

BRIEF REPORT: TREATMENT OF A LABORATORY-ACQUIRED SABIÁ VIRUS INFECTION

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ARENAVIRUSES are a group of RNA viruses several of which have the potential to cause a deadly syndrome of hemorrhagic fever. In humans these viruses are usually transmitted by exposure to infected rodent excreta; occasional laboratory or nosocomial infections have been reported.¹ Sabiá virus is an arenavirus that was first isolated in São Paulo, Brazil, in 1990 from an agricultural engineer who presented with a hemorrhagic fever syndrome and ultimately died. Necrosis of the liver was found at autopsy. The virus was subsequently characterized as a new member of the Tacaribe complex of the family Arenaviridae.² A laboratory technician in Brazil who was involved in the characterization of the agent was also infected and had a severe nonfatal illness. Neither patient was treated with ribavirin.²

We now report a third case of Sabiá virus infection, which was successfully treated with ribavirin. We also discuss occupational exposure, the clinical course of the illness, and biosafety management in a university-hospital setting.

CASE REPORT

Occupational Exposure

On August 8, 1994, a 46-year-old virologist working alone in a biosafety-level-3 laboratory used a high-speed centrifuge to clarify a harvest of infected Vero cells containing Sabiá virus. The centrifuge contained six 250-ml bottles in a rotor with an intact O-ring to seal the contents during centrifugation. Each screw-capped polycarbonate bottle contained approximately 200 ml of tissue-culture fluid. The centrifuge was run at 10,000 rpm for 10 minutes (10,200×g) at a temperature setting of 4°C. The virologist observed no indication of a problem during the centrifugation process. On opening the lid of the rotor to remove the centrifuge bottles, he noted that the outside of one bottle was wet and that fluid had leaked into the bottom of the rotor. No obvious break was identified at the time, and the virologist was wearing a surgical mask, a disposable solid-front gown, and gloves. He had no abrasions or scratches on his hands.

The virologist used a second pair of gloves during the decontamination of the rotor, but did not wear a positive air-purifying respirator, although it was available. He decontaminated the spillage by pouring a concentrated solution of sodium hypochlorite (5.25 percent) directly into the rotor bucket as well as inside and outside the bottle that had leaked. The combined bleach and liquid in the rotor were then absorbed with paper towels. After the incident, the virologist continued

working alone in the laboratory for another three to four hours. All his protective garments as well as other contaminated material in the laboratory were autoclaved. Initially, he did not report the incident because he believed that no exposure to virus had occurred.

Case Presentation and Clinical Course

On August 16, 1994, the virologist noted myalgias, a mild headache, a stiff neck, and fever while driving home to New Haven, Connecticut, after a weekend visit to Boston. He treated himself with ibuprofen for two days before seeking medical care. On questioning, he described recrudescences of *Plasmodium vivax* infection that had never been treated with primaquine. He was concerned that this fever could represent a relapse of malaria. He initially did not recall any serious laboratory exposures. On physical examination he appeared mildly ill, with a temperature of 37.6°C (99.8°F), a pulse of 89 beats per minute, and a blood pressure of 130/80 mm Hg. The only remarkable features were mild conjunctival injection and shotty cervical nodes in the anterior chain. Laboratory studies performed that afternoon revealed a hematocrit of 42 percent, a white-cell count of 2600 per cubic millimeter, a platelet count of 138,000 per cubic millimeter, and an alanine aminotransferase level of 63 U per liter; urinalysis revealed moderate proteinuria (2+). After a smear proved negative for malaria, further review of possible infectious exposures led the patient to recall the August 8 laboratory incident with Sabiá virus.

The patient was immediately hospitalized and treated with intravenous ribavirin at a dosage used by the Centers for Disease Control and Prevention (CDC) for other arenavirus infections (a loading dose of 30 mg per kilogram of body weight, followed by a dose of 15 mg per kilogram every six hours for four days, and then by a dose of 7.5 mg per kilogram three times daily for six days). Pretreatment blood samples were sent for viral culture and examination by the polymerase chain reaction (PCR) for the presence of Sabiá virus RNA. PCR testing for Sabiá virus was reported to be positive on hospital day 2. The reverse-transcription PCR technique produces a fragment of 180 base pairs by using one primer specific for arenavirus in combination with one specific for Sabiá virus. Controls consisted of Sabiá virus RNA extracted from infected cell monolayers and normal human serum (Rico-Hesse R; personal communication).

Figure 1 shows the patient's temperature curve and laboratory values during hospitalization. No hemorrhagic manifestations were observed. His white-cell and platelet counts reached a nadir on hospital day 2: total white-cell count, 1300 per cubic millimeter; absolute neutrophil count, 650 per cubic millimeter; and platelet count, 98,000 per cubic millimeter. His serum alanine aminotransferase values peaked at 128 U per liter on day 10 of hospitalization. During treatment, his hematocrit dropped, and aminotransferase levels increased. He also reported a severe headache. The patient was sent home with a white-cell count of 3800 per cubic millimeter on August 29 after 10 days of intravenous ribavirin.

Blood samples were collected daily from the patient before ribavirin therapy was begun and during the rest of his hospitalization. The serum samples were used to inoculate cultures of Vero E-6 cells maintained at 37°C, and the cultures were examined for the presence of Sabiá virus antigen 10 days after inoculation with the use of an indirect fluorescent-antibody technique with mouse ascitic fluids that were reactive to the Tacaribe group and specific for Machupo virus and a commercial fluorescein-labeled goat antimouse IgG antibody. Uninfected Vero cells served as controls. Cells inoculated with a serum sample obtained on admission to the hospital and before ribavirin therapy was begun were positive for viral antigen. IgM and IgG antibodies to Sabiá virus antigen (infected Vero cells) were subsequently detected by indirect fluorescent-antibody testing in a serum sample drawn during convalescence 35 days after the clinical onset of the illness. Serum samples obtained before this time were negative for Sabiá virus antibodies and served as negative controls. Attempts to isolate the virus from urine, semen, and blood were repeatedly negative after ribavirin therapy was begun.

Biosafety Management and Contact Surveillance

After PCR confirmation of infection with Sabiá virus, the patient was transferred to a negative-pressure isolation suite.³ The suite con-

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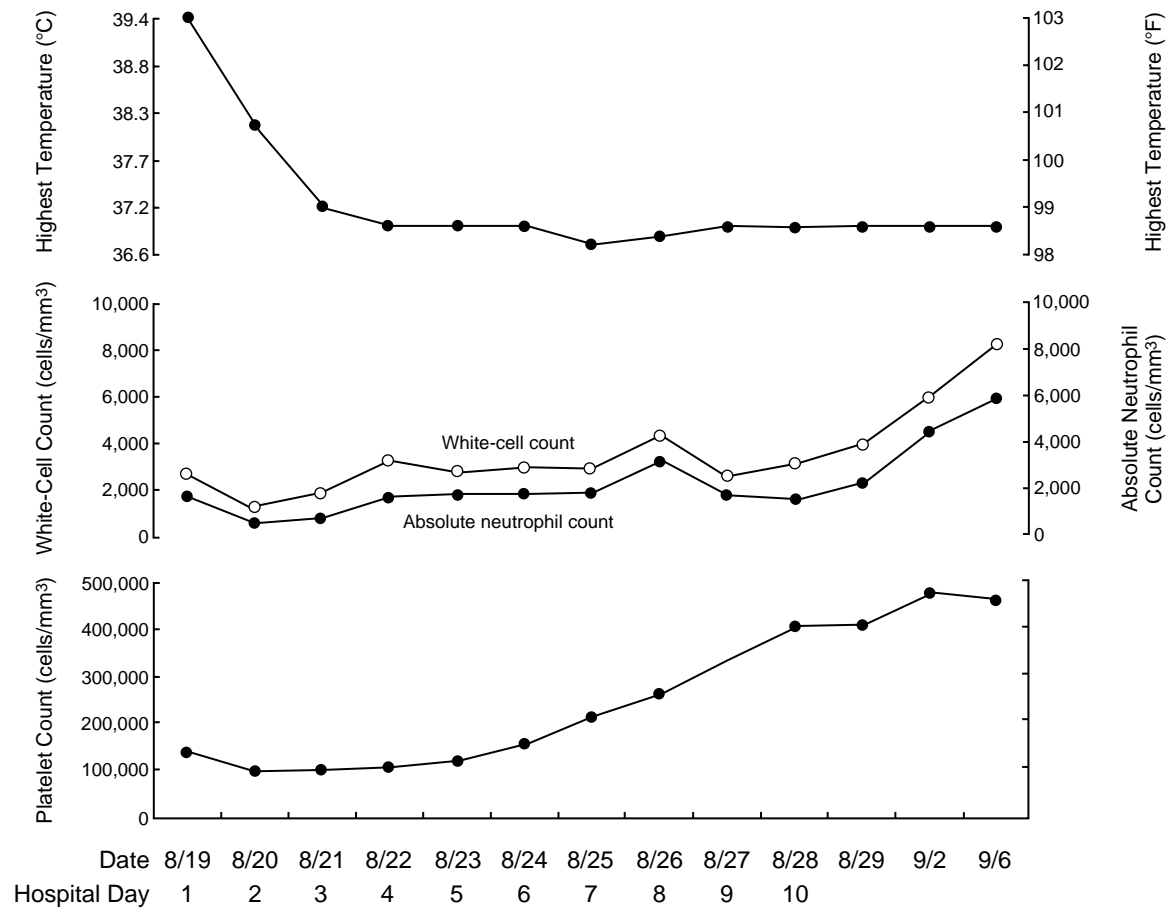


Figure 1. Laboratory Findings in a Patient Infected with Sabiá Virus.

The patient was exposed to Sabiá virus on August 8, 1994, and became symptomatic on August 16. To convert values for total bilirubin to micromoles per liter, multiply by 17.1; to convert values for calcium to millimoles per liter, multiply by 0.25; and to convert values for phosphate to millimoles per liter, multiply by 0.3229.

sisted of an anteroom with lower pressure than the surrounding hallway and two patient rooms with lower pressure than the anteroom. The patient remained in the same room throughout his hospital stay; the other patient room was used for the temporary storage of waste and for decontamination.

Once materials entered the negative-pressure isolation suite, they were considered to be contaminated. Items were double bagged and held in the adjacent negative-pressure storage room before being transported in an enclosed cart to the autoclave. Larger items were wiped with disinfectant before removal. Disposable items were bagged on removal from the room, autoclaved, and incinerated.

Throughout the patient's hospitalization universal precautions were used by all health care workers who came in contact with him or his specimens.⁴ Precautions were upgraded after PCR confirmation to include the use of fit-tested masks with high-efficiency par-

ticulate air filters, gowns, and gloves by all persons entering the patient's room and laboratory workers processing the patient's specimens. The number of health care workers who came in contact with the patient or his specimens was restricted.

Additional precautions were implemented in the laboratories to reduce the risk of potential exposure to aerosols from infected body fluids. All specimens were double bagged and carried by hand to the laboratories by the patient's physician. Chemistry specimens were processed in a negative-pressure room. Samples were spun in a sealed centrifuge, and serum was treated with Triton X-100 (10 µl of 10 percent Triton X-100 per milliliter of serum) to inactivate the virus. Hematologic specimens were processed in a Coulter counter that did not require removal of the top of the tube, and the effluents were treated with sodium hypochlorite and then autoclaved. The Coulter counter was also cleaned after use with several cycles of di-

luted bleach. Other laboratory tests were carried out within a bio-safety cabinet.

All contacts of the patient were identified and stratified into risk groups. One hundred thirty-nine contacts were identified, consisting of workers providing patient care, hospital laboratory workers, colleagues, friends, and family members. No exposure had occurred that would have placed any contact at high risk of contracting the virus. However, all contacts were notified that a possible exposure to Sabiá virus had occurred, and they were enrolled in a medical-surveillance program. As part of the surveillance program, all contacts were interviewed, answered a questionnaire, and provided a blood sample for testing at the CDC; six weeks later, they completed a follow-up questionnaire and provided a second blood sample.

The contacts were also instructed to take their temperatures twice daily over the six-week period and to monitor for early symptoms of the disease. They were given a telephone number that was staffed 24 hours a day by physicians with expertise in occupational medicine and infectious diseases and who were familiar with the index case. No symptoms suggestive of Sabiá virus infection occurred in the group under surveillance during the six-week follow-up period; however, one subject had a viral syndrome with leukopenia and mild hepatitis just after the end of the six-week observation period. A serologic test (enzyme-linked immunosorbent assay) for arenavirus infection and PCR testing for Sabiá virus RNA were negative, and a serum sample obtained during convalescence was also negative for IgM and IgG antibodies to Sabiá virus antigen.

A protocol was generated with the emergency department for the triage and care of any secondary cases. The plans included restricting the number of health care workers who came in contact with people who might have been secondarily infected; immediately notifying the clinical laboratories, the hospital epidemiology department, the state health department, and the CDC of any additional suspected cases; isolating other potentially infected persons in a negative-pressure isolation suite; and having physicians familiar with the index patient clinically evaluate such persons.

DISCUSSION

We describe the third confirmed case of Sabiá virus infection. The two previous cases were identified in Brazil.² Four other arenaviruses (Lassa, Junin, Machupo, and Guanarito) have been associated with a hemorrhagic disease in humans.¹ Each of these agents has a distinct geographic distribution, although they cause similar clinical manifestations and high mortality.¹ Wild rodents serve as reservoirs for these arenaviruses; humans usually become infected by contact with contaminated rodent excreta. Little is known about the mode of transmission of Sabiá virus or its source in nature.

We believe that Sabiá virus was most likely transmitted to the investigator by aerosol, probably when the centrifugation rotor was opened and the leak was observed or during the decontamination process. The 8-day incubation period in this case is consistent with that of other arenaviral illnesses (range, 6 to 21 days).^{1,3} We chose to treat the patient early with intravenous ribavirin, a guanosine analogue, since clinical studies have shown it to be effective in diminishing mortality in Lassa fever if given early in the course of illness.⁵ Clinically, our patient had an excellent response; he became afebrile and asymptomatic within 48 hours, in contrast to the other two patients with Sabiá virus infection, who had a prolonged course with hemorrhagic symptoms. His severe leukopenia and thrombocytopenia rapidly improved, and no further virus was detected in his blood by culture after treatment was initiated. The mild hemolysis was thought to be due to ribavirin

therapy and is a well-described effect of the drug.⁶ The late peak in aminotransferase values, although not dramatic, was unusual, since it was asynchronous with the recovery from leukopenia and thrombocytopenia. It could have been caused by an adverse effect of the ribavirin not previously described, infection, or immunologic recovery from Sabiá virus. Hepatocellular damage and hepatitis have been described with Sabiá virus and other arenaviruses.^{1,2} The patient's late immunologic response, as reflected by the finding of IgG and IgM antibodies to Sabiá virus, may have been modified by treatment with ribavirin, or it may represent the natural response to Sabiá virus infection.

Since a number of nosocomial outbreaks have been described among hospital personnel attending acutely ill patients with arenavirus infections, a high level of precaution was instituted at our hospital, as recommended by the CDC.³ Although extensive experience in West Africa with Lassa fever has shown that universal precautions and nursing using barrier precautions generally prevent the transmission of Lassa virus in hospitals, we also instituted negative-pressure isolation, limited the number of hospital personnel caring for the patient, and required personnel potentially exposed to blood aerosols to wear respirators with high-efficiency particulate air filters.^{3,4} Clinical laboratory specimens were processed to minimize workers' exposure to aerosolized virus, and routine automated equipment for chemistry specimens was not used unless the specimen was first treated with Triton X-100. Contact surveillance with a follow-up of six weeks (well within the incubation period of arenaviral illnesses) identified no secondary cases.

Researchers need to recognize the risk of occupational exposure to Sabiá virus by aerosol. Ribavirin treatment may modify the clinical course of Sabiá virus infection. Although standard universal precautions may be adequate to prevent nosocomial spread, additional biosafety precautions should be considered when one is dealing with arenaviruses and other dangerous viruses whose mechanisms of transmission are unknown.

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