Serious adverse events associated with yellow fever 17DD vaccine in Brazil: a report of two cases


Summary

Background The yellow fever vaccine is regarded as one of the safest attenuated virus vaccines, with few side-effects or adverse events. We report the occurrence of two fatal cases of haemorrhagic fever associated with yellow fever 17DD substrain vaccine in Brazil.

Methods We obtained epidemiological, serological, virological, pathological, immunocytochemical, and molecular biological data on the two cases to determine the cause of the illnesses.

Findings The first case, in a 5-year-old white girl, was characterised by sudden onset of fever accompanied by headache, malaise, and vomiting 3 days after receiving yellow fever and measles-mumps-rubella vaccines. Afterwards she decompensated with icterus, renal failure, and haemorrhagic signs and died after a 5-day illness. The second patient—a 22-year-old black woman—developed a sore throat and fever accompanied by headache, myalgia, nausea, and vomiting 4 days after yellow fever vaccination. She then developed icterus, renal failure, and haemorrhagic diathesis, and died after 6 days of illness. Yellow fever virus was recovered in suckling mice and C6/36 cells from blood in both cases, as well as from fragments of liver, spleen, skin, and heart from the first case and from these and other viscera fragments in case 2. RNA of yellow fever virus was identical to that previously described for 17D genomic sequences. IgM ELISA tests for yellow fever virus were negative in case 1 and positive in case 2; similar tests for dengue, hantaviruses, arenaviruses, Leptospira, and hepatitis viruses A–D were negative. Tissue injuries from both patients were typical of wild-type yellow fever.

Interpretation These serious and hitherto unknown complications of yellow fever vaccination are extremely rare, but the safety of yellow fever 17DD vaccine needs to be reviewed. Host factors, probably idiosyncratic reactions, might have had a substantial contributed to the unexpected outcome.

Introduction Brazil, like several other countries in South America, has a region of extensive Amazonian forest, in which jungle yellow fever is endemic, and a coastal zone, in which yellow fever does not occur. The boundary between these zones can be affected by periodic expansions in epizootic activity. The densely populated coastal zone has become reinfested with the urban vector of yellow fever virus, Aedes aegypti, and is thus receptive to the introduction and spread of yellow fever from the endemic area.

The endemic area includes 12 states in the western two-thirds of the country, which is inhabited by 29.3 million people. Beginning in the first quarter of 1998 and continuing to 2000, one of the largest epizootics in history occurred, leading to the occurrence of 192 registered human cases of jungle yellow fever, 88 (46%) of which had a fatal outcome. The epizootic is believed to be linked to excessive rainfall caused by the recent El Niño/southern oscillation event. The cases of yellow fever occurred over a wide area from Roraima along the Venezuelan border in the west, to Pará state in the north, Minas Gerais and Bahia in the east, and São Paulo state in the south. The remaining receptive, non-endemic coastal zone includes 15 states and is inhabited by 126.3 million people.

Due to the upsurge in yellow fever activity and the increasing mobility of the human population, cases of jungle yellow fever acquired in the endemic zone have been exported to cities of the coastal zone including Rio de Janeiro, São Paulo, and Campinas. The risk of urban yellow fever is increased by the expanding distribution and density of A. aegypti, which now includes all 27 states of the country.

In response to the increase in epizootic activity and the threat of urban yellow fever, use of yellow fever vaccine has increased strikingly in Brazil. Additionally, a new policy was established in 1998 to include the vaccine in the national programme of childhood immunisation. Between 1990 and

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2000, about 85 million doses of yellow fever vaccine were given across the whole country. Vaccine coverage in 1999 was estimated to exceed 90% in most states in the endemic zone. However, theoretical coverage estimated by the number of doses as a function of total population does not provide an accurate picture, because revaccination is commonplace and populations with easy access to fixed centres are overimmunised, whereas pockets of the population in more rural, remote, and inaccessible areas escape immunisation. For this reason, the use of mobile vaccinating teams has recently been reinstated.

The live, attenuated yellow fever 17D virus is one of the most successful vaccines developed to date. Its production in embryonated chicken eggs is well defined and efficient, a seed-lot system is used to control passage level, and the vaccine is subject to strict quality control including monkey neurovirulence testing of seed viruses. It induces long-lasting immunity in almost 100% of people vaccinated with a single dose, and it is also inexpensive. Its use has been estimated at more than 400 million doses, and it has an excellent record of safety. Only 21 cases of post-vaccinal encephalitis have been reported since the implementation of the seed-lot system in 1945; the rate of encephalitis in very young infants is 0·5–4·0 per 1000 in those younger than 9 months, and 1 per 8 million in those 9 months or older.18

Yellow fever vaccines approved by WHO are manufactured in Brazil, the USA, England, France, Russia, and Senegal. Brazil uses the 17DD substrain, whereas other manufacturers use 17D. The two substrains represent independent passage lineages from the original 17D virus developed by Theiler and Smith.19 Vaccination with 17DD virus has been associated with three reports of adverse reactions in Brazil, all of which were neurological symptoms similar to those previously reported after vaccination with other the 17D substrains.15 There was one case of encephalitis and two of paralysis, giving a prevalence of 0·09 per million (unpublished data), which is not different from the historical prevalence of post-vaccinal encephalitis—an expected, rare complication of yellow fever vaccination.

Recently, however, two fatal adverse events with an unexpected clinical syndrome occurred during a period of intensified yellow fever vaccination in Brazil. We report the clinical details of these two cases associated with use of the yellow fever 17DD vaccine virus.

Patients and methods

Patients

Case 1 was a 5-year-old white girl from Goiânia, Goiás state, who was given simultaneous but separate injections of yellow fever 17DD (lot 98UFB0882Z) and measles-mumpsrubella vaccines on Oct 8, 1999. 3 days later, she developed fever and diarrhoea, and the next day anorexia, vomiting, and vulval pruritus and exudate, at which point she was seen in the emergency room. On examination, she was febrile (temperature 39·6°C), tachypnoeic, with a hyperaemic pharynx without exudate, and no visceromegaly. She was anaemic (haematocrit 34%) and had raised serum aminotransferase concentrations (aspartate aminotransferase 114 IU/L, alanine aminotransferase 160 IU/L). The bilirubin concentration was slightly raised (18·8 μmol/L). The diagnoses of possible hepatitis A and urinary-tract infection were made.

On the fourth day of illness, she decompensated, with prostration, respiratory distress, increased vomiting and diarrhoea, dehydration, and scleral icterus. Abdominal examination revealed an enlarged, tender liver. Possible meningitis was noted, and pneumonia and meningitis were added to the differential diagnosis. The cerebrospinal fluid examination was normal. Chest radiography revealed diffuse interstitial infiltrate in the left lung. The haematocrit fell to 30% despite dehydration, and the total leucocyte count was 12 000/L (4% bands, 88% neutrophils, 8% lymphocytes). She was hydrated and treated with antibiotics. The next day, she returned to the hospital in extremis and was admitted to intensive care. On admission, she was hypotensive. On resuscitation, cyanotic, and in acute respiratory distress. She had a cardiorespiratory arrest, from which she was resuscitated, but died within the next hour.

The patient had not travelled before the event. A review of the past medical history of the patient revealed that she was a low-birthweight baby, who had had repeated episodes of diarrhoea and bronchiitis during childhood. 3 months before the current illness, she had been admitted to hospital with aseptic meningitis. Her parents were in good health and underwent testing for HIV, with negative results.

Case 2 was a 22-year-old black woman from Americana, São Paulo state, who was vaccinated against yellow fever (lot 995F029Z) on Feb 17, 2000. 4 days later, she experienced pain in the left arm (the site of vaccination) and fever. She was seen in the emergency room and analgesics were prescribed. 3 days after illness onset, she sought medical attention at a clinic in the same city for fever, headache, generalised myalgia, and sore throat. She was given symptomatic treatment. That evening she again sought medical attention at the emergency room; examination revealed intense pharyngitis. She was given penicillin and released. The next morning (4 days after onset) she returned to the clinic complaining of epigastric pain; she was given cimetidine and released. That night, she returned to the emergency room with complaints of feeling increasingly ill, fever, and myalgia. She was noted to be jaundiced and was admitted with an initial diagnosis of hepatitis. On admission, her haematocrit was 43%, white-blood-cell count 9600/L (7% bands, 77% neutrophils, 8% lymphocytes), platelet count 54 000/L, creatinine concentration 247·5 μmol/L, aspartate aminotransferase concentration 430 IU/L, and alanine aminotransferase concentration 190 IU/L. The urine analysis showed 1+ protein, 2+ urobilinogen, 103 000 white blood cells per L, and 36 000 red blood cells per L. She was treated with antibiotics and released.

The next day (5 days after onset), she again sought medical attention and was referred to a regional hospital, where she was admitted into intensive care with a tentative diagnosis of leptospirosis, dengue, or an adverse reaction to yellow fever vaccine. She had noted diminishing urine output and oedema of the arms and legs during the 24 h before admission. On admission she was acutely ill, alert, icteric, and had oedema of the arms and legs, and hepatomegaly. Her blood pressure was 110/60 mm Hg. She was treated with intravenous penicillin, ranitidine, furosamide, mannitol, and dopamine. Her white cell count was 38 000/L, total bilirubin concentration 27 700/L with a striking left-shift, haematocrit 37·7%, increased WBC to 36 000 red blood cells per L, and underwent testing for HIV, with negative results.
underwent cardiopulmonary arrest and was resuscitated, intubated, and mechanically ventilated. She was transferred to the intensive-care unit and treated with antibiotics (for possible sepsis), fresh frozen plasma, furosamide, dopamine, and norepinephrine. A central line was placed. The patient had a large haemothorax, which was drained by chest tube. She had a significant decrease in her haematocrit to 17.9%; other laboratory values were not substantially different from those noted above. She had another cardiac arrest and died. A complete necropsy was done.

The patient had been in good health and had no recent travel history. She had received no other concomitant vaccines. There was a history of hepatitis A and nephritis as a child. There was a history of rats in the house, but an inspection revealed none. The patient lived in a metropolitan urban area in which no cases of yellow fever had been reported.

**Procedures**

Blood and tissue samples were collected after obtaining consent from the parents of the patients. Specimens for virus isolation and serology were stored at −70°C until used; those for histological studies were preserved at room temperature in 10% buffered formalin.

Serum samples were initially tested by haemagglutination inhibition against yellow fever antigen (strain BeH111).

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**Figure 1: Histopathological examination of case 1**

A: Liver showing intense mid-zonal necrosis and hepatocytes preserved near centrilobular area (haematoxylin-eosin stain, original magnification ×200).
B: Higher magnification (×400) of liver showing vesicular fatty degeneration and bodies of Councilman. C: Liver showing yellow fever virus antigens by immunohistochemistry (×400). D: Spleen showing intense congestion and focal haemorrhage in red pulp area (haematoxylin-eosin, ×400). E: Spleen showing yellow fever virus antigens by immunohistochemistry, especially in white area (×400). F: Heart showing intense interstitial oedema and focal haemorrhage (haematoxylin-eosin, ×400).
Tests were done as described by Clarke and Casals with acetone-extracted serum samples and a microtitre assay. Serum samples were also tested for IgM antibodies with the MAC-ELISA procedure described by Kuno and colleagues.

For virus isolation, tissue fragments were homogenised in phosphate-buffered saline (pH 7.4) and clarified by centrifugation (2000 g, 10 min, 4°C). 0·1 mL of a 10% (w/v) suspension was inoculated into Vero cells and C6/36 A aegypti cells. The methods used for virus isolation have been previously published. Samples from case 1 were cultured at Instituto Evandro Chagas, Belém, and those of case 2 at Instituto Adolfo Lutz, São Paulo. Culture supernatants were harvested 6 days after inoculation. Simultaneously, 3-day-old suckling mice (outbred, Swiss strain) were inoculated by the intracerebral route with 0·02 mL suspension. Mice were observed for signs of illness.

Samples of frozen tissues or cell-culture supernatants were homogenised in Trizol (Life Technologies, Rockville, MD, USA) for RNA extraction. Total RNA (1 µg) was used for cDNA synthesis and amplification with oligonucleotides specific for yellow fever virus DNA. PCR products were gel purified (Quiagen gel extraction kit, Qiagen, Valencia, CA, USA) and sequenced with an ABI PRISM dye terminator kit and ABI 373 or 377 instruments (Perkin Elmer, Shelton, CT, USA).

All virus strains isolated were identified by immunofluorescence staining of cells by use of polyclonal and monoclonal antisera. Virus in heart tissue suspension contained the yield virus in one or both systems. The liver isolate was negative. Culture supernatants were harvested 6 days after inoculation. Simultaneously, 3-day-old suckling mice (outbred, Swiss strain) were inoculated by the intracerebral route with 0·02 mL suspension. Mice were observed for signs of illness.

Results

Case 2

Serum collected on the fifth day after vaccination (second day after onset of illness) was negative by haemagglutination inhibition tests for antibodies to yellow fever virus, other flaviviruses (St Louis encephalitis, Ilhéus, Rocío, Bussuquara, dengue 3, and dengue 4 viruses), alphaviruses (Eastern, Western, and Venezuelan equine encephalitis viruses, and Mayaro virus), and bunyaviruses (Oropouche, Catu, Caraparu, Guara, Maguari, and Tacatuama viruses). ELISA for IgM antibodies against yellow fever virus, dengue 1 and 2 viruses, hepatitis B core protein, hepatitis A virus, hepatitis B surface antigen, hantaviruses (sin nombre virus), arenavirus (Sabia virus), and Lepospira were negative. Virus isolation in suckling mice and C6/36 cells were negative.

Post-mortem biopsy specimens of liver, heart, spleen, and skin were examined, as well as blood obtained by from cardiac puncture. Virus was isolated from blood, heart, liver, spleen, and skin in suckling mice and C6/36 cells. Reisolation of yellow fever virus was obtained from blood and viscera fragments in Vero and C6/36 cells. The viruses recovered from blood and tissues were identified by immunofluorescence with polyclonal and monoclonal antisera. Virus in heart tissue suspension contained the highest titre in suckling mice (median lethal dose log 9–10/0·1 mL) and Vero cells (log 4·7 plaque-forming units/mL).

Histopathological examination revealed changes consistent with those seen in wild-type yellow fever. The liver showed midzonal necrosis, microvesicular fatty change, and eosinophilic degeneration of hepatocytes (Councilman bodies). The spleen showed congestion, haemorrhage, and hypoplasia of the white pulp. The heart showed congestion and focal haemorrhage. Immunocytochemical staining with polyclonal antibody against yellow fever virus showed large amounts of antigen in hepatocytes, Councilman bodies, and Kupffer cells (figure 1). Yellow fever antigen was also detected in spleen, but not in the heart tissue.

Reverse transcription PCR on RNA extracted directly from liver, spleen, and heart fragments was positive for yellow fever virus (figure 2). Sequence analysis of the amplicons corresponding to the 3' untranslated region (figure 3) revealed that the virus recovered from case 1 was the 17D vaccine virus and not wild-type virus (neither of the two genotypes present in South America). This observation is supported by the fact that nucleotides 28–93 are absent in the 3' untranslated region of South American wild-type viruses, but present in the vaccine viruses and African genotype I virus from which the 17D virus was derived. Sequence analysis also excluded African genotype II from being the causative agent, given the absence of nucleotides 32–79, the presence of nucleotides 114–116, and the overall genetic difference. The cytosine (C) residue at position 66 (figure 3) instead of a thymidine (T) suggests that the virus from case 1 corresponded to 17D virus. Further sequencing of the envelope protein gene revealed it to be 17DD virus.

Case 2

Serological tests on serum obtained 10 days after vaccination (6 days after onset) were positive by IgM ELISA for yellow fever virus, and negative for dengue 1 and 2 viruses. Haemagglutination inhibition tests for antibodies to yellow fever, St Louis encephalitis, Ilhéus, Rocío, and Eastern equine encephalitis viruses were negative. IgM ELISA and agglutination tests for Lepospira, IgM ELISA for hantavirus (sin nombre virus), and IgM for hepatitis A virus, hepatitis B core protein, and hepatitis B surface antigen were negative. Blood cultures for bacteria were negative. Virus isolation in suckling mice and C6/36 cells was attempted from heart blood, as well as from brain, spinal cord, cerebellum, kidney, liver, spleen, lung, and heart fragments. All tissues yielded virus in one or both systems. The liver isolate was passed in suckling mice, which became ill on day 5. The virus was identified by indirect immunofluorescence (in C6/36) or complement fixation (mouse brain) as a flavivirus, most probably yellow fever virus. The final identification was made by sequencing a PCR amplicon of the 3' untranslated region and the E gene. The virus was identical to the isolate from case 1 (figure 3).
Histopathological examination of liver showed changes were consistent with those seen in cases of wild-type yellow fever and were similar to those of case 1, although somewhat milder. Immunocytochemistry was positive for yellow fever antigen in hepatocytes, Councilman bodies, and Kupffer cells.

Epidemiological investigations
An extensive epidemiological investigation was done in the residential neighbourhood of the affected families both in Goiânia and Americana. Case 1’s family resided in a lower socioeconomic suburb of Goiânia, near an area of residual forest. A serosurvey of 1791 residents showed no evidence of recent yellow fever by immunofluorescence assay and IgM ELISA. An entomological survey revealed a low density of \textit{A aegypti} and \textit{Haemagogus} spp; virus isolation attempts on mosquito pools were negative. There was judged to be no evidence of transmission of wild-type yellow fever virus.

Case 2’s family lived in a middle-class district of Americana. No cases of yellow fever have been reported in São Paulo state since 1952, and there have been none in this region of the state for more than 100 years. No cases of jungle yellow fever have occurred within 500 km of this area.

Discussion
Wild-type yellow fever virus strains have the potential to cause severe damage to liver lobules in humans and monkeys. This so-called viscerotropism is characterised by diffuse disorganisation of the parenchyma, mid-zonal necrosis, and degenerative lesions including fatty infiltration and hyaline intracellular degeneration (Councilman bodies). By contrast, yellow fever 17D viruses do not have...
these invasive and destructive capabilities.\textsuperscript{19} Until now, neither preclinical safety tests in monkeys nor clinical experience have indicated that 17D vaccine has residual viscerotropism. A meeting of yellow fever experts was convened by the Pan American Health Organization in Brasilia in May, 2000, to consider the clinical, laboratory, and epidemiological features of the two cases. The panel unanimously concluded that the vaccine virus was the probable cause of fatal infections, which closely resembled wild-type yellow fever. A viscerotropic infection resembling wild-type yellow fever represents a hitherto unknown complication of vaccination with yellow fever 17D viruses, and has never been reported in animals, including monkeys, which are more susceptible hosts than humans to yellow fever.\textsuperscript{4}

This conclusion regarding causality was based on the following points: (1) onset of illness in close temporal proximity (3–4 days) to immunisation; (2) nearly identical clinical course—an initial non-specific febrile illness followed by rapid progression with jaundice, renal failure, hypotension, shock, and death on the fifth to sixth day after onset; (3) clinical laboratory findings with raised serum aminotransferase concentrations (aspartate aminotransferase predominant as in wild-type yellow fever), bilirubin concentrations, creatinine concentrations, and thrombocytopenia; (4) histopathological changes in the liver (and heart and spleen in one case examined) pathognomonic for yellow fever, with yellow fever antigen present by immunohistochemistry; (5) yellow fever 17DD virus isolated from multiple tissues; (6) no epidemiological evidence of jungle yellow fever transmission in the cities where the two patients lived, no history of trips to endemic areas, nor history of exposure to potentially risky situations for jungle yellow fever transmission; and (7) absence of any other clear explanation for the illnesses.

Certain clinical features of these cases remain controversial and poorly understood. The aminotransferase concentrations were lower than those typically seen in wild-type yellow fever. This observation suggests that tissue injury is less severe than in the wild-type disease, but nevertheless deaths occurred rapidly—perhaps more rapidly than in typical wild-type virus infections in which death occurs 5–10 days after illness onset. The rapid evolution could explain the relatively low concentrations of serum aminotransferase enzymes. We could not exclude the possibility that concomitant infection, possibly bacterial sepsis, might have contributed to illness and death. Both patients had possible sources for bacterial infection (urinary tract or lung), raised white-cell counts, and a left shift (especially case 2). Although these alterations could be caused by bacterial sepsis, marked leucocytosis is also a frequent late event in wild-type yellow fever. Moreover, histological examination of tissues did not reveal evidence of bacteria.

There were no changes in manufacturing methods of 17DD vaccine in Brazil that could account for the adverse events. The secondary seed lot 102/84 SC 285 produced in 1984 has been used for production of all vaccine lots since 1985, and 370 million doses have been produced. This secondary seed was tested in the monkey safety (neurovirulence) test in 1984, and has been used subsequently as the reference material in five other monkey neurovirulence tests between 1987 and 1999. In each case, the safety profile (viraemia as indicative for viscerotropism, clinical scores, and neuropathological scores) were similar and within WHO specifications.\textsuperscript{28} The lyophilised vaccine contains the same stabiliser that has been used for many years. The potency of virus in the final filled containers has not changed, and is log 4·4 (SD 0·2) plaque-forming units.

The lots associated with the two fatal cases were released at titres of log 4·56 and log 4·63 plaque-forming units per dose. No significant variation is seen in potency of the final bulk or the dilution factor used for final formulation of the vaccine.

One explanation is the occurrence of genetic variation during replication of the vaccine virus in the host, or the selection of a genetic variant already present in the vaccine virus population (quasispecies). Nucleotide sequence analysis of the PCR products in the E gene and the 3′ end untranslated region revealed no changes in the virus genome, as was seen with 17D virus isolated from a fatal case of encephalitis.\textsuperscript{21} We have now concluded more extensive sequence analyses on the secondary seed lot virus, the viruses from both vaccine lots, and the viruses from both cases. These analyses proved that the viruses are identical and that no major alteration took place in the host. Further evaluation of virulence of these viruses in monkeys and rodents has also suggested that the viruses are similar since no alterations on pathology were seen (unpublished data).

Despite the likely vaccine association, the possible contribution of host factors to the severity of the cases should be discussed. The two cases occurred as isolated events, and the vaccine recipients were not identifiable—neither preclinical safety tests in monkeys nor clinical neurovirulence tests between 1987 and 1999. In each case, the vaccine virus was the probable cause of fatal infections, which closely resembled wild-type yellow fever. A viscerotropic infection resembling wild-type yellow fever represents a hitherto unknown complication of vaccination with yellow fever 17D viruses, and has never been reported in animals, including monkeys, which are more susceptible hosts than humans to yellow fever.\textsuperscript{4}

This conclusion regarding causality was based on the following points: (1) onset of illness in close temporal proximity (3–4 days) to immunisation; (2) nearly identical clinical course—an initial non-specific febrile illness followed by rapid progression with jaundice, renal failure, hypotension, shock, and death on the fifth to sixth day after onset; (3) clinical laboratory findings with raised serum aminotransferase concentrations (aspartate aminotransferase predominant as in wild-type yellow fever), bilirubin concentrations, creatinine concentrations, and thrombocytopenia; (4) histopathological changes in the liver (and heart and spleen in one case examined) pathognomonic for yellow fever, with yellow fever antigen present by immunohistochemistry; (5) yellow fever 17DD virus isolated from multiple tissues; (6) no epidemiological evidence of jungle yellow fever transmission in the cities where the two patients lived, no history of trips to endemic areas, nor history of exposure to potentially risky situations for jungle yellow fever transmission; and (7) absence of any other clear explanation for the illnesses.

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prohibitively large. Increased passive surveillance for syndromes meeting one or more case definitions (eg, febrile illness with jaundice) might be an alternative. Surveillance could include hospital admissions and death registries. Education of health-care providers with respect to reporting cases and obtaining appropriate specimens for diagnosis is required, especially if other fatal cases were to be reported. Collection of specimens for investigation must be a priority.

On the basis of the new vaccine safety concerns, a universal vaccination policy in Brazil or other countries in the South American continent where yellow-fever risk is clearly partitioned between endemic and non-endemic zones is no longer advisable. In areas at no risk of jungle yellow fever, such as the coastal zones, vaccination should be substituted by an effective policy of surveillance for yellow fever and readiness for emergency intervention including A aegypti control.

Contributors
Pedro Vasconcelos was responsible for preparation of the paper, data organisation and interpretation, virus isolation and identification case 1, and epidemiological studies; Expedito Luna contributed to preparation of the paper, and data organisation and interpretation; Ricardo Gallero was involved with paper preparation, data organisation and interpretation, PCR detection and sequence determination of the virus from case 1, and comparative analysis of sequences from both cases; Luiz Silveira contributed to the preparation of the paper, data organisation and interpretation, isolation and identification of virus from case 2, and epidemiological studies; Terezinha Coimbra isolated virus from case 2 in mice, and did cell culture; Vera Barros was responsible for the histopathological diagnosis of yellow fever in case 1, and for overseeing the histological diagnosis in both cases; Thomas Monath organised and interpreted data, and helped prepare the paper; Sueli Rodrigues contributed to isolation of virus from case 1, diagnosis by PCR, and nucleotide sequence determination; Cristina Laval was responsible for treatment of and collection of clinical data from case 1; Maria Vilela did epidemiological studies; Cecilia Santos identified the virus from case 2 by PCR, and determined the nucleotide sequence; Cristina Laval contributed to data organisation and interpretation, isolation and identification of virus from case 2; Helena Sato did clinical and epidemiological studies, diagnosis, and virus identification for case 2; Elisabeth Rosa contributed to case 1 virus isolation and characterisation by serological assays; Gustavo Froguas was case 2's clinician, and provided early fever in case 1, and for overseeing the histological diagnosis in both cases; Luiz Silva contributed to isolation of virus from case 1, diagnostic analysis of sequences from both cases; Vera Barros was responsible for the histopathological diagnosis of yellow fever in case 1, and for overseeing the histological diagnosis in both cases; Thomas Monath organised and interpreted data, and helped prepare the paper; Sueli Rodrigues contributed to isolation of virus from case 1, diagnosis by PCR, and nucleotide sequence determination; Cristina Laval was responsible for treatment of and collection of clinical data from case 1; Maria Vilela did epidemiological studies; Cecilia Santos identified the virus from case 2 by PCR, and determined the nucleotide sequence; Cristina Papaioanou did the necropsy and histological examination on case 2; Venancio Alves did the histochemical assays for case 2 virus, and provided an overview of histological diagnosis for both cases; Liliana Andrade was responsible for clinical characterisation of case 2; Helena Sato did clinical and epidemiological studies, diagnosis, and virus identification for case 2; Elisabeth Rosa contributed to case 1 virus isolation and characterisation by serological assays; Gustavo Froguas was case 2's clinician, and provided early description and data collection; Ethel Lacava and Leda Almeida did epidemiological studies for case 2; Ana Cruz helped with case 1 virus isolation, diagnosis by PCR, and nucleotide sequence determination; Irey Rosco contributed to case 2 virus isolation and characterisation by serological assays; Raimunda Santos analysed serological data for differential diagnosis; and Otavio Oliva contributed to data organisation and interpretation, and to the preparation of the paper.

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