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*Case Report*

# Septic Tularemia in 2 Cottontop Tamarins (*Sanguinus oedipus*)

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**Two captive cottontop tamarins (*Sanguinus oedipus*) died within 5 d of each other from systemic infection by *Francisella tularensis* (tularemia). One tamarin experienced mild clinical signs, including malaise, anorexia, and a mucoid nasal discharge for 4 d before death, whereas the other experienced a more rapid progression of disease that lasted less than 24 h. Differential diagnoses included gram-negative septicemia by an organism such as *Escherichia coli*, *Salmonella*, or *Yersinia*; protozoal infection such as *Toxoplasma gondii* or an acute viral infection such as lymphocytic choriomeningitis. *F. tularensis* infection was identified by *F. tularensis*-specific PCR in both primates. Possible sources of infection include aerosol, biting arthropod vectors, and transmission via a rodent reservoir. This case report highlights the importance of tularemia as a differential diagnosis in acute febrile illness in captive nonhuman primates.**

Tularemia is a life-threatening zoonotic disease caused by *Francisella tularensis*, a small, pleomorphic, facultative, gram-negative coccobacillus.[1,20] The organism is found widely throughout the northern hemisphere.[1,11] *F. tularensis* is highly virulent, and disease can be induced with as few as 10 organisms.[4,11] The incubation period is usually 3 to 5 d but can range from 1 to 14 d.[11] *F. tularensis* has been divided into 2 major subspecies (biovars)—*F. tularensis* biovar *tularensis* (Jellison; type A) and *F. tularensis* biovar *palaearctica* (type B; formerly *holarctica*)—on the basis of virulence testing and biochemical reactions.[4] In humans, transmission often is associated with an arthropod vector but also can include oral and respiratory routes, bites by infected vertebrates, and exposure to infected tissues or fluids.[6] The 7 clinicopathologic forms of tularemia in humans are ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, typhoidal, and septicemic.[6] The clinical progression of disease in humans includes a sudden-onset fever, generalized ache, inflammation of the upper respiratory tract with nasal discharge, lymphadenomegaly, vomiting, malaise, and anorexia.[6] The cases we describe here are consistent with septic tularemia, due to the nature of the acute, febrile, systemic illness and the severe and fatal nature of the disease.

Experimentally induced tularemia has been reported in common marmosets, rhesus macaques, and African green monkeys.[3,8,15,17,18,20] In captivity, tularemia in primates is rare, with reports of sporadic, naturally acquired infection in common squirrel monkeys (*Saimiri sciureus*), cynomolgus monkeys (*Macaca fascicularis*), orangutans (*Pongo pygmaeus*), black-and-red tamarins (*Sanguinus nigricollis*), talapoins (*Cercopithecus talapoin*), vervet monkeys (*Chlorocebus aethiops*), patas monkeys (*Erythrocebus patas*), golden-headed lion tamarins (*Leontopithecus chrysomelas*), and common marmosets (*Callithrix jacchus*).[1,2,6,7,11,13,14,19] A study in common marmosets revealed that disease progression in nonhuman primates appears to be consistent with human features of tularemia.[15] Cases of tularemia have been reported infrequently in callitrichids.[7,12,14-16,19]

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## Case Report

Two cottontop tamarins, a male and a female, both 11 y 7 mo old, died within 5 d of one another due to tularemia. These animals were captive-born and had been at the zoo for 7 and 5 y, respectively. They were housed together in an indoor exhibit with a 2-toed sloth (*Choloepus didactylus*) that remained clinically normal during the period of the tularemia outbreak. The 3 other cottontop tamarins at the zoo were housed elsewhere and were unaffected by tularemia.

**Clinical findings.** The male tamarin showed clinical signs of shivering, malaise, mucoid nasal discharge, and anorexia for 4 d prior to his death. Despite his illness, he eluded capture for examination and treatment. Four days after the death of the male tamarin, the female was seen shivering in the exhibit and later was found on the ground. She was brought to the veterinary hospital, where she was anesthetized with isoflurane and oxygen. Physical exam revealed an approximately 5-mm diameter bruise on the skin of her inguinal area, but was otherwise within normal limits. Based on her clinical signs and the preliminary necropsy results of her cagemate indicating sepsis from a gram-negative rod bacteria, she was treated empirically for a bacterial infection. There was no diagnosis of tularemia for the male tamarin yet due to delays at the diagnostic laboratory. She received meloxicam (0.1 mg SC; Metacam Boehringer Ingelheim, St Joseph, MO), enrofloxacin (9 mg SC; Baytril, Bayer Animal Health, Shawnee

Mission, KS), ampicillin (10 mg SC; Claris Lifesciences, North Brunswick, NJ), and clindamycin (7.5 mg SC; Hospira, Lake Forest, IL) and subcutaneous lactated Ringer solution.

**Clinical pathology.** Blood work from the female tamarin included a CBC, which revealed a prominent leukopenia (total WBC count, 1120/µL; reference interval, 6167 to 15,673/µL) characterized by severe neutropenia (absolute neutrophil count, 40/µL; reference interval, 2819 to 10,915/µL).[9] Neutrophils were moderately toxic, with basophilic, foamy cytoplasm. In addition, numerous extracellular and intracellular small, rod-shaped bacteria were identified on the blood smear (Figure 1). The neutropenia and toxic changes were attributed to overwhelming tissue demand secondary to bacterial sepsis. A moderate thrombocytopenia (automated platelet count, $88.6 \times 10^3$/µL; reference interval, 206 to $442 \times 10^3$/µL) was attributed to septicemia, decreased platelet production, and decreased platelet survival.

Serum biochemistry revealed a mild elevation in ALP (358 U/L; reference interval, 46 to 266 U/L) and a moderate elevation in GGT (108 U/L; reference interval, 6 to 30 U/L). ALT was mildly elevated (154 U/L; reference interval, 5 to 71 U/L). The increases in ALP, GGT, and ALT suggest both cholestatic and hepatocellular disease and were likely secondary to bacterial hepatitis. AST was elevated markedly (AST, 1142 U/L; reference interval, 97 to 257 U/L), and creatine kinase was increased (8198 U/L; reference interval, 0 to 982 U/L). Elevations in AST and creatine kinase indicate muscle damage secondary to trauma, recumbency, or myositis. Moderate hypoproteinemia (total protein, 3.2 g/dL; reference interval, 6.0 to 7.6 g/dL) characterized by moderate hypoalbuminemia (1.2 g/dL; reference interval, 3.2 to 4.6 g/dL) and mild hypoglobulinemia (2.0 g/dL; reference interval, 2.2 to 3.6 g/dL) was present. The panhypoproteinemia was likely multifactorial; possible causes include inflammation, protein loss through the kidney or gastrointestinal tract, or decreased protein synthesis. BUN and creatinine were mildly elevated (BUN: 48 mg/dL; reference interval, 8 to 22 mg/dL; creatinine: 1.6 mg/dL; reference interval; 0.3 to 0.9 mg/dL), indicating prerenal or renal azotemia. Mildly low blood glucose (76 mg/dL; reference interval, 87 to 265 mg/dL) was likely secondary to septicemia and possibly hepatic insufficiency and starvation.

**Postmortem examination.** Necropsies were performed on both tamarins within 24 h after death; both carcasses were in good nutritional and postmortem condition. Numerous 2- to 5-mm-diameter white to gray foci effaced both the capsule and cut surfaces of the liver and spleen and were present throughout the mesenteric fat of both tamarins. In the male tamarin, the lungs were diffusely mottled dark red to purple. In the female tamarin, the kidneys had pinpoint hemorrhages throughout the parenchyma.

**Bacteriology.** Numerous *Proteus* spp. were cultured from the liver, lung, and heart of the male tamarin; no anaerobic bacteria were isolated. The overgrowth of *Proteus* may have prevented the isolation of the *F. tularensis* from these samples. *E. coli* was cultured from the spleen of the female tamarin; no anaerobic bacteria were isolated. This initial testing was performed locally at West Vet Diagnostic Laboratory (Meridian, ID). Cultures of the blood, spleen, and liver from the female tamarin that were performed on chocolate agar at the Washington Animal Disease Diagnostic Laboratory yielded moderate to numerous slow-growing white colonies on chocolate agar. The isolates were oxidase-negative, β-lactamase-positive, weakly catalase-positive, gram-negative,

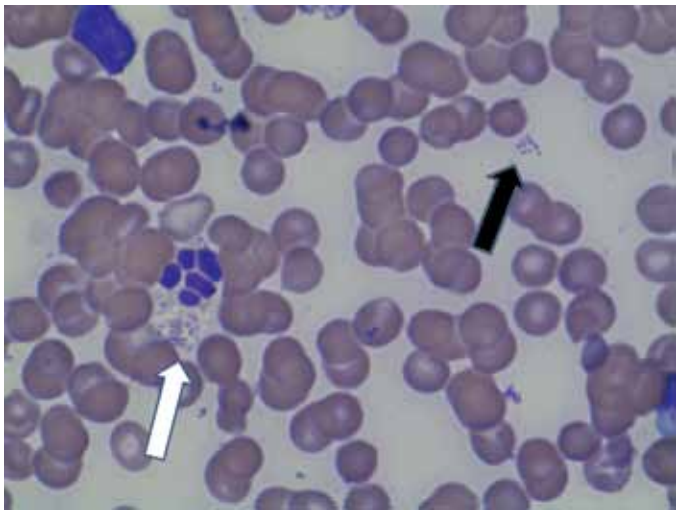

**Figure 1.** Blood smear. Degenerate neutrophil with intracellular bacterial rods (arrow). Black arrow shows numerous rod-shaped extracellular bacteria. Wright stain; magnification, 100×.

tiny coccobacilli. Colony characteristics and the failure to grow on blood agar or MacConkey agar were suggestive for *F. tularensis*.

**Histopathology.** Microscopic examination of samples from the male tamarin revealed mild to moderate, multifocal foci of lytic necrosis associated with suppurative inflammation in the spleen and liver, along with fibrin accumulation in the liver. Multifocal necrosis and hemorrhage was present throughout the cortex of the adrenal gland. Gram staining and Gomori methenamine silver stain failed to reveal the presence of bacteria or fungi within these lesions.

The tissue samples from the female tamarin similarly revealed multifocal lytic necrosis in the spleen and liver (Figure 2), and when combined with the CBC results, these findings suggested a systemic bacterial infection (bacteremia). In addition, superficial necrosis of the colonic mucosa was present, with less severe necrosis in sections of the pancreas, mesenteric lymph nodes and ovary. Histopathology also revealed evidence of primary renal disease, including renal tubular necrosis, interstitial fibrosis, and glomerular sclerosis.

**Molecular diagnostics.** DNA was extracted from a fixed tissue block of liver and spleen (male tamarin) and fresh samples of spleen (female tamarin) by using a commercial kit (DNeasy, Qiagen, Valencia, CA) as previously described.[5] *F. tularensis*-specific multiplex PCR was performed by using previously described primers.[10] Briefly, *Francisella* genus-specific primers FtulC1F and FtulC4R yielded a 300-bp amplicon in a multiplex PCR including the *F. tularensis*-specific primers TUL4-435 and TUL4-863, which generated a 429-bp amplicon. PCR was performed in a 50-µL reaction mixture containing 10 µL 5× buffer with $NH_2SO_4$ (Fermentas), 2 mM $MgCl_2$ (Invitrogen, Carlsbad, CA), 1.0 mM each primer (Invitrogen), 200 mM each dNTP (Invitrogen), 1 U *Taq* polymerase (Fermentas, Thermo Fisher Scientific, Waltham, MA), and 9.5 µL extracted DNA. The reaction mixtures underwent 40 cycles of amplification in a thermal cycler (model 2720, Applied Biosystems, Foster City, CA), with cycles consisting of denaturation for 1 min at 95 °C, annealing for 1 min at 54 °C, and extension for 1 min at 72 °C, with a final extension for 7 min at 72 °C. DNA from the *F. tularensis* vaccine strain was used as a

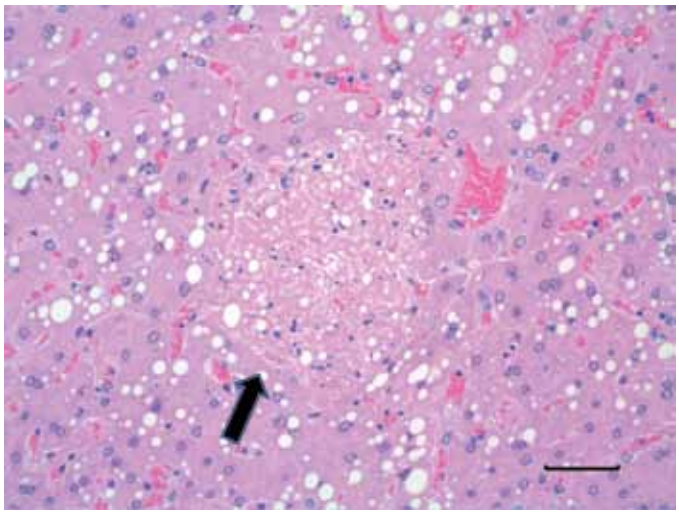

**Figure 2.** Histopathology from the liver of the female tamarin showing a focus of hepatocellular necrosis consistent with septicemia. Fatty changes in hepatocytes are incidental. Hematoxylin and eosin stain; bar, 25 µm.

positive amplification control. Extracted sterile, deionized, diethylpyrocarbonate-treated water was used as a negative extraction control, and water was used as a no-template control. Amplicons from the PCR assay were visualized on 1.5% agarose gels containing ethidium bromide. Both samples yielded amplicons of the correct sizes for *F. tularensis*. Specificity was confirmed by sequencing the PCR amplicons and sequence analysis as previously described.[5] The 17-kDa lipoprotein gene *F. tularensis*-specific target sequence obtained from both animals matched (that is, 100% sequence identity) that of *F. tularensis* subsp. *holarctica* (biovar *palearctica*; GenBank accession no., BK006741). The smaller 300-bp RNA helicase gene sequence from the multiplex PCR also completely matched the same GenBank accession. Fresh spleen from the female tamarin was sent to the Centers for Disease Control (Atlanta, GA), where the results were confirmed by culture and direct fluorescent antibody testing as positive for *F. tularensis*. The bacteria were identified as *F. tularensis* type B (biovar *palearctica*) at the Centers for Disease Control.

## Discussion

Tularemia is uncommon in zoos but has been reported in a variety of nonhuman primate species. Transmission to captive animals can occur via small mammals such as voles, squirrels, mice, and rabbits, which act as natural reservoirs of infection.[4,6,13] In addition, biting arthropod vectors such as ticks, flies, and horseflies can transmit disease.[4,6,13] In addition, nonhuman primates may ingest blood-feeding arthropods during episodes of social grooming.[1]

Tularemia may be under-recognized in captive nonhuman primates but should be considered as a differential diagnosis during febrile illnesses, including pneumonia and hepatitis.[11] If diagnosed or suspected early, tularemia can be treated successfully with antibiotics. Streptomycin and doxycycline have been used successfully to treat nonhuman primates that had a confirmed diagnosis of tularemia.[2,10] In humans, streptomycin is considered the drug of choice; gentamicin is an alternative.[4] Tetracyclines and chloramphenicol are used in humans but are associated with more frequent relapse and treatment failures.[4] Ciprofloxacin has been shown to be a successful therapy against tularemia in vitro and in humans.[4]

Other differential diagnoses for acute febrile illness in nonhuman primates include septicemia by another gram-negative bacterium such as *E. coli*, *Yersenia pseudotuberculosis*, or *Salmonella* spp. Additional differentials include *Toxoplasma gondii* and lymphocytic choriomeningitis virus. These infections were ruled out by the absence of organisms or inclusions in tissues and the positive culture results for *F. tularensis*.

Veterinarians and other animal care personnel are at high risk of zoonotic tularemia, given the ease of infection through skin contact with infected material or via aerosol.[1,10,11] Multiple veterinarians have acquired infection from bite wounds or after performing necropsies on infected primates.[10,13,16] If *F. tularensis* infection is suspected, all personnel should take appropriate biosafety precautions. None of the staff, laboratory personnel, or other animals in the collection was infected during this disease episode.

Because tularemia is a reportable disease, investigations were performed by the local health department and the Centers for Disease Control after initial reporting by the diagnostic laboratory (Washington Animal Disease Diagnostic Laboratory). No definitive source of infection was determined. Possibilities for exposure included arthropod or rodent vectors and inhalation of contaminated spores. Another possible source was cross-contamination from frozen rodents fed to other animals at the zoo. No other animals at the zoo were clinically affected by tularemia during this episode or since; these cases may highlight a unique susceptibility of cottontop tamarins to tularemia.

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