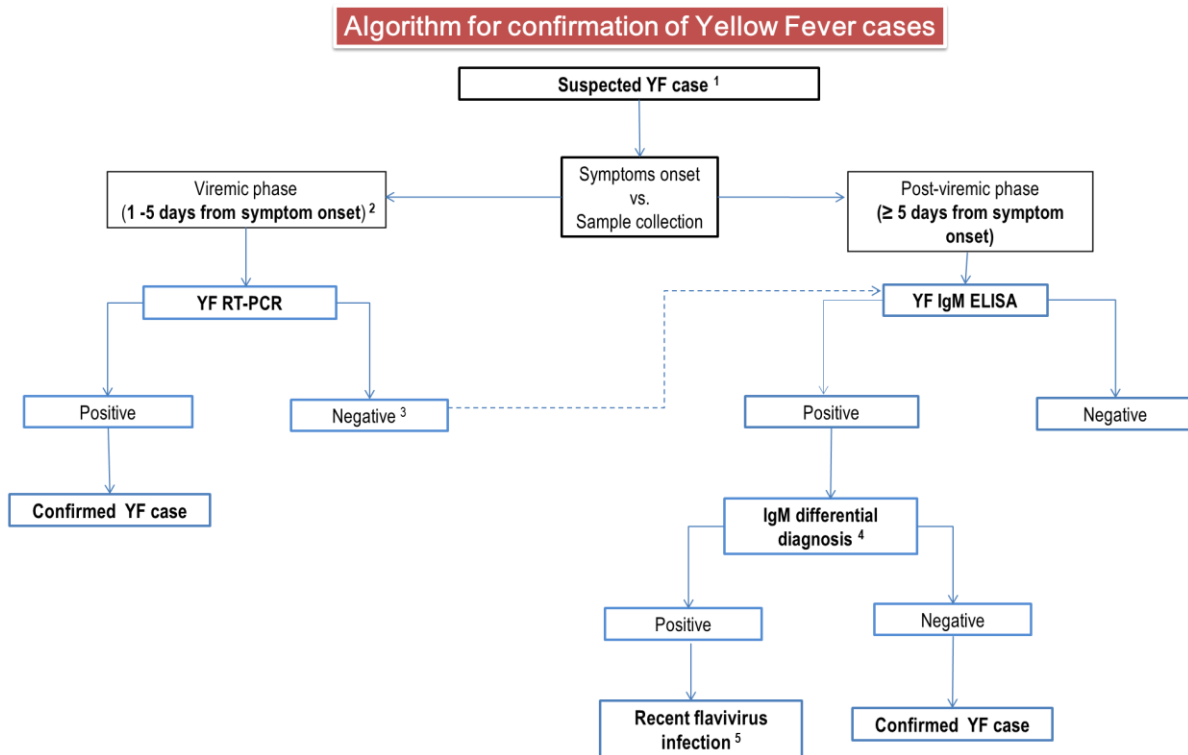
The confirmation of a case of yellow fever by IgM ELISA will depend on the epidemiological situation and the results of the laboratory differential diagnosis. In areas where other flaviviruses co-circulate (especially dengue and Zika), the probability of cross-reactivity is higher (Figure 4).

Other serological techniques include the detection of IgG antibodies by ELISA and of neutralizing antibodies by plaque reduction neutralization test (PRNT). IgG ELISA is useful with paired samples (collected at least 1 week apart), while PRNT90 may be useful with paired samples, or with a single post-viremic sample if the assay includes multiple flaviviruses.

A seroconversion (negative results in the first sample and positive result in the second sample), a more than 4-fold increase in antibody titers in paired samples, or a detectable antibody titer

against yellow fever in a post-viremic sample by PRNT90 is presumptive of yellow fever virus infection. Confirmation of a yellow fever case using these techniques will depend on the epidemiological situation and the results of the laboratory differential diagnosis. In areas with co-circulation of other flaviviruses the probability of cross reactivity is higher (see figure 2). Additionally, in those areas where active vaccination campaigns are ongoing, detection of vaccine induced antibodies may occur and then diagnosis should be carefully interpreted (see below the section *Post-vaccination immune response*).

Figure 4. Algorithm for confirmation of yellow fever cases



¹ Suspected YF case (unvaccinated or unknown vaccination history)
² Occasionally, viral RNA can be detected up to 7 days from symptom onset
³ If the PCR is negative, check sample quality, collection and transport conditions. Also, consider collecting a post-viremic sample for serological testing.
⁴ Must include at least dengue (and other flaviviruses depending on the epidemiological situation of the region / country).
⁵ If samples are available, consider performing PRNT on paired samples in a reference laboratory

Interpretation of serology results and differential diagnosis

Serological techniques are often cross-reactive among flavivirus infections (in particular, in secondary flavivirus infections). This should be considered in areas where the co-circulation of yellow fever virus with other flaviviruses (dengue, St. Louis encephalitis, Zika, and others of the Japanese encephalitis complex) is documented and the population is likely to have been previously infected with these viruses. Also, it should be noted that in individuals vaccinated against yellow fever, vaccine-induced IgM can be detected for several months or even years.

Therefore, it is recommended to carry out the parallel detection of antibodies to other flaviviruses and to carefully interpret the results taking into consideration the individual vaccination history as well as the available epidemiological information.

In general, the PRNT offers greater specificity than the detection of IgM and IgG. However, cross-reactivity has also been documented for the neutralization assays. Thus, it is also recommended that this technique be performed using antigens for several flaviviruses.

Moreover, the differential diagnosis of yellow fever should include other febrile and febrile icteric syndromes – such as dengue, leptospirosis, malaria, viral hepatitis, among others – depending on the epidemiological profile of the affected country or area.

A case of yellow fever will be confirmed by serological techniques only if the differential laboratory diagnosis, taking into consideration the epidemiological situation of the country, is negative for another flavivirus (Figure 4).

Post-vaccination immune response

Vaccination induces a relatively low viremia that decreases after 4 to 7 days. Concurrently, an IgM response develops. This response cannot be differentiated from the IgM response induced by a natural infection. Approximately 10 days after vaccination, the vaccinee is considered to be protected against a natural infection. The IgM response may be detected from around day 5 onwards with a peak occurring generally 2 weeks after vaccination. Subsequently, antibody levels tend to decrease. However, in a significant proportion of individuals, the IgM response can be detected one month and in some cases (mainly travelers) up to 3-4 years. In addition, neutralizing antibodies induced by vaccination can be detected for several decades. Therefore, the interpretation of serological results in vaccinated individuals is complex, particularly in those who have recently been vaccinated and results should be carefully assessed.

Sample conservation

- Whole blood (in EDTA tube) or serum (red-top tube) should be kept refrigerated (2 - 8 °C) if processed (or sent to a reference laboratory) within 48 hours.
- Serum should be kept frozen (-10 to -20 °C) if processed after 48 hours but in a period of no more than 7 days.
- Serum should be kept frozen (-70 °C) if processed more than a week after. Serum samples can be stored at -70 °C for extended periods of time.
- Multiple freeze-thaw cycles should be avoided.
- Fresh tissue samples (approximately 1 cm³) can be used for molecular diagnosis. Freeze at -70 °C and send to a reference laboratory on dry ice. If not possible, store fresh tissue in sterile saline or refrigerated PBS (2-8 °C) and ship with refrigerant gels.
- For histopathological and immunohistochemistry analyses, tissue samples (approximately 1 cm³) must be fixed in buffered formalin and sent to a pathology laboratory at room temperature. Liver is the tissue of choice for histopathological and immunohistochemistry analyses. Spleen and kidney samples may also be useful.

Shipping of samples to the reference laboratory by air

The following are some aspects to consider for shipping samples by air:

- The cold chain should be maintained with dry ice (if possible) or with refrigerant gel. Triple packaging should always be used.
- Samples should be shipped, if possible, within the first 48 hours.
- The original samples must be packaged, marked, labeled (if dry ice is used) and registered as **category B**.
- The shipment must be accompanied by the complete clinical and epidemiological record.

Related Links

- PAHO/WHO Yellow Fever. Available at:
http://www.paho.org/hq/index.php?option=com_topics&view=rdmore&cid=5514&Itemid=40784&lang=en
- PAHO/WHO Guidance on Laboratory Diagnosis of Yellow Fever Virus Infection, February 2017, Available at:
http://www.paho.org/hq/index.php?option=com_docman&task=doc_download&Itemid=270&gid=38104&lang=en

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