Viscerotropic Disease Associated with Vaccination against Yellow Fever

Case Studies

GUIDE FOR PARTICIPANT

Pan American Health Organization
Regional Office of the World Health Organization

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1902 - 2012

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Area of Family and Community Health
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The case studies presented in this guide should be implemented with the guidance of the Pan American Health Organization and under the supervision of experts in immunization, epidemiological surveillance, and the laboratory and clinical aspects of yellow fever.

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ACRONYMS

ALT (SGPT)	alanine transaminase (serum glutamic pyruvic transaminase)
ARDS	adult respiratory distress syndrome
AST (SGOT)	aspartate transaminase (serum glutamic-oxaloacetic transaminase)
CDC	Centers for Disease Control and Prevention (CDC)
cm	centimeter
CPK	creatinine phosphokinase
DGE	General Directorate of Epidemiology (Peru)
DIRESA	Regional Health Directorate (Peru)
dL	deciliter
ESAVI	event supposedly attributable to vaccination or immunization
IgG	immunoglobulin G
IgM	immunoglobulin M
IHR	International Health Regulations
INR	international normalized ratio (prothrombin time)
INS	National Institute of Health (Peruvian agency responsible for health research)
L	liter
mg	milligram
mL	milliliter
mm	millimeter
MoH	Ministry of Health
NAMRU-6	Naval Medical Research Unit 6 (formerly NMRCD, United States)
PAHO	Pan American Health Organization
PCR	polymerase chain reaction
PFU	plaque forming units
Q-PCR	quantitative polymerase chain reaction
RNA	ribonucleic acid
RT-PCR	reverse transcription polymerase chain reaction
U/L	units per liter
VTD	viscerotropic disease
WHO	World Health Organization

GLOSSARY

Active surveillance of ESAVIs: Surveillance based on the routine and proactive investigation of cases conducted on a case-by-case basis by health workers.

Brighton Collaboration: Global network of vaccine-safety experts. The organization's website can be accessed at http://Brightoncollaboration.org/public.

Crisis related to vaccination: A situation caused by a real or perceived adverse event that produces the real or potential loss of confidence in vaccines and immunization services.

Immunoglobulin M (IgM) and immunoglobulin G (IgG): Antibodies produced by B-lymphocytes responsible for acute humoral immunity (IgM) and for immunological memory (IgG). A person with a recent infection tends to present IgM in the serum, while IgG appears later.

National ESAVI Committee: Committee responsible for assessing serious ESAVI cases in order to guide case investigation and provide final classifications. The committee includes professionals from various fields, including experts in epidemiology, public health, and biostatistics and members of the national regulatory authority (NRA).

Neurologic disease associated with yellow fever vaccination (YEL-AND): A neurological syndrome associated with the yellow fever vaccine that presents one or more of the following signs and symptoms: fever, focal neurological deficits, changes in mental status, seizures, or pleocytosis or excessive protein in cerebrospinal fluid. Neurologic disease can be due to direct vaccine viral invasion into the central nervous system or due to autoimmune manifestation triggered by the vaccine.

Passive surveillance: Surveillance based on the spontaneous reporting of ESAVI cases conducted on a case-by-case basis by health workers.

Polymerase chain reaction (PCR): Test used to detect genetic material (e.g., nucleic acids) in serum or tissue specimens.

Real-time or quantitative PCR (Q-PCR): Test that allows for the quantification of genetic material at any time during amplification. For cases of viscerotropic disease associated with yellow fever vaccine, Q-PCR makes it possible to identify viral concentrations in biological samples.

Reverse transcription polymerase chain reaction (RT-PCR): Test that detects and amplifies genetic material via retrotranscription of ribonucleic acid (RNA) into deoxyribonucleic acid (DNA).

Risk communication: Decision-making process that considers the risk of potential dangers in formulating, studying, and comparing risk control measures that are intended to protect the population in the event of a probable danger.

Sentinel surveillance: Surveillance based on reports by a group of sources (sentinel units) within the health system in which a sample of a population group is studied.

Viscerotropic disease associated with yellow fever vaccination: A disease associated with vaccination against yellow fever and characterized by systemic multiple organ failure and clinical symptoms similar to those of wild yellow fever.

INTRODUCTION

The purpose of the following case studies is to familiarize participants with case definitions and with the epidemiological investigation of viscerotropic disease (VTD) associated with vaccination against yellow fever. We present here a cluster of events supposedly attributable to vaccination or immunization (ESAVIs) that occurred in Peru in 2007, during a yellow fever vaccination campaign implemented in a non-endemic area.

For whom is this document intended

1. Health professionals from the national, subnational, and local levels who participate in the investigation of ESAVI cases.
2. Clinicians, laboratorians, and other professionals (academics, investigators, etc.) who participate in vaccination activities or pharmacovigilance.
3. Staff from the national regulatory authority, particularly those responsible for pharmacovigilance and vaccine regulation.

Study objectives

1. To understand and apply case definitions of VTD.
2. To analyze and implement reporting, investigation, and classification procedures for cases of VTD in a timely manner.
3. To review response measures in the event of a case of VTD associated with yellow fever vaccine.
4. To understand and apply principles of causality assessment for cases of VTD.
5. To understand the importance of planning for establishing strategic alliances with media sources and other important stakeholders.

General instructions

1. Participants will work as members of a rapid response team assigned to investigate multiple cases of VTD associated with yellow fever vaccine.
2. Participants will be assigned to teams of approximately 6-8 persons to answer questions related to each case study. Each group will have a facilitator and a rapporteur. After reading the case studies, group members will discuss their responses, sharing their experiences and raising concerns.
3. Group work allows for the exchange of experiences and for participants to learn new information and opinions.
4. In group work, the facilitator should help to stimulate the conversation and assist participants with responses as needed, while maintaining the conceptual framework.

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Evaluation

To measure participant learning during the case studies, evaluations of the participants' knowledge will be conducted at the beginning and end of the exercises (Annex 1). It is recommended to present a summary of these results and the progress made during the case studies to participants.

Prior knowledge

In preparation for working with the case studies, participants are encouraged to review the following documents:

1. Pan American Health Organization. *Control of yellow fever.* Washington, D.C.: PAHO; 2005.
2. Pan American Health Organization. *Safe vaccination training modules. Module III: Safe injection practices. Module IV: Technical and clinical aspects of events supposedly attributable to vaccination or immunization.* Washington, D.C.: PAHO; 2007.
3. World Health Organization. Yellow fever. *Surveillance of adverse events following immunization against yellow fever. Field guide.* Geneva: WHO; 2010.
4. Gershman MD, Staples JE, Bentsi-Enchill AD, Breugelmans JG, Brito GS, Camacho LA, et al. Viscerotropic disease: Case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Vaccine.* 2012; 30(33):5038-5058. Epub 2012 May 4.
5. Whittembury A, Ramirez G, Hernández H, Ropero AM, Waterman S, Ticona M, et al. Viscerotropic disease following yellow fever vaccination in Peru. *Vaccine.* 2009; 27(43): 5974-81. Epub 2009 Aug 11.
6. Pan American Health Organization. Serious events supposedly attributable to vaccination or immunization associated with receipt of yellow fever vaccine during a mass immunization campaign, Peru, 2007. *Findings and recommendations of an expert panel.* Washington, D.C.: PAHO; 2008.

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ABOUT YELLOW FEVER AND ESAVIs

Yellow fever

Yellow fever is a disease endemic to the tropical areas of Africa and South America. Approximately 200,000 cases and 30,000 deaths from the disease occur each year (*1*).These cases primarily affect young adult males who, for occupational reasons, must enter enzootic areas. In the Region of the Americas, yellow fever is endemic mainly in Bolivia, Brazil (eastern-central region), Colombia, Ecuador, French Guiana, Guyana, Peru, Suriname, Trinidad and Tobago, and Venezuela. Sporadic cases have also been reported in other countries, such as Argentina, Panama, and Paraguay (*2*). Yellow fever virus transmission in the Americas predominantly occurs in a jungle cycle. Enzootic areas are rural, isolated from urban areas, and mostly situated in the Amazon jungle (*3*).Nonetheless, in 2008, yellow fever cases occurred in a peri-urban area in Paraguay (*4*).

Yellow fever can be prevented with live-attenuated yellow fever vaccine 17D, which is considered safe and effective. Yellow fever vaccination strategies implemented in the Region of the Americas include: 1) introduction of yellow fever vaccine into national immunization programs in all endemic countries for children aged 1 year; 2) vaccination campaigns during inter-epidemic periods; 3) vaccination campaigns in response to outbreaks or epizootics; and 4) vaccination of travelers entering enzootic areas, except when contraindicated.

As of 2011, all countries in the Americas with enzootic areas have included yellow fever vaccine in their national vaccination schedules. In Argentina, Brazil, and Suriname, the vaccine is administered exclusively in areas considered at risk. Vaccination coverage of children aged 1 year was approximately 70% for the period 2007-2011 and has been significantly affected by limited vaccine availability.

The International Health Regulations (IHR) indicate that proof of yellow fever vaccination can be demanded from travelers as a requirement for admission into a country (*5*).

Events supposedly attributable to vaccination or immunization (ESAVIs)

An ESAVI is defined as a set of clinical symptoms that occur following immunization, which may or may not be attributable to the vaccine, and which causes great concern among the population (*6*).

The following ESAVIs must be reported, investigated, and classified in a timely manner: 1) serious events that require hospitalization, are life-threatening for the patient, cause disability, or are fatal; 2) events that affect a group of people (disease clusters); 3) events related to the immunization program (programmatic errors); and 4) events that generate rumors or confusion among the population.

It is difficult to determine whether an ESAVI is truly the result of a vaccine. Consequently, the objectives of ESAVI investigation are to confirm or rule out the notified event as vaccine-related, determine if the event might have another cause, determine whether the event is isolated or related to other events, and notify all interested parties of the results of the investigation.

Following a thorough review by vaccine-safety experts, the classification of ESAVIs was recently modified as follows (*7*):

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Vaccine product-related event:

An event that is caused or precipitated by a vaccine due to one or more of the inherent properties of the vaccine product.

Example: Extensive limb inflammation (edema) following diphtheria-tetanus-pertussis (DPT) vaccination.

Vaccine quality defect-related event:

An event that is caused or precipitated by a vaccine that is due to one or more quality defects of the vaccine product including its administration device as provided by the manufacturer.

Example: Failure of the manufacturer to completely inactivate a lot of inactivated polio vaccine (IPV) leads to cases of paralytic polio.

Immunization error-related event:

An event that is caused by inappropriate vaccine handling, prescribing, or administration and thus by nature is preventable.

Example: Sepsis, toxic shock syndrome, infection (e.g., localized abscess at the injection site), or death due to a non-sterile injection.

Immunization anxiety-related event:

An event that arises from anxiety about the vaccine.

Example: Vaso-vagal syncope in adolescents following immunization against the Human Papillomavirus (HPV).

Coincidental events:

An event that is caused by something other than the vaccine product, immunization error, or immunization anxiety.

Example: Fever occurs at the time of vaccination (temporal association) but is caused by another agent such as malaria.

Inconclusive cases:

An event whose cause remains unknown despite a thorough investigation. In such cases, it must be explained why a conclusion was not reached and how far the investigation progressed.

ESAVIs associated with yellow fever vaccine

In general, yellow fever vaccine is considered to be among the safest vaccines in existence. More than 500 million people have received the vaccine, with very good results in terms of safety and tolerance. Nevertheless, side effects, such as fever, headache, and back pain, have been observed since the first studies on the 17D vaccine were conducted in the 1930s (*8*).

Between 1953-1994, 10 clinical trials were conducted to study reactions to the vaccine. A small percentage of those vaccinated experienced mild, self-limited reactions. The most common of these were pain and erythema at the injection site as well as systemic reactions, including fever, chills, headache, myalgia, and malaise. These symptoms typically appeared 3–7 days following vaccination. Reactogenicity was observed to be less in infants than in adults (*9*).

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Serious ESAVIs associated with yellow fever vaccine are rare. According to available data, the incidence of reported ESAVIs is 1.6 cases per 100,000 vaccine doses (*10*).The most frequently reported serious ESAVIs in the scientific literature are cases of VTD, neurologic disease, and severe hypersensitivity reactions.

Viscerotropic disease was first identified as an adverse event of yellow fever vaccination in 2001 (*11*). The disease occurs by dissemination and widespread replication of the 17D live attenuated virus of the vaccine. Based primarily on a retrospective review of ESAVI reports, 65 cases of VTD following yellow fever vaccination were identified as occurring worldwide between 1973 and March 2011.

Although the incidence of the disease is unknown largely because of underreporting and passive ESAVI surveillance systems, data from travelers in the United States and Europe show a risk of 0.3-0.4 VTD cases per 100,000 yellow fever vaccine doses distributed. Furthermore, based on data from 2008 and 2009 vaccination campaigns in Brazil, the estimated risk of VTD following yellow fever vaccination in that country is similar to those reported in the United States and Europe (*12*). The mortality rate is estimated to be 60% in notified cases, though this may be an overestimate because fatal cases are more likely to be reported than nonfatal cases (*12*).

Although it has not been possible to determine the physiopathological mechanism that causes VTD following yellow fever vaccination, some population groups, such as those aged ≥60 years, have demonstrated a greater risk of developing the disease. As compared to the 19-29 year age group, the case ratio of the ≥60-year age group is 5.9, with an estimated relative risk of 4.4-13.4. In addition, a history of thymectomy for benign or malignant thymus disease is considered a risk factor for VTD associated with the yellow fever vaccine (*12*).

In the United States, 0.4-0.8 cases of neurologic disease following yellow fever vaccination (YEL-AND) have been reported per 100,000 vaccine doses administered. The highest rate is found among individuals aged ≥60 years (1.4-1.8 cases per 100,000 doses administered). Australia and the United Kingdom reported similar data, suggesting an increased risk for the elderly. Cases of post-vaccination encephalitis have also been attributed to the 17D yellow fever vaccine virus in infants aged <4 months (0.8 cases per 100,000 doses administered). As a result, the vaccine is contraindicated for infants aged <6 months and thereby has a greater safety margin. Neurological sequelae are unusual and deaths are rare. Neurological events reported as related to yellow fever vaccine include acute disseminated encephalomyelitis and Guillain-Barré Syndrome (*9*).

Lastly, serious hypersensitivity reactions are extremely rare. The yellow fever vaccine is contraindicated for individuals allergic to eggs. The most frequent hypersensitivity reactions are skin rashes, asthma (1 case per 130,000-250,000 doses administered) (*9*), and anaphylactic shock (0.8 cases per 100,000 doses administered) (*13*).

SUBSTRAINS OF THE 17D VACCINE

The 17D vaccine against yellow fever is obtained from wild-type yellow fever virus (Asibi strain) isolated in Ghana in 1927 and attenuated by serial passage. Numerous mutations in the structural and non-structural viral genes have resulted in the attenuated variant 17D. The attenuated virus exists as two substrains (17D-204 and 17DD), whose sequences are homologous at 99.9%. Both substrains are used in vaccines (*14*).

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BACKGROUND

On 15 August 2007, a severe earthquake struck Peru. The earthquake had a magnitude of 8.0 on the Richter scale and its epicenter was located 40 km from Ica, a city south of the country's capital, Lima. The Ica Region lies along the coast, and its population at the time was estimated to be 693,411 inhabitants in five provinces: Chincha, Ica, Nazca, Palpa, and Pisco.

Although Ica is a non-endemic area for yellow fever, Peru's Ministry of Health (MoH) began a vaccination campaign targeting individuals aged 15-59 years on 23 September 2007. The campaign used two vaccine lots, 050VFA121Z (121Z) and 050VFA123Z (123Z), of 17DD vaccine manufactured at Bio-Manguinhos in Brazil. From the first lot, 42,742 doses were administered; from the second, 20,432 were administered (*15*).These lots were used solely in the Ica Region.

As part of the campaign, the Ica Regional Health Directorate (DIRESA in Spanish) strengthened regional surveillance of adverse events and established daily negative reporting of serious ESAVI cases. A few months before, the MoH's General Directorate of Epidemiology (DGE in Spanish) had organized a training workshop on ESAVI surveillance for healthcare workers of the Ica DIRESA. The training was part of ongoing evaluation and training activities in the country's surveillance network.

The vaccination campaign in Ica was one of several activities carried out to mitigate the earthquake's effects and complemented the National Plan for Accelerated Yellow Fever Control started in 2004 (*15*).The plan included routine vaccination of children aged 1 year throughout the country, and vaccination campaigns for individuals aged 2-59 years in two phases: 1) vaccination of the population living in endemic areas and 2) vaccination of populations in migratory areas, where many people leave non-endemic areas and enter endemic areas during the harvest season. As part of the plan, approximately 12 million doses of yellow fever vaccine were administered between 2004-2007 in endemic and migratory areas. During this period, no cases of VTD associated with yellow fever vaccine were reported. Vaccination campaigns continued throughout 2007 in the regions of Amazonas, Cusco, San Martín, and Piura. Figure 1 shows the assessment of yellow fever risk areas in Peru.

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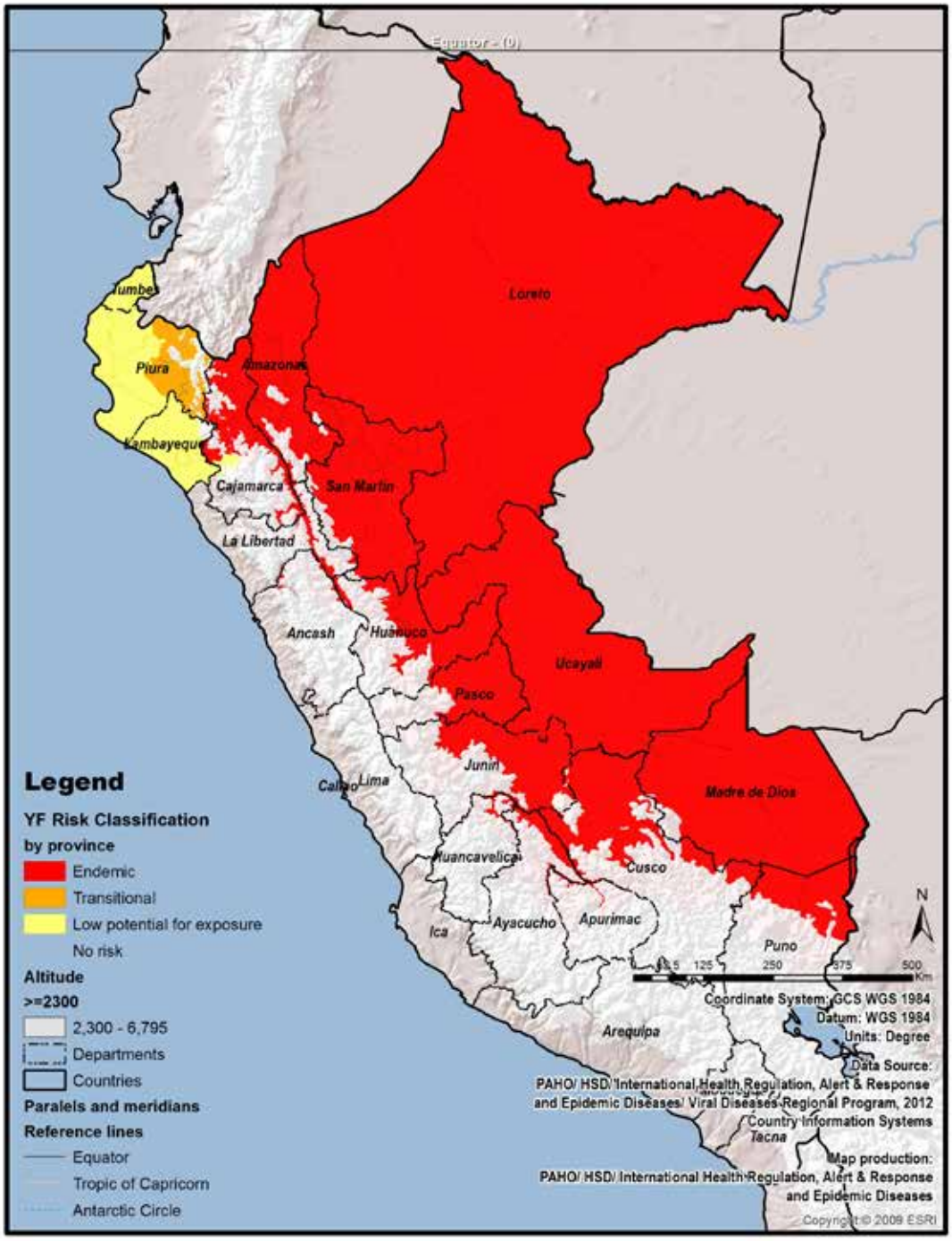
FIGURE 1. ASSESSMENT OF YELLOW FEVER RISK AREAS, PERU, 2012



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TIME AVAILABLE: 90 MINUTES

A. First case: medical student

On 6 October 2007 at 1:30 a.m., the epidemiologist in charge of the surveillance of vaccine-preventable diseases at the MoH received a call from his counterpart at the Ica Regional Hospital, reporting a serious ESAVI. The Ica epidemiologist indicated that he had already notified officials at the regional level, but given the situation, he also wanted to immediately notify the MoH. The patient was a female medical student aged 23 years and a former student of the Ica epidemiologist. The epidemiologist himself had seen the patient in the hospital's emergency room on the morning of 5 October. The patient arrived with clinical symptoms of approximately a week's duration, including fever, nausea, vomiting, and diarrhea. What most alarmed the attending physician was how rapidly the patient's health had deteriorated. She had arrived at the hospital on her own, lucid, and without any apparent serious clinical symptoms. Twelve hours later, she had to be admitted to the intensive care unit due to liver and renal failure. The only notable precursor to the patient's illness had been her yellow fever vaccination on Thursday, September 27, a fact confirmed by her vaccination card.

The MoH epidemiologist advised his counterpart at the Ica Regional Hospital to monitor the patient's progress and to notify him of any changes. At 6:00 a.m., the hospital epidemiologist reported that the patient's symptoms had worsened and that her prognosis was poor. The diagnosis was multiple organ failure. The patient was provided inotropic support and mechanical ventilation.

The epidemiologist at the Ica Hospital was advised to obtain a blood specimen from the patient to send to the National Institute of Health (INS in Spanish) in order to determine the etiological diagnosis. The patient's clinical symptoms were similar to those of a rare adverse reaction documented in several medical journal articles: viscerotropic disease associated with yellow fever vaccine. Based on this information, two epidemiologists were immediately dispatched to investigate the case. Additionally, the National ESAVI Committee was urgently convened and PAHO's focal point for yellow fever immunization was informed of the case.

1. **Did the Ica Hospital epidemiologist notify the case in a timely manner? Explain.**

2. **Use IHR regulations to determine the mechanism for official international notification of this case. (See adapted version of the IHR Annex 2 algorithm in Figure 2 below.)**

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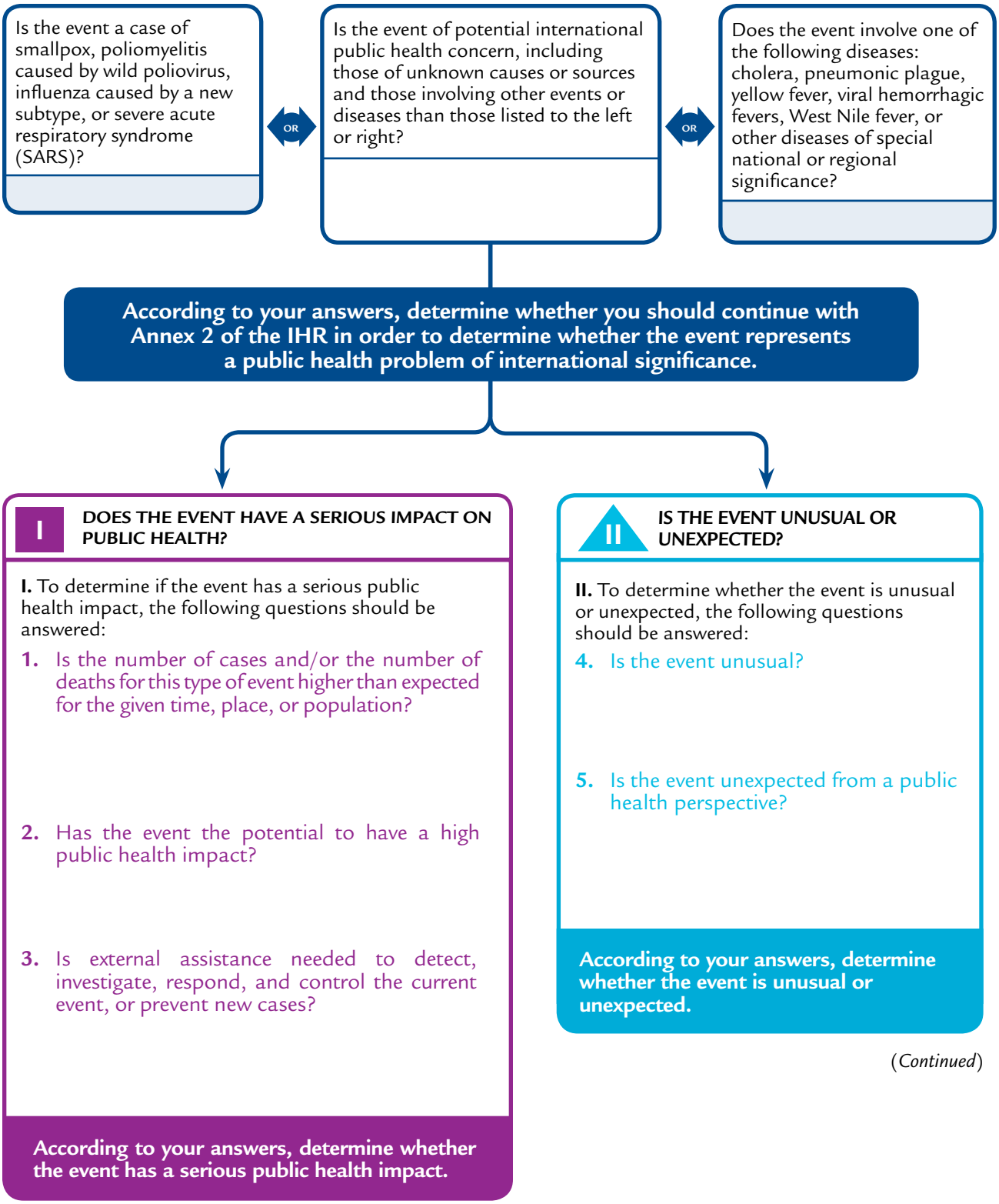
FIGURE 2. ANNEX 2 OF THE INTERNATIONAL HEALTH REGULATIONS



(*Continued*)

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FIGURE 2. (*Continued*)

III **IS THERE A SIGNIFICANT RISK FOR INTERNATIONAL SPREAD?**

III. To determine whether the event poses a significant risk of international spread, the following questions should be answered:

6. Is there evidence of an epidemiological link to similar events in other countries?

7. Is there any factor that indicates the potential for cross border movement of the agent, vehicle, or host?

According to your answers, determine whether the event poses a significant risk of international spread.

IV **DOES THE CASE IMPLY SIGNIFICANT RISKS FOR INTERNATIONAL RESTRICTIONS ON TRAVEL OR TRADE?**

IV. To determine whether there is a significant risk of international restrictions on travel or trade, the following questions should be answered:

8. Have similar events in the past resulted in international restrictions on trade and/or travel?

9. Is the source suspected or known to be a food product, water, or any other good that might be contaminated that has been exported/imported to/from other countries?

10. Has the event occurred in association with an international gathering or in an area of intense international tourism?

11. Has the event caused requests for more information by foreign officials or international media?

According to your answers, determine whether the event poses a significant risk of international trade or travel restrictions.

What is your final conclusion?

In Ica, the patient continued to evolve unfavorably. Complementary testing showed the following results: metabolic acidosis; moderate increases in transaminases; AST (SGOT):[1] 78 U/L; ALT (SGPT): 65 U/L; bilirubin: 0.85 mg/dL; leukocytes: 66,400 mL; lymphocytes: 8%; monocytes: 2%; neutrophils: 90%; platelets: 54,000 mL; and creatinine: 4.1 mg/dL. Despite the administration of inotropic drugs, the patient had a systolic blood pressure of 60 mm Hg and a central venous pressure of 15 cm H_2O. Chest X-rays showed pulmonary congestion with prominent hilar markings and bilateral pleural effusions, while abdominal ultrasonography showed hepatosplenomegaly and ascites (>500 mL). The patient also presented oliguresis (10 cm^3/hour).

Due to the patient's fragile health, she could not be transferred to a hospital in Lima and remained at the Ica hospital. The patient died on the same day of her admission (October 6) at 10:00 p.m.

[1] All acronyms related to diagnostic results are explained in the list of abbreviations provided at the beginning of the case studies.

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3. **What information should the epidemiologists at the Ica Regional Hospital obtain to complete the case investigation?**

B. Suspension of the vaccination campaign

The same day of the patient's death and in response to increasing media pressure, the director of DIRESA announced at a press conference that vaccination against yellow fever would be suspended throughout the region, until the results of the investigation of the death of the young student who received one dose of vaccine became available (*15*).

On 8 October, the MoH convened two emergency committee meetings: the National ESAVI Committee and the Crisis Committee, chaired by the vice minister of health. Both committees agreed that, throughout the crisis, the Ministry's spokespersons would be the president of the ESAVI Committee and the president of the National Immunization Technical Advisory Group (NITAG). They also concurred that these officers would respond to media requests for information on the campaign's suspension in Ica.

1. **What arguments exist in favor of suspending the vaccination campaign?**

2. **Should yellow fever vaccination have been suspended in other parts of the country? Support your answer.**

3. **What is the function of the Crisis Committee, and who should be part of it? Briefly mention the components that should be included in a crisis plan for a situation like the one described above.**

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TIME AVAILABLE: 90 MINUTES

A. Second case: a young mother

On 8 October 2007, a 24-year-old female sought emergency services at the Chincha provincial hospital, located in the Ica Region. The patient had been referred by her private physician, who had evaluated her hours earlier at her home. Three months before, she had given birth to a healthy child. According to the patient, she had been vaccinated against yellow fever approximately 10 days earlier; however, she did not have her vaccination card on hand. On the day of vaccination, she experienced pain at the injection site. The next day, she experienced headache, malaise, myalgia, and fever. These symptoms lasted 3-4 days. On 30 September, a private physician diagnosed her with a urinary tract infection and prescribed ciprofloxacin, dexamethasone, and amikacin.

By 3 October, in addition to her original symptoms, the patient developed watery stools, nausea, and vomiting. She had as many as 20 bowel movements on 7 October. Following her medical evaluation, the patient was diagnosed with hypovolemic shock, severe dehydration, and renal failure. The epidemiologist at the hospital immediately reported the case to the Ica DIRESA, which in turn notified the DGE. The DGE arranged the patient's transfer to the Dos de Mayo National Hospital in Lima, where she arrived on the night of 8 October.

Clinical examination upon admission showed: blood pressure: 80/50 mm Hg; heart rate: 100/min; respiratory rate: 30/min; and mydriatic pupils, 4/4 with response to light. Other signs and symptoms included slow capillary refill time, tachypnea, dyspnea, and vesicular murmur passing properly in both lungs. The patient was moaning, agitated, and able to move her four limbs. On the night of her admission to the hospital, two epidemiologists visited the Dos de Mayo Hospital to obtain the patient's background and clinical information and to request that a blood specimen be sent to the INS.

1. **What specimens and laboratory tests should be used to classify the case?**

2. **What should the Ica DIRESA epidemiologist investigate while visiting the patient's home?**

B. Field investigation

The Ica DIRESA and Chincha hospital epidemiologists interviewed the patient's husband, who indicated that she had given birth to a full-term healthy infant three months earlier, a fact corroborated by her clinical records. The patient's vaccination history could not be verified, as

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the husband did not have her vaccination card. Nonetheless, he indicated that she had been vaccinated at the Chincha hospital. The patient had not traveled in previous months. Unsuccessful attempts were made to locate the patient's clinical records corresponding to the private care she received, which would have indicated diagnosis of a urinary tract infection days before her hospital admission. However, several empty amikacin containers found at the patient's home confirmed that she had received treatment for such an infection. As time passed and the case gained notoriety in the media, it became impossible to again speak with the patient's husband, who refused further contact with investigators.

The epidemiologists, in coordination with the head of the hospital's immunizations program, verified the patient's vaccination records at the Chincha hospital. They then interviewed the health care worker who had vaccinated the patient and evaluated injection practices used in the hospital. The patient had been immunized with vaccine from lot 121Z on 27 September—the same lot responsible for the medical student's death on 6 October.

1. **What aspects of vaccination safety should the investigating team have evaluated during its visit to the vaccination center?**

2. **What hypothesis is suggested by the fact that both patients received vaccine from the same lot?**

3. **What would you do if the patient's family member refused to cooperate in the investigation?**

C. Evaluation of the vaccination center and the cold chain

Using a structured questionnaire designed specifically to evaluate aspects of vaccination safety, epidemiologists assessed injection practices and the experience and knowledge of the person who administered the vaccine associated with the young mother's death. Investigators also observed the immunization of other patients at the vaccination center. Based on this evaluation, the nurse's performance was considered adequate and the health center was determined to be well organized. The cold chain was in good working order and possessed sufficient cooling capacity. Nevertheless, given the rush in implementing the campaign, the nurse had not been

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specifically trained in yellow fever vaccination. The vaccination center also lacked equipment to properly dispose of vaccine waste products.

The investigating team visited Ica DIRESA to evaluate the quality of the cold chain, which met technical specifications for biological storage and transportation. Around this time, upon learning that the patient had received vaccine from the same lot as the medical student, the MoH evaluated the storage and transportation of vaccines at all levels and requested that samples from the vaccine vials of the lot in question be sent to the INS for safety and potency evaluations. The MoH then requested technical assistance from PAHO, which offered to investigate the lot's production in its country and laboratory of origin (Bio-Manguinhos, Brazil).

1. **Do you agree with the need to perform potency and safety tests? Justify your response.**

2. **What are the recommendations for shipping vaccine samples?**

D. Clinical and laboratory assessment

While admitted at the Dos de Mayo Hospital, the patient received treatment with intravenous fluids, vasopressors, hydrocortisone, meropenem, vancomycin, and insulin, and was placed on mechanical ventilation. She experienced fever (40.2°C), jaundice, edema, and thrombocytopenia (24,000 blood platelets/mL). Results of liver function tests were as follows: AST (SGOT): 735 U/L; ALT (SGPT): 167 U/L; bilirubin: 6.3 mg/dL; CPK: 3,173 U/L; and INR: 3. The patient also presented renal function deficiency, with serum creatinine of 3.2 mg/dL and urea of 154 mg/dL. The patient's stool culture tested positive for *Escherichia coli* O86. Likewise, in her first blood culture, Staphylococcus strains were isolated, although this was attributed to a contaminated specimen. A second blood culture tested positive for *Candida* spp. The patient died on her third day of hospitalization. The autopsy was conducted at the Dos de Mayo Hospital, and was attended by the epidemiologist of the Lima Health Directorate that has jurisdiction over the hospital.

The serum specimen analyzed by the INS yielded the following results:

- Serum IgM positive for yellow fever.
- Serum IgM negative for hepatitis B, Oropouche virus, Mayaro virus, Hantavirus, Venezuelan equine encephalitis, and rickettsiae.
- ELISA negative for HIV.

As part of the autopsy, a macroscopic anatomopathological analysis was performed and specimens were obtained for the microscopic anatomopathological and toxicological analyses, which were performed by the INS and Central Morgue. Results were as follows:

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- *Candida* in the pharynx, esophagus, and upper portion of the stomach.
- Liver: examination with hematoloxylin-eosin showed preserved liver structure, serious liver microvacuolization with midzonal predominance, limited lymphoplasmocytic inflammatory component with predominance of portal spaces, and liver steatosis.
- Kidney: tubular necrosis.
- Brain: cerebral edema.
- Immunohistochemistry: positive for yellow fever antigen in the brain, kidney, liver, and lung.

1. Does the patient meet the case definition for VTD? Justify your response.

2. What specimens should be obtained during the autopsy?

Given the tests results, increasing media pressure, and limited information on viscerotropic disease, the MoH requested support from PAHO, the United States Naval Medical Research Unit 6 (NMRU-6, known as NMRCD at time of the outbreak) in Peru, and the U.S. Centers for Disease Prevention and Control (CDC). Serum and tissue specimens were subsequently provided to NMRU-6 and the CDC. Final results from the CDC became available in February 2008. These included: viral culture: negative; RT-PCR and Q-PCR: positive in the serum, urine, lung, kidney, liver, and brain, with viral concentrations ranging from 2.4×10^2 to 1.1×10^4 PFUeq/mL; and immunohistochemistry: positive and limited in the lung, liver, and kidney.

Additionally, anatomo-pathology tests performed by the INS and the Central Morgue yielded the following results:

- Thyroid: follicular thyroid neoplasm and chronic thyroiditis.
- Liver: hematoloxylin-eosin examination showed midzonal necrosis, limited lymphoplasmocytic inflammatory component with predominance of portal spaces, serious liver microvacuolization, and intracellular cholestasis.
- Kidney: vascular congestion, hemorrhage foci, and tubular necrosis.
- Lung: edema, congestion, and vascular expansion.
- Brain: cerebral edema and discreet gliosis.
- Immunohistochemistry for yellow fever: positive (polyclonal and monoclonal antibodies) in the lung, liver, kidneys, and brain.
- Toxicology tests: negative.

3. Based on this information, how do you believe the ESAVI Committee classified the case?

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A. State of emergency services, the health system, and the patient referral system

The earthquake that struck Peru in August 2007 rendered useless many of the health centers in Ica. To make matters worse, the death of two patients following vaccination against yellow fever generated considerable concern among the local population. As a result, a large number of vaccinated individuals sought care from emergency departments that already faced high demand and logistical constraints. These circumstances, as well as limited knowledge on VTD, impaired the ability of healthcare workers to accurately diagnose and treat the disease.

To improve care, health officials developed a provisional case definition of suspected VTD. Patients meeting the definition were required to be observed for at least 12 hours in the hospital and for an additional 72 hours in their homes under the responsibility of the Ica DIRESA. For all cases, patient vaccination histories had to be obtained. To that end, the immunization program created a database to monitor all individuals vaccinated during the campaign. In a 10-day period, 139 people sought care for fever, diarrhea, nausea, and other symptoms similar to those of the deceased patients.

1. **Develop a case definition for suspected VTD associated with the yellow fever vaccine that may be used to rapidly detect cases in a situation similar to the one described above.**

2. **What other measures could be taken to decrease demand of health services?**

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B. Third case: a vaccinated 79-year-old man

On 10 October 2007, a new ESAVI case was reported in a 79-year-old man, who had been transferred to the Ica Regional Hospital from a hospital in Nazca (a province south of Ica). The patient presented abdominal pain syndrome, acute diarrheal disease, and hypovolemic shock. He was diagnosed with congestive heart failure, acute dysenteric diarrheal disease, and shock of undetermined etiology. Despite not being part of the campaign's target age group, the patient had received yellow fever vaccine on 1 October. While hospitalized, the patient deteriorated rapidly. On 11 October, he was placed on mechanical ventilation, but, despite the efforts of health workers, he died the same day. The patient had a history of allergy to sulfa drugs and had undergone prostatectomy in October 2002.

The results of the patient's tests at the Ica hospital were: AST (SGOT): 416 U/L; ALT (SGPT): 231 U/L; total bilirubin: 2.93 mg/dL; direct billirubin: 2.25 mg/dL; and prostatic specific antigen: 76 ng/mL. The results of RT-PCR and Q-PCR showed partial viral sequences 100% homologous to the 17DD vaccine virus in the serum, lung, liver, kidney, and brain, with viral concentrations ranging from 1.9×10^2 to 3.5×10^4 PFUeq/mL.

1. **How would you classify this case? Justify your answer.**

2. **Do aspects of this case suggest a programmatic error? Explain.**

3. **Who else may be at risk for developing VTD associated with yellow fever vaccine? How long should follow-up activities last?**

C. Identification and follow-up of other persons at risk

To identify and follow-up with other patients at risk, investigators reviewed lists of patients who had received vaccine from the same vial as the deceased patient in order to determine if they had developed an ESAVI. After a few days, most vaccinated individuals had been located, and it was reported that none had developed important clinical symptoms.

Since the first case of VTD following yellow fever vaccination occurred in a university student who had been vaccinated with many of her classmates, the entire cohort of vaccinated students was evaluated. Although some experienced mild or moderate events (among them the boyfriend of the deceased patient), none presented significant clinical symptoms. Additionally, no one vaccinated at the same facility as the known cases reported significant adverse events.

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1. **What strategies would you recommend to detect other cases of VTD associated with yellow fever vaccine?**

2. **What could be done to capture vaccinated individuals in a timely manner? What minimum variables should be collected?**

3. **How would you structure an active case-finding strategy to detect VTD cases, taking into account the largely urban population?**

D. Retrospective epidemiological investigation

The surveillance team proposed implementing an active, institutional, and retrospective case-finding system in order to determine whether other cases of VTD associated with yellow fever vaccine had occurred. Since the disease has a high fatality rate and clinical symptoms requiring hospitalization, investigators reviewed emergency service records and discharge databases of all Ica hospitals that cared for patients following the earthquake. Municipal death records were also evaluated.

To identify potential cases, both searches used a time period stretching from the start of the campaign to 30 days after its completion. Reviewing a large number of registries would have required a multi-stage case strategy, and in an initial, rapid search of cases, suspected patients with VTD would have been identified by their clinical symptoms. Due to limited knowledge of the disease, however, it was not clear which cases could be classified as probable or confirmed. For this reason, the country requested support from PAHO and the CDC in implementing a field investigation.

E. Methods and results of the investigation

The PAHO/CDC team of experts arrived in Lima at the end of October. Shortly thereafter, the team analyzed the cases and reviewed the outbreak investigation. The team decided to use the following definitions:

a. **Suspected case:**

Any patient presenting fever >38°C (or who feels feverish) for >24 hours and one or more of the following signs or symptoms:

- Intense headache.
- Sensory disturbance.
- Tonic-clonic seizure.

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- Nausea, vomiting.
- Watery stools.
- Myalgia lasting >24 hours.
- Arthralgia lasting >24 hours.
- Increased respiratory rate (>20 breaths/min).

b. Probable case:

Any suspected case that was vaccinated less than 15 days before the onset of symptoms, with no evidence of other etiologies to explain clinical symptoms, and with fever and one or more of the following signs and symptoms:

Nausea, vomiting, malaise, watery stools, myalgia, arthralgia, dyspnea, **and** one or more of the following:

- Elevated serum transaminases ≥3 times normal level.
- Elevated total serum bilirubin ≥1.5 times normal level.
- Serum creatinine ≥1.5 times normal level.
- Total CPK: ≥5 times normal level.
- Thrombocytopenia (blood platelets <100,000/mL).
- Myocarditis (compatible abnormalities detected by electrocardiogram, echocardiogram, or cardiac enzymatic changes, or inflammation confirmed by cardiac tissue biopsy).
- Elevation of prothrombin time or activated partial thromboplastin time or elevated INR.
- Histopathology compatible with yellow fever (e.g., midzonal hepatic necrosis or Councilman's bodies).

c. Confirmed case:

Any probable case that has one or more of the following:

- Isolation in blood of 17D[2] yellow fever virus >7 days after vaccination, and/or through PCR >11 days following vaccination.
- Specific antigen for yellow fever in visceral tissue demonstrated by immunohistochemistry.
- Isolation of 17D[2] yellow fever virus in visceral tissue.
- Amplification of the 17D[2] yellow fever RNA virus in visceral tissue.

Presently, the case definitions developed by the *Brighton Collaboration* are recommended for making case classifications (Annexes 3-5).

1. **Compare the case definition used in Peru with those developed by the *Brighton Collaboration* Working Group.**

[2] Presence of virus 17D (and of all 17D vaccine-derived viruses) confirmed by nucleotide sequencing.

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2. **Reviewing the causality criteria below, which are consistent with VTD following yellow fever vaccination?**

   The field investigation was conducted on 13-23 November 2007 and included all five provinces of Ica: Chincha, Ica, Nazca, Palpates, and Pisco. Investigators sought medical records for all individuals who received care or died between 23 September and 6 November. A total of 28,788 medical records were reviewed, among which 311 cases met the criteria for suspected cases. Of those, five were classified as probable cases of VTD following yellow fever vaccination. Four of these had been captured by the ESAVI surveillance system and associated with yellow fever vaccination. The fifth was discarded because the patient had not received yellow fever vaccination. No suspected cases of neurologic diseases associated with the yellow fever vaccine were identified.

3. **If you find yourself in a similar situation in your country, how would you conduct a prospective search for cases?**

TIME AVAILABLE: 90 MINUTES

A. Fourth case: a patient with an autoimmune disorder

On 23 October 2007, a case was reported in a 49-year-old female patient at the emergency room of the Social Security Hospital of Chincha. The patient's symptoms included intense headache, oliguresis, generalized edema, mild jaundice, and signs of dehydration. She had a history of hypertension, rheumatoid arthritis, systemic lupus erythematosus, and chronic renal failure. She also had difficulty moving due to stroke sequelae.

The patient had been vaccinated on 24 September at the insistence of a nurse in the Ica region, despite the patient's and her family members' initial reluctance. An analysis of the patient's vaccination history revealed she had received a vaccine from lot 121Z. Three days prior to vaccination, the patient had developed pain in her hip that hindered her ability to walk. When the pain increased, she went to the Chincha hospital on 28 September, where the attending physician prescribed treatment with methotrexate and tenoxicam, both in tablet form, as well as diclofenac and dexamethasone in ampoules. Days later, the patient experienced various episodes of melena and three episodes of vaginal bleeding. On 12 October, she was again seen at the Social Security Hospital for intense headache and malaise. This was the patient's last contact with the health care system prior to 23 October, and she was prescribed analgesics and prednisone.

On 24 October, the patient was transferred to the Rebagliati National Hospital in Lima. She arrived in poor health and was diagnosed with metabolic encephalopathy, metabolic acidosis, decompensated chronic renal insufficiency, electrolyte disorder, icteric syndrome of unknown etiology, systemic lupus erythematosus, and exacerbation of lupus activity. Despite receiving treatment in the emergency intensive care unit, the patient died on the afternoon of 24 October.

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Laboratory tests performed at the Chincha hospital showed AST (SGOT): 91 U/L; ALT (SGPT): 128 U/L; total bilirubin: 5.2 mg/dL; direct bilirubin: 4.2 mg/dL; and indirect bilirubin: 1.0 mg/dL. Yellow fever immunohistochemistry (monoclonal and polyclonal antibodies) was slightly positive in the liver and kidney. Additionally, results from the RT-PCR and Q-PCR were positive in the kidney and slightly positive in the liver, with a viral concentration of 1 x 10^4 PFUeq/mL in the kidney.

1. **Would you have vaccinated this patient? Explain.**

2. **What are the indications, contraindications, and precautions of yellow fever vaccine (*2,17*)?**

3. **Could this case be considered the result of a programmatic error? Support your answer.**

B. Risk communication

Shortly before the international team's arrival, the parents of a 1-year-old girl who died in Lima following yellow fever vaccination confronted ministry spokespersons. While it was later determined that the cause of the child's death was not VTD, the time required to complete the investigation and the MoH's poor relationship with the press meant that the true cause of the child's death was not widely reported. The child's case was the last reported serious ESAVI following yellow fever vaccination, and it was officially classified as a coincidental event after the patient's death from sepsis and hemolytic anemia.

The PAHO/CDC team of technical experts arrived in the country at the end of October and remained until mid-November (Annex 6). The team supported the MoH in two fundamental ways: 1) by participating in field investigations and helping to classify suspected cases of VTD associated with yellow fever vaccine and 2) by responding to media inquiries, reinforcing the messages of ministry spokespersons, and thereby improving communication with the public.

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1. **What traits should a spokesperson possess?**

2. **How should a spokesperson prepare for an interview with mass media outlets?**

C. Evaluation of the vaccine lot

The vaccine administered in Peru was donated by the government of Bolivia, which, in turn, had received it as a donation from the Bolivarian Republic of Venezuela. In Venezuela, 73,000 doses of lot 121Z had been administered, with no reported serious ESAVI cases. In Bolivia, vaccines from lot 121Z had not been administered. However, 23,000 doses were stored in the country's central warehouse.

Test results on the potency and quality of the vaccine performed by the INS of Peru showed no abnormalities. Production procedures were evaluated at the manufacturing laboratory, even though the WHO had prequalified the vaccine. Using a validated assay, a laboratory contracted by WHO analyzed retention samples from the manufacturer and samples of vaccine lot 121Z used in Ica at the end of its storage period. No differences in vaccine potency were observed between distributed samples and those obtained at the end of the storage period. These results were consistent with those from the manufacturer, INS, and CDC, which showed no reductions in the dose or potency of the vaccine lot.

The manufacturer also performed tests to detect bacteria, fungi, mycoplasma, and several known polluting avian viral agents (although not mammalian viruses) in the vaccine's preparation products. Results were negative.

All other evaluations also yielded satisfactory results, and no defects in product quality were found to explain the four cases in Ica.

D. Virological evaluation

Studies revealed high viremia, elevated viral load, and broad viral tissue distribution (including in many vital organs) in those who had died following vaccination. These results are consistent with previous reports of fatal cases of VTD following yellow fever vaccination.

Stability studies of lot 121Z indicated there had not been a significant loss of viral viability, despite the fact that the vaccine was nearing its expiration date (October 2007) and had been transported on multiple occasions among countries. Nor was a variant of the virus found in tissue

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specimens of those had died following receipt of lot 121Z. The consensus nucleotide sequence of the 17DD vaccine virus isolated was essentially identical to the sequence that the laboratory has used for vaccine production since 1984. In short, laboratory studies did not reveal changes in the vaccine virus that could explain the ESAVIs.

1. **Do you believe the following hypothesis can be rejected: lot 121Z used in Ica was inherently "more dangerous than other lots?" Please provide arguments supporting and refuting the hypothesis.**

CONCLUSIONS

In conjunction with national authorities, a panel of international experts (Annex 6) was formed to analyze the reported cases and determine what causal relationship existed between the cases and the yellow fever vaccine. The panel reached the following conclusions (*18*):

- The occurrence of cases of viscerotropic disease associated with yellow fever vaccine clustered in time and space with a single lot of yellow fever vaccine (050VFA-12Z, Bio-Manguinhos, Brazil) had no precedent.
- The incidence of VTD following yellow fever vaccination was 7.9-11.7 cases per 100,000 doses administered—over 20 times the incidence previously notified. The incidence among individuals who received the vaccine from lot 121Z was not statistically greater ($p<0.05$) than the incidence observed among people who received a vaccine from a different lot (123Z) during the mass campaign.[3]
- Clinical, virological, and pathological evidence was sufficient to classify four cases as VTD following yellow fever vaccination (all fatal) and one case as probable (survived). The cause of death was a generalized infection with the 17DD vaccine virus, probably associated with severe immunity response syndrome.
- The consensus sequence of the genome of vaccine lot 121Z, in both the parental seed lot and in viral RNA collected in patient specimens, indicated there were no genetic changes in the vaccine virus that could have caused the cases of VTD associated with yellow fever vaccine.
- No sign of virological or other factors related to the vaccine production process were found that pointed to anomalies inherent to vaccine lot 121Z that could explain the higher frequency of VTD following yellow fever vaccination among individuals vaccinated from the lot.
- Peru's surveillance system for detecting adverse events proved sensitive. No other fatal cases of VTD following yellow fever vaccination in Ica were detected after the retrospective review of hospital and death records.
- Factors related to the host may have increased the risk of severe infection with viral strain 17DD. These include old age in one case and autoimmune disease (lupus) in another. In two cases, potentially immunosuppressive medication was administered following vaccination. Prior to these cases, autoimmune disease was not recognized as a risk factor for VTD following yellow fever vaccination. However, greater attention should be paid to autoimmune diseases in the future. The apparent association of diarrhea in cases of VTD suggests the direct participation of the yellow fever virus in gastrointestinal tract infection, or as an aggregate cofactor with another infectious agent (Annex 7).

[3] Given an expected rate of 0.4 cases of viscerotropic disease associated with yellow fever vaccine per 100,000 vaccine doses, it is extremely unlikely that four cases would occur after 42,742 doses of lot 121Z by chance alone ($p<0.001$) (*15*).

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- In this outbreak, factors related to the population were probably significant. Among these are the wide use of yellow fever vaccine among adults (many with risk factors, such as old age) in a non-endemic area and a population that had not been previously vaccinated against yellow fever and thus lacked protective immunity against the disease.

Today, the search continues for cases of viscerotropic disease following yellow fever vaccination or factors that increase predisposition to this adverse event. International research protocols have been developed, as have case definitions to identify and investigate suspected cases. The comprehensive study of all cases has the potential to make a great difference in our understanding of this adverse event and the strategies needed to prevent it.

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ANNEX 1: PRE- AND POST-TESTS ON YELLOW FEVER CASE STUDIES

Pseudonym: _____

1. What countries are endemic for yellow fever in the Region of the Americas?
   a. Argentina, Chile, and Paraguay.
   b. Brazil, Bolivia, and Venezuela.
   c. Costa Rica, Guyana, and Suriname.
   d. Ecuador, French Guiana, and Peru.
   e. b and d are correct.

2. Which of the following is a **contraindication** against yellow fever vaccination?
   a. Laboratory personnel exposed to the yellow fever virus.
   b. Pregnant women, except in epidemiological emergencies when the risk of yellow fever is very high.
   c. Women who are breastfeeding and will be traveling to endemic areas.
   d. b and c are correct.

3. Which of the following is a **precaution** against yellow fever vaccination?
   a. People of any age with a thymus disorder.
   b. Asymptomatic patients infected with HIV.
   c. People with severe febrile diseases compromising their general health status.
   d. Women who are breastfeeding and will be traveling to endemic areas.
   e. b and d are correct.

4. Which of the following is/are not a serious ESAVI associated with the yellow fever vaccine?
   a. Viscerotropic disease.
   b. Guillain-Barré Syndrome (GBS).
   c. Fever, chills, headache, myalgia.
   d. Hypersensitivity reactions.
   e. a and d are correct.

5. Why should this cluster of cases of viscerotropic disease associated with vaccination against yellow fever be reported immediately to the World Health Organization (WHO), as stipulated by the International Health Regulations (IHR)?
   a. The event is unusual.
   b. The number of cases is higher than expected, since the incidence was 1 case per 42,742 doses.
   c. The vaccine is being massively administered to a susceptible population.
   d. b and c are correct.
   e. a and b are correct.

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6. Which of the following criteria would you consider in evaluating a case of viscerotropic disease?
   a. Clinical: *Faget* sign (bradycardia and fever).
   b. Clinical: jaundice and hemorrhage.
   c. Timing post-vaccination: the patient was vaccinated 7-30 days after the onset of symptoms.
   d. b and c are correct.
   e. All of the above are correct.

7. Which of the following is a **major criterion** used in the definition of viscerotropic disease?
   a. Jaundice.
   b. Petechiae or purpura.
   c. Melena.
   d. Need for mechanical ventilation.
   e. c and d are correct.

8. Which of the following criteria must be met in order to establish definitive causality between viscerotropic disease and the yellow fever vaccine?
   a. Yellow fever 17D virus concentration in blood ≥2 $\log_{10}$ PFU/mL, but <3 $\log_{10}$ PFU/mL on any day 1-10 days post-vaccination.
   b. Isolation of yellow fever 17D virus from blood >18 days post-vaccination.
   c. Isolation of yellow fever 17D virus from blood >10 days post-vaccination.
   d. a and b are correct.
   e. All of the above are correct.

9. During an ESAVI investigation, it is recommended to request the reference laboratory to conduct potency tests of the vaccine in question. What is your opinion of this recommendation?
   a. It is always correct.
   b. It is never correct.
   c. It depends on the results of the investigation.
   d. It depends on the results of the sterility, toxicity, and identity tests.

10. During the investigation of a serious ESAVI that was widely publicized in the media, it was not possible to determine the cause of the event. What actions should health workers take?
    a. Report the findings of the investigation to interested parties.
    b. Clearly communicate the results and include mass media outlets.
    c. Do not make any statement, since no conclusion was reached.
    d. Avoid mentioning the issue with the media in order to maintain the public's trust in the immunization program.
    e. a and b are correct.

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ANNEX 2: RECOMMENDED LABORATORY TESTS FOR SERIOUS ESAVIS FOLLOWING YELLOW FEVER VACCINATION

A. All serious ESAVIs

First set of laboratory tests

SPECIMEN	LABORATORY TEST	CLINICAL REASONING
Blood	Complete blood count and platelet count	Establish clinical baseline, rule out bacterial infection
Blood	Thick blood film	Rule out malaria, infection by *Borrelia*
Urine	Urinalysis	Evaluate proteinuria, bleeding
Cerebrospinal fluid	Turbidity	Establish clinical baseline, rule out bacterial infection
	Cell count	Establish clinical baseline, rule out bacterial infection
	Protein	Rule out meningitis

B1. Suspected cases of serious viscerotropic disease

Second set of laboratory tests for clinical evaluation and differential diagnosis

SPECIMEN	LABORATORY TEST	CLINICAL REASONING
Blood	Complete blood count and platelet count	Rule out other etiologies
	Blood culture	Rule out bacteremia
Serum	Transaminases	Evaluate liver enzymes and function
	Direct and indirect bilirubin	Evaluate liver enzymes and function
	Alkaline phosphatase	Evaluate liver enzymes and function
	Tests for Hepatitis B and C	Rule out other hepatitis viruses
	Urea nitrogen	Evaluate renal function
	Creatine	Evaluate renal function
	Amylase	Evaluate pancreas function
	Creatinine phosphokinase	Rhabdomyolysis evaluation
	Partial prothrombin time Partial thromboplastin time	Coagulation panel
Urine	Urinalysis	Rhabdomyolysis evaluation
	Urea antigen	Rule out leptospirosis
Saliva	PCR	Detect yellow fever virus
Feces	PCR	Detect yellow fever virus
Other fluids	PCR	Detect yellow fever virus
Serum	IgM, IgG antigens against yellow fever (acute and convalescent)	Confirm infection by or vaccination against yellow fever
	PCR/viral culture	Rule out wild-type yellow fever

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B2. Suspected cases of neurologic disease

Second set of laboratory tests

SPECIMEN	LABORATORY TEST	CLINICAL REASONING
Cerebrospinal fluid	Gram stain and culture	Rule out bacterial infection
	Blood cell count: red blood cells, white blood cells, and differential	Rule out bacterial infection
	Glucose	Rule out bacterial infection
	Protein	Rule out bacterial infection
Blood	Thick blood film	Rule out malaria
Cerebrospinal fluid (paired serum specimen)	PCR/culture within first 7 days	Confirm presence of yellow fever virus
	IgM against yellow fever	Confirm presence of yellow fever virus
Serum	PCR/culture	Confirm presence of yellow fever virus
	Antibody tests	Rule out infection with other viruses
Feces	Viral culture	Rule out polio, other enteroviruses

Source: Pan American Health Organization. *Serious adverse events associated with administration of yellow fever vaccine during a mass vaccination campaign*. Peru, 2007. Findings from an expert panel. Washington D.C.: PAHO, 2008.

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ANNEX 3: CASE DEFINITION OF VISCEROTROPIC DISEASE,[1] ACCORDING TO THE *BRIGHTON COLLABORATION*[2]

Level 1 of diagnostic certainty

≥3 major criteria[3]

Level 2 of diagnostic certainty

2 major criteria[3]

or

1 major criterion and ≥2 minor criteria[3]

Level 3 of diagnostic certainty

≥3 minor criteria

or

1 major criterion and 1 minor criterion[3]

Major and minor criteria used in the case definition of viscerotropic disease

MAJOR CRITERIA	
Hepatic:	Total bilirubin >1.5X ULN[4] [≥1.5X patient's baseline value if known] **OR** ALT or AST ≥3X ULN[4] [≥3X patient's baseline value if known]
Renal:	Creatinine ≥1.5X ULN[4] [≥1.5X patient's baseline value if known]
Musculoskeletal:	CPK ≥5X ULN[4]
Respiratory:	Oxygen saturation ≤88% on room air **OR** Requirement for mechanical ventilation
Platelet disorder:	Platelets <100,000/µl

(*Continued*)

[1] The case definition should be applied when there is temporal association with vaccination and no clear alternative diagnosis to account for the symptoms.

[2] Previously published WHO guidelines regarding the case definition and data collection for VTD associated with yellow fever vaccine preceded the development of these guidelines. However, the more recent and detailed *Brighton Collaboration* case definitions and guidelines are preferred.

[3] Whenever ≥1 major criteria or both a major and minor criteria are used to meet the case definition, they must each represent different organ systems (e.g., hepatic or renal).

[4] ULN = Upper limit of normal for the reference range of normal values reported by the clinical laboratory performing the indicated test.

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Annex 3 Table. (*Continued*).

MAJOR CRITERIA	
Hypertension:	Requirement for vasopressor drugs to maintain systolic blood pressure.
Coagulopathy:	INR ≥1.5 or prothrombin time ≥1.5X ULN[4] or activated partial thromboplastin ≥1.5X ULN[4] or elevated FDP[5] or hemorrhage from more than one site.[6]
Hypertension:	Requirement for vasopressor drugs to maintain systolic blood pressure.
Coagulopathy:	INR ≥1.5 or prothrombin time ≥ 1.5X ULN[4] or activated partial thromboplastin ≥1.5X ULN[4] or elevated FDP[5] or hemorrhage from more than one site.[6]
MINOR CRITERIA	
Hepatic:	Jaundice
Renal:	Urine output <500 mL urine/24 h for adults Urine output <0.5 mL/kg/h for children[7]
Musculoskeletal:	Positive urine dipstick test for blood with a negative urine microscopy exam for red blood cells
Respiratory:	Increased respiratory rate for age[8]
Platelet disorder:	Petechiae or purpura present
Hypertension:	Systolic BP <90 mm Hg for adults Systolic BP <5th percentile for children aged <16 years
Coagulopathy:	Clinically evident hemorrhage (one of the following):[9] • Epistaxis • Hematemesis • Melena • Hematochezia • Hemoptysis • Metrorrhagia or menorrhagia • Gingival hemorrhage • Persistent bleeding from needle puncture sites

[5] FDP = Fibrin degradation products.

[6] See coagulopathy criteria below for list of included hemorrhage sites.

[7] Applies to children aged <13 years.

[8] Age-specific thresholds for increased respiratory rate (breaths/min): 6-11 months: >50; 1-5 years: >40; and >6 years: >20. (Modified from WHO. *The management of acute respiratory infections in children: practical guidelines for outpatient care.* 1995.) Available at: http://whqlibdoc.who.int/publications/1995/924154477^eng.pdf.

[9] Due to the relatively high baseline of hematuria due to various causes, hematuria is too nonspecific to be used as an indicator of coagulopathy for the VTD case definition.

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ANNEX 4: VISCEROTROPIC DISEASE (VTD) VTD-CAUSALITY CRITERIA FOR YELLOW FEVER VACCINE, ACCORDING TO THE *BRIGHTON COLLABORATION*

I. Definite yellow fever vaccine-associated causality

One or more of the following are present:

1. Yellow fever 17D[1] virus isolation from blood >10 days post-vaccination.
2. Yellow fever 17D[1] virus concentration in blood ≥3 $\log_{10}$ PFU/mL on any day.
3. Yellow fever 17D[1] viral amplification from blood ≥14 days post-vaccination.
4. Isolation of yellow fever 17D[1] virus **OR** amplification of yellow fever 17D[1] viral RNA from tissue and histopathology compatible with yellow fever (e.g., liver midzonal necrosis, Councilman's bodies).
5. Yellow fever virus-specific antigen in tissue with characteristic vaccine-associated distribution (extrahepatic or mesenchymal cell involvement) demonstrated by immunohistochemistry[2] **AND** histopathology consistent with yellow fever (e.g., liver midzonal necrosis, Councilman bodies) **AND NO** history of being in a yellow fever-endemic or -epidemic area within 10 days of symptom onset.

II. Probable yellow fever vaccine-associated causality

One or more of the following are present:

1. Yellow fever 17D[1] virus isolation from blood 8-10 days post-vaccination.
2. Yellow fever 17D[1] virus concentration in blood ≥2 $\log_{10}$ PFU/mL, but <3 $\log_{10}$ PFU/mL on any day 1-10 days post-vaccination.
3. Yellow fever 17D[1] viral RNA amplification from blood ≥14 days post-vaccination..
4. Isolation of yellow fever 17D[1] virus **OR** amplification of yellow fever-endemic or 17D[1] viral RNA from tissues.
5. Histopathology consistent with yellow fever (e.g., liver midzonal necrosis, Councilman bodies) **AND** no history of being in a yellow fever-endemic or -epidemic area within 10 days of symptom onset.[3]

III. Suspect yellow-fever vaccine-associated causality

One or more of the following are present:

1. Histopathology consistent with yellow fever (e.g., liver midzonal necrosis, Councilman bodies) **AND** history of being in a yellow fever-endemic or -epidemic area within 10 days of symptom onset.
2. Yellow fever virus-specific antigen in tissue demonstrated by immunohistochemistry[2] **AND** history of being in a yellow fever-endemic or -epidemic area within 10 days of symptom onset.

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[1] Confirmed as 17D virus by nucleotide sequencing.

[2] Immunohistochemistry (IHC) performed by using polyclonal antibody to yellow fever viral antigen that reacts to both 17D yellow fever virus and wild type yellow fever virus.

[3] If both the fourth and fifth criteria for probable yellow fever vaccine-associated causality are present, this is equivalent to the fourth criteria for definite yellow fever vaccine-associated causality. In this case, the condition of "no history of recently being in a yellow-fever endemic/epidemic area within 10 days of symptom onset" is unnecessary, because the possibility of wild-type yellow fever virus infection has been eliminated by the identification of the 17D yellow fever vaccine virus in the tissues.

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IV. Insufficient data to determine yellow fever vaccine-associated causality

One or more of the following:

1. No yellow fever testing done.

   OR

2. Yellow fever testing done and results do not meet any of the criteria for causality levels 1, 2, or 3 as previously indicated.[4]

[4] The presence or absence of serum yellow fever virus-specific antibodies (IgM or IgG) has not been demonstrated to correlate with or be predictive of yellow fever vaccine causality in cases of VTD.

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ANNEX 5. EVENT CLASSIFICATION ACCORDING TO THE *BRIGHTON COLLABORATION*

A. The case definition applies when there is temporal association and there is no clear alternative diagnosis to explain the symptoms.

B. Once this is determined, **it should be determined if the clinical picture meets the case definition:**

1. The event meets the case definition (Annex 3): that is, the case meets one of the three levels of diagnostic certainty:
   a. Level 1 of diagnostic certainty: ≥3 major criteria.
   b. Level 2 of diagnostic certainty: 2 major criteria or 1 major criterion and 2 or more minor criteria.
   c. Level 3 of diagnostic certainty: ≥3 minor criteria or 1 major criterion and 1 minor criterion.

   The case definition levels differ in diagnostic certainty, not in clinical severity of VTD. Similarly, levels of diagnostic certainty do not reflect causal association with a given vaccine.

2. If an adverse event reported as VTD does not meet levels 1, 2, or 3 of the VTD case definition, then additional diagnostic studies should be done to further search for diagnoses not previously considered that could explain the clinical picture of the ESAVI.

3. Reported VTD with insufficient evidence to meet the case definition. If the evidence available for the event is insufficient to permit classification by any level of diagnostic certainty (e.g., because of missing information), such an event should be categorized as "reported VTD with insufficient evidence to meet the case definition." Notations should be made as to what evidence is missing.

4. If there is adequate evidence that an event does not meet the case definition, the event should be rejected and should be reported as "not a case of VTD." Such evidence is considered adequate if the investigation reveals negative findings for all necessary criteria (necessary conditions) for diagnosis.

C. **Once it is determined that an event meets the case definition** (levels 1-3) or is classified as reported VTD with insufficient evidence to meet the case definition, the event should be **classified according to its causality with the yellow fever vaccine** in one of the categories of Annex 4.

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ANNEX 6: INTERNATIONAL TECHNICAL COOPERATION

Following the report to PAHO/WHO of four fatal adverse events associated with yellow fever vaccination (sub-strain 17DD), technical collaboration activities began with Peru's MoH, which had already started investigating the cases. A panel of experts on hemorrhagic yellow fever, virology, epidemiology, yellow fever vaccination, and vaccine quality was established. In conjunction with national authorities, the panel aimed to analyze the reported cases and determine if a causal relationship with the vaccine existed. The panel included experts from WHO, PAHO, CDC, the University of Texas in Galveston, the Butantan Institute of Brazil, and Kleiner, Perkins, Caufield, & Byers (KPCB) Pandemic and Biodefense Fund of the United States.

As a part of its work, the panel issued alerts to countries in the Americas endemic for yellow fever. The first message, sent on 2 November 2007, provided an update of the outbreak in Peru and requested that countries:

- Immediately suspend use of yellow fever vaccine lot 05OVFA121Z from Bio-Manguinhos, and lots related to it in production, specifically lots 05OVFA118Z, 05OVFA119Z, 05OVFA120Z, 05OVFA122Z, 05OVFA123Z, 05OVFA124Z, 05OVFA125Z, and 050VFA126.
- Intensify surveillance to detect serious adverse events following vaccination.

The message was followed by conference calls held with all target countries, with the aim of reviewing and classifying serious ESAVI and VTD cases. Other measures taken by the international team included:

- Sending a team of experts from PAHO/WHO and the CDC to the field to continue gathering the epidemiological, virological, molecular, and pathological data needed to classify reported cases. The regional network of reference laboratories facilitated technical cooperation between countries in the use of technologies not available in many national reference laboratories.
- Performing independent analyses of manufacturing processes, vaccine quality, and the distribution and use of the aforementioned lots.

Additionally, laboratory tests on vaccine samples were conducted to determine whether the notified events had an association with the lots in question. One set of vaccine vials remains stored in the CDC and in Peru, so that they may be reexamined when more advanced technology becomes available.

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ANNEX 7: CLINICAL CHARACTERISTICS OF CONFIRMED CASES OF VTD ASSOCIATED WITH YELLOW FEVER VACCINE IN PERU

CASE	AGE/ SEX	PRE-EXISTING CONDITIONS	DAYS BETWEEN VACCINATION AND		SIGNS OR SYMPTOMS	LABORATORY ABNORMALITIES	RESULTS AND FINDINGS OF THE AUTOPSY
			ONSET	DEATH			
1	23 / F	Acne rosacea	1	9	Fever, headache, myalgia, malaise, nausea, vomiting diarrhea; 8 days after vaccination, developed shock, adult respiratory distress syndrome (ARDS), encephalopathy, multi-organ failure. Death.	Leukocytes: 66,400 mm^3 Platelets: 54,000/ mm^3 AST: 78 U/L ALT: 65 U/L Creatinine: 4.1 mg/dL CPK: 4,055 U/L	Midzonal necrosis, steatosis (liver), acute tubular necrosis, thyroid neoplasia, and chronic thyroiditis.
2	24 / F	None known	<1	14	Fever, headache, malaise, myalgia, nausea, vomiting, diarrhea. Eleven days after vaccination, presented shock, encephalopathy, acidosis, gastrointestinal bleeding, jaundice, ARDS, multi-organ failure. Death.	Hematocrit 15.5% Leukocytes: 16,300/mm^3 Platelets: 15,000/ mm^3 AST: 735 U/L ALT: 167 U/L Bilirubin: 6.2 mg/dL Blood urea nitrogen: 112 mg/dL CPK: 3173 U/L	Steatosis, focal necrosis (liver), cerebral edema, pulmonary edema, and severe *Candida* infection (larynx, trachea, stomach).
3	79 / M	Cardiac disease, prostate cancer	3	11	Fever, malaise, dyspnea, abdominal pain, vomiting, diarrhea. Nine days after vaccination, presented progressive shocks, ARDS, acidosis, renal failure. Death.	Leukocytes: 17,400/mm^3 Platelets: 249,000/ mm^3 AST: 416 U/L ALT: 231 U/L Creatinine: 2.8 mg/dL	Midzonal necrosis, steatosis (liver), acute tubular necrosis, and depletion of white pulp (spleen).

(*Continued*)

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Annex 7 (*Continued*).

CASE	AGE/ SEX	PRE-EXISTING CONDITIONS	DAYS BETWEEN VACCINATION AND		SIGNS OR SYMPTOMS	LABORATORY ABNORMALITIES	RESULTS AND FINDINGS OF THE AUTOPSY
			ONSET	DEATH			
4	49 / F	Systemic lupus erythematosus, rheumatoid arthritis	Unclear (7-18 days)	30	Headache, malaise, arthralgia; 29 days after vaccination, hospitalized with generalized edema, jaundice, altered mental status, acidosis bleeding, difficulty breathing. Death.	Hematocrit: 31% Leukocytes: 5,530/mm^3 Platelets: 57,000/ mm^3 AST: 91 U/L ALT: 128 U/L Bilirubin: 5.2 mg/ dL Creatinine: 3.3 mg/dL	

Source: Adapted from Whittembury A, Ramirez G, Hernández H, Ropero AM, Waterman S, Ticona M, et al. Viscerotropic disease following yellow fever vaccination in Peru. *Vaccine.* 2009;27(43):5974-81. Epub 2009 Aug 11.

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Pan American Health Organization

Regional Office of the World Health Organization