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**A case of yellow fever in a brown howler (Alouatta fusca) in Southern Brazil**

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**Abstract.** Many brown howlers (*Alouatta fusca*) have died in a 3-month period in a subtropical forest in Southern Brazil. One was examined after a systemic illness. According to clinical signs, and necropsy and histopathology findings, yellow fever virus (YFV) infection was suspected. Tissue sections from liver, kidney, and lymphoid organs were screened by immunohistochemistry for YFV antigens. Cells within those tissues stained positively with a polyclonal antibody against YFV antigens (1:1,600 dilution), and yellow fever was diagnosed for the first time in the brown howler in the area.

Yellow fever virus (YFV) infection is an acute arthropod-borne Flavivirus (family Togaviridae) infection that causes fever, jaundice, albuminuria, and hemorrhage. It occurs in 2 forms: urban and sylvan. There are 2 types of endemic areas: humid forests and emerging zones where urban and sylvan forms intermingle. The vectors are *Aedes aegypti* in urban areas, *A. albopictus* in suburban areas, and tree-hole-breeding mosquitoes (*Haemagogus* spp.) in the forests. The virus circulates in the forests in mosquito vectors causing scattered epizootics in nonimmune monkeys. Alternatively, transmission may be vertical from the female mosquito to her offspring, allowing virus survival from one rainy season to the next in *A. aegypti* eggs. Yellow fever remains endemic in many regions of Africa and South America, despite the existence of an effective vaccine. Currently, YFV infection may be confused with other similar hemorrhagic conditions.

In humans, severe necrotic lesions are seen at autopsy in many organs, particularly the liver. Microscopically, YFV infection is characterized by midzonal necrosis of the liver with microvesicular fatty change of hepatocytes, the presence of apoptotic bodies (Councilman bodies), renal tubular degeneration, and splenic lymphoid necrosis.

Up to 80 brown howlers (*Alouatta fusca*) are suspected to have died from March 2001 to May 2001 at the border between Brazil and Argentina. People of the subtropical forest of the area reported that many dead monkeys had fallen from the trees. They observed that the monkeys had yellow mucosae and skin. A yellow fever outbreak was suspected. One of the dead monkeys was necropsied in May 2001 at the Department of Pathology, Universidade Federal de Santa Maria, Southern Brazil.

The necropsied brown howler was a thin, young adult female monkey in poor body condition. All mucosae and large vessel intimae and the liver were jaundiced. The right kidney had an irregular area at the surface, and the spleen had uneven borders. The stomach was full of nondigested green leaves, fibers, and seeds. The intestines contained feces coated in dry, yellow mucus. The urinary bladder contained yellow urine with white floccular strands. Formalin-fixed, paraffin-embedded tissues were sectioned at 5 μm and stained with hematoxylin and eosin (HE). By light microscopy, massive coagulation necrosis of most hepatocytes and fatty degeneration of the remaining cells was observed in the liver (Fig. 1). Scattered apoptotic hepatocytes were present. Renal tubular cells had de-
Generative changes, and hyaline casts were noted within the lumen of many tubules. An extensive area of subacute interstitial nephritis in the right kidney corresponded to the irregular area seen grossly. The lymphoid follicles of the splenic white pulp had variable central necrosis (Fig. 2). No lesions were found in other organs.

The history, gross findings, and histological changes were highly suggestive of yellow fever. Paraffin-embedded sections of liver, kidney, and spleen were submitted for immunohistochemistry to the Evandro Chagas Institute at Belém, Pará, Northern Brazil. A mouse polyclonal antibody (dilution 1:1,600) and a commercially available Streptavidin–alkaline phosphatase complex that reacted with the biotin linked with the secondary antibody were used for YFV antigen detection. The specific substrate for the alkaline phosphatase was Histomark Red. The slides were counterstained with Mayer hematoxylin and mounted in Entellan. Positive and negative controls were run with every test. Renal tubular epithelial cells and widespread hepatocytes stained positive for YFV (Fig. 3).

Cases of yellow fever and dengue, both Flavivirus infections, overlap geographically in some areas of South America and, clinical differentiation of these infections is usually difficult. The severe hepatic lesions of yellow fever were once considered pathognomonic. Historically, in dengue, the low production of viral progeny by infected hepatocytes usually resulted in limited foci of infection, whereas foci tend to be massive in yellow fever infection. However, in recent years, dengue has been shown to be capable of more extensive cytopathic effect. An immunohistochemical method is required for confirmation of either diagnosis.

In the monkey studied in this report, jaundice was marked, but other typical gross lesions, e.g., hemorrhages and serous effusions were absent. Histopathological changes in the liver were severe and consistent with the extensive hepatic destruction reported for yellow fever.

In Brazil, urban yellow fever has been curtailed for more than 50 years by controlling the vector, Aedes sp. The sylvatic cycle still occurs in outbreaks that reflect vector movement and changing climatic conditions. After diagnosis in this brown howler, sanitary measures were immediately taken to halt the vector spread to the surrounding urban areas, and the human population around the forest was vaccinated because of the potential zoonotic hazard.

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Sources and manufacturers
a. GIBCO-BRL Life Tech. Inc., Gaithersburg, MD.
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In vitro evaluation of the susceptibility of Edwardsiella ictaluri, etiological agent of enteric septicemia in channel catfish, Ictalurus punctatus (Rafinesque), to florfenicol

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Abstract. In vitro studies were conducted to assess the sensitivity of Edwardsiella ictaluri, the etiological agent of enteric septicemia of catfish (ESC), to the antibacterial drug florfenicol (FFC). Twelve different E. ictaluri isolates from cases submitted between 1994 and 1997 to the Thad Cochran National Warmwater Aquaculture Center fish diagnostic laboratory (Stoneville, MS) were used for testing. These isolates originated from channel catfish infected experimentally with E. ictaluri. In some of these experimental infections, FFC was used for treatment. These cultures of E. ictaluri were identified by morphological and biochemical tests. Kirby-Bauer zones of inhibition (in mm) for FFC against E. ictaluri were determined using standard methods. The minimum inhibitory concentration (MIC) of FFC for each isolate was determined. The MIC for FFC tested with E. ictaluri was consistently 0.25 μg/ml. Edwardsiella ictaluri tested in these studies were highly sensitive to FFC in vitro.

Edwardsiella ictaluri, first isolated in 1976, is the etiological agent of enteric septicemia of catfish (ESC). The disease is capable of causing high mortalities in channel catfish (Ictalurus punctatus) and is considered the most significant factor affecting commercial catfish aquaculture. Outbreaks of ESC peak when water temperatures are 22–28°C.13,14 For successful treatment of fish exhibiting signs of ESC, immediate diagnosis and treatment with oral antibiotics are recommended while the majority of the fish are still feeding.12 Currently, the only antibiotics approved for use with food fish in the United States are oxytetracycline (Terramycin®) and a sulphadimethoxine and ormetoprim combination (Romet-30®). However, there are reports of bacterial resistance to these antibiotics.7,11,12 In addition, palatability problems have been reported with sulphadimethoxine and ormetoprim combinations.8 An alternative treatment for ESC would be beneficial to the industry.

Previous in vitro research with fish pathogens indicates that most fish pathogenic bacteria are sensitive to the antibiotic florfenicol (FFC). Studies involving the bacterial species Photobacterium damselae (subsp. Piscicida), Vibrio anguillarum, Aeromonas hydrophila, Aeromonas salmonicida, and Edwardsiella tarda determined that the minimum inhibitory concentrations (MICs) were less than or equal to 1.6 μg/ml for...