Tularemia as a cause of fever in a squirrel monkey

Catherine S. Beckwith, DVM, PhD, DAACLAM

**Case Description**—A 3-year-old female squirrel monkey (Saimiri sciureus sciureus) was examined because of sudden onset of lethargy and fever.

**Clinical Findings**—On initial examination, the monkey was weak and febrile and had petechiae on both thoracic limbs. Following collection, blood samples were slow to clot. During the next week, the monkey developed anemia and thrombocytopenia; *Francisella tularensis* was isolated from blood samples.

**Treatment and Outcome**—Treatment with gentamicin resulted in the monkey's gradual return to health, but inguinal lymphadenopathy developed after drug administration was discontinued. *Francisella tularensis* was isolated from a fine-needle aspirate of an enlarged lymph node. Treatment with streptomycin resulted in resolution of infection. By use of biochemical and molecular tests, the microbial isolate was characterized as *F. tularensis* subsp. *holarctica*. Results of a microagglutination assay confirmed that the monkey had developed serum antibodies against *F. tularensis*.

**Clinical Relevance**—With timely diagnosis, treatment of tularemia in the squirrel monkey was successful. *Francisella tularensis* is the cause of a highly infectious zoonotic disease, and infection with this microorganism is enzootic in wildlife throughout the Northern Hemisphere. Tularemia should be considered in the differential diagnosis of febrile disease in animals of any species. Even limited or indirect exposure of humans or other animals to outdoor environments in which reservoir hosts and arthropod vectors are present can lead to transmission of *F. tularensis*. *Francisella tularensis* is a class A agent of bioterrorism, and all cases of tularemia (regardless of species) should be reported to public health officials. (*J Am Vet Med Assoc* 2006; 229: 269–273)

A 3-year-old female Guyanese squirrel monkey (*Saimiri sciureus sciureus*) was examined at the veterinary center because of sudden onset of lethargy and fever. The captive-born monkey had been housed in a pen with 7 other squirrel monkeys at a satellite research facility (approved by the Association for Assessment and Accreditation of Laboratory Animal Care International) located in a semirural area in northern California. The pen was inside a shelter with garage-type doors that are opened in pleasant weather to provide ventilation and environmental enrichment for the monkeys; the doors were open in June when the monkey became sick. On physical examination (day 1), the monkey was weak and had a rectal temperature of 41.5°C (106.7°F; upper reference limit, 39.5°C [103.1°F]). A moderate amount of nasal discharge was present, but findings on thoracic auscultation were unremarkable. There were petechiae on the medial aspect of each thoracic limb. The monkey was thin and weighed 594 g (1.3 lb); it was estimated to be 5% dehydrated on the basis of skin turgor.

Differential diagnoses were developed to account for the presence of fever and petechiae. Infectious disease was given the highest consideration because the monkey had been exposed to the outdoor environment. Previous experience with other monkeys at the facility that had become ill on occasion directed clinical suspicion toward systemic bacterial infection. Blood samples were collected aseptically for bacterial culture and hematologic and serum biochemical analyses. A sample of urine could not be obtained at this time. Treatment with broad-spectrum antimicrobials, including an aminoglycoside, was instituted as soon as blood collection was completed. The monkey was treated on day 1 with gentamicin (4.4 mg/kg [2 mg/lb], IM, q 12 h on day 1 and q 24 h thereafter) and sterile ticarcillin disodium-clavulanate potassium (30 mg/kg [22.7 mg/lb], IM, q 12 h). Supportive care included SC administration of lactated Ringer's solution and provision of an external heat source.

Few abnormalities were detected via a CBC. The monkey's total leukocyte count and differential were within reference limits, although Hct (37%; reference range, 38.2% to 49.4%) and platelet count (265 × 10³ platelets/µL; reference range, 378 × 10³ to 561 × 10³ platelets/µL) were slightly low. Samples of blood were slow to clot, and an insufficient volume of serum was obtained for biochemical analyses.

The monkey's condition improved gradually, and its fever diminished over several days. Petechiae were no longer visible by day 4, although the platelet count remained mildly low (246 × 10³ platelets/µL). On day 4, a CBC revealed that the monkey was anemic (Hct, 28%; RBC count, 5.08 × 10¹² cells/L [reference range, 6.30 × 10¹² cells/L to 8.20 × 10¹² cells/L]) with high serum creatine kinase activity (2,604 U/L; reference range, 54 to 830 U/L). Rectal temperature was within reference limits by the fifth day; at this time, it was reported that bacterial culture of blood had yielded heavy growth of *Francisella tularensis*. Presumptive microbial identification was based on the organism's morphologic features and growth on modified Thayer-Martin medium and cysteine heart agar with rabbit blood with penicillin and polymyxin B (media that promote *F. tularensis* growth). Identification was subsequently confirmed via agglutination of the organisms with *F. tularensis*-specific antiserum. Additionally, blood smears that had been pre-

From The Department of Comparative Medicine, Stanford University School of Medicine, Stanford, CA 94305-5410. The author thanks Dr. Marry Schnieffer and Brook Yockey for providing reagents for and assisting with microagglutination, direct fluorescent, and PCR assays; Drs. David Fisher and Linda L. Werner for cytologic examinations; Dr. Corrine Davis for histologic evaluations; and Janis Atuk-Jones for graphics assistance.
pared on day 1 yielded positive results via a direct fluorescent antibody test with fluorescein-5-isothiocyanate-conjugated rabbit anti–*F. tularensis* antibodies. The isolate was further characterized by biochemical analysis. The isolate was classified as glycerol negative, a characteristic of *F. tularensis* subsp. holarctica (also known as type B). Treatment with gentamicin was continued, and administration of other antimicrobials was discontinued. Results of antimicrobial susceptibility testing later confirmed that the organism was susceptible to gentamicin.

On day 6, the monkey had pronounced anemia and thrombocytopenia (Hct, 25.1%; RBC count, 4.57 × 10⁶ cells/µL; platelet count, 55.6 × 10³ platelets/µL); poikilocytosis and anisocytosis were detected. The total leukocyte count was low (3.62 × 10³ WBCs/µL [reference range, 4.5 × 10³ to 17.3 × 10³ WBCs/µL]), and neutrophils with toxic granulation were present. Serum concentrations of BUN and creatinine and activities of hepatic enzymes were within reference ranges. The monkey’s activity level and appetite were good. However, on day 8, low Hct and WBC, RBC, and platelet counts persisted and its activity level began to decrease. On day 9, the monkey became febrile and lethargic again; it developed respiratory difficulty with open-mouth breathing, became ataxic, and was observed head pressing on the cage floor. By day 11, the monkey’s clinical appearance improved again, although anemia (Hct, 26.3%; RBC count, 4.57 × 10⁶ cells/µL), anisocytosis, and thrombocytopenia (55.5 × 10³ platelets/µL) persisted and many large platelets were observed microscopically. The total leukocyte count was within reference range; mild relative neutrophilia was evident, but there was no evidence of toxic changes in those cells. The monkey’s condition gradually returned to normal. After 18 days of administration, treatment with gentamicin was discontinued. On day 26, mild relative neutrophilia remained but there were no other detectable clinicopathologic abnormalities. The monkey was transferred and housed singly in a room with other squirrel monkeys at the main research animal facility where there is no outdoor exposure.

Two weeks later, the monkey had gained 20 g (0.044 lb) in body weight. Its appetite and activity were normal, but perineal fecal staining and diarrheic feces in the cage pan were observed. Lymph nodes in both inguinal regions were visibly enlarged (Figure 1). Fecal flotation and bacterial culture revealed no parasite ova or enteric pathogens. Cytologic examination of a fine-needle aspirate of an inguinal lymph node revealed lymphoid hyperplasia with mild inflammation consistent with antigenic stimulation; no microorganisms were detected. Four days later, bacteria identified as *F. tularensis* were grown in cultures of the aspirated material. Treatment with streptomycin was initiated (10 mg/kg [4.5 mg/lb], IM, q 12 h) and continued for 3 weeks. Throughout this treatment, the monkey remained in good condition. The size of the lymph nodes decreased until they were no longer visibly apparent, although they remained palpable. One inguinal lymph node was resected 3 months later. Cytologic and histologic evaluation of the excised tissue revealed mild to moderate reactive lymphoid hyperplasia. Bacterial culture of lymph node tissue yielded no microbial growth.

Aliquots of serum acquired during the monkey’s treatment were assessed by use of a microagglutination assay for reactivity with formalin-killed *F. tularensis.* Serum collected on day 5 lacked anti–*F. tularensis* antibodies, but the monkey seroconverted by day 8, and the titer increased precipitously (Figure 2). Serum concentration of antibodies against *F. tularensis* decreased over time but remained much greater than diagnostic concentration at day 313. By PCR assay amplification of a specific outer membrane protein gene (tuH⁴) from purified bacterial DNA, identification of *F. tularensis* was confirmed. Another PCR assay was performed on day 156 yielded no growth. The serum antibody titer increased precipitously by day 11. Relapse was diagnosed on day 42 when *F. tularensis* was cultured from a lymph node specimen; however, a lymph node that was excised on day 156 yielded no growth. The serum antibody titer remained greater than diagnostic concentration at day 313.
with a primer set capable of distinguishing \( F.\) \( \text{tularensis} \) subsp \( \text{tularensis} \) and \( F.\) \( \text{tularensis} \) subsp \( \text{holarctica} \) by presence or absence, respectively, of the insertion sequence \( \text{IS}F\text{tu2} \). The isolate lacked \( \text{IS}F\text{tu2} \), which confirmed the previous identification (based on results of biochemical testing) of the organism as \( F.\) \( \text{tularensis} \) subsp \( \text{holarctica} \).

**Discussion**

The gram-negative coccobacillus \( F.\) \( \text{tularensis} \) is the cause of tularemia, a zoonotic disease. Tularemia is primarily a disease of wildlife; \( F.\) \( \text{tularensis} \) naturally infects hundreds of species of animals in large regions of the globe but almost exclusively in the Northern Hemisphere. In North America, the most important reservoir hosts are lagomorphs (rabbits and hares) and rodents (voles, squirrels, muskrats, and beavers). Many other mammals can also be infected, and there have been infrequent reports of infection in birds, amphibians, and reptiles. Among wildlife, transmission occurs via the bite of tick, fly, and mosquito vectors or by exposure to infected carcases or contaminated water or soil. Infection may develop after ingestion or inhalation of \( F.\) \( \text{tularensis} \), and the organism can survive for months in contaminated soil. The alternate names for tularemia, deer-fly fever and rabbit fever, reflect 2 common sources of infection for humans. Between 1990 and 2000, more than 100 human cases of tularemia were reported each year in the United States; the largest numbers of those cases were located in Arkansas, Missouri, South Dakota, and Oklahoma. There have been localized outbreaks in Massachusetts. In recent years in the state of California, 1 to 2 cases of human tularemia have been reported each year. The causative organism was originally named \( \text{Bacterium tularensi} \) for Tulare County, California, where it was first isolated from a California ground squirrel (\( \text{Spermophilus beecheyi} \)) in 1910.

Two subspecies of \( F.\) \( \text{tularensis} \) predominate in North America, both of which have been found in northern California. \( \text{Francisella tularensis} \) subsp \( \text{tularensis} \) (also called type A) commonly infects rabbits and is transmitted by ticks. It is capable of causing severe disease in humans, which can result in death if not treated. \( \text{Francisella tularensis} \) subsp \( \text{holarctica} \) (type B) is associated with rodents and contaminated water and causes milder or chronic disease in humans. \( \text{Francisella tularensis} \) is a highly infective facultative intracellular organism; as few as 10 colony-forming units of \( F.\) \( \text{tularensis} \) subsp \( \text{tularensis} \) can cause illness in humans.

In humans, 6 classical forms of tularemia (distinguished on the basis of the route of inoculation) are recognized but there is overlap of many of the clinical signs. Sudden onset of fever is common to all forms, and lymphadenopathy in the region of exposure is also a nearly universal feature. In the ulceroglandular form, a cutaneous papule progresses to an ulcer in the area drained by the affected lymph node. The glandular form is similar but lacks apparent involvement of the skin. Oculoglandular and pharyngeal forms develop following exposure through the conjunctiva and the oropharynx, respectively. Pneumonic tularemia results from either inhalation of the bacteria or hematogenous spread of the organism to the lungs. Typhoidal tularemia lacks prominent lymphadenopathy or other localizing features.

To the author’s knowledge, this is the first report of the timely diagnosis, documented seroconversion, and successful treatment of tularemia in a squirrel monkey. Critical factors in the successful outcome of this case were prompt recognition of the monkey’s illness and clinical suspicion based on previous cases of tularemia from the same facility, which guided diagnostic efforts and treatment. On initial examination, the monkey’s condition resembled typhoidal tularemia in humans. No papules, ulcers, or ectoparasites were present to indicate cutaneous exposure to \( F.\) \( \text{tularensis} \), and there was no evidence of lymphadenopathy. The petechiae and delayed clotting of blood samples collected at the initial examination followed by progressive decreases in Hct, RBC count, and platelet count and changes in RBC morphology suggest that disseminated intravascular coagulation or immune-mediated destruction of RBCs and platelets developed as a consequence of gram-negative sepsis.

In the monkey of this report, there was notable clinical improvement after gentamicin was administered and the level of bacteremia was controlled. However, gentamicin does not reach bacteria that are located within cells, and the second episode of fever with respiratory signs was likely a result of the ongoing presence and systemic release of bacteria. Relapse of the glandular form was suspected when inguinal lymphadenopathy developed 2 weeks after discontinuation of antimicrobial treatment. \( \text{Francisella tularensis} \) was cultured from aspirated lymph node material, which confirmed that small numbers of live organisms persisted. The cause of diarrhea that developed at that time was not determined, but diarrhea can be associated with typhoidal tularemia in humans. Historically, streptomycin has been the drug of choice for treatment of tularemia in humans but was not in stock at the veterinary center when the monkey became ill. Gentamicin is also effective in humans but, as illustrated by the monkey of this report, is associated with a higher relapse rate, compared with streptomycin. At the time of this report, more than 2 years had elapsed after treatment with streptomycin and the monkey had had no further relapses. In recent years, ciprofloxacin has been used with success to treat tularemia in humans; relapses have also occurred with its use, but overall, the results have been favorable. Because ciprofloxacin is both bacteriocidal and penetrates the intracellular compartment, its use may reduce the chances of relapse. \( \text{Francisella tularensis} \) has fastidious nutritional requirements, and it can fail to grow if only routine media are used for culture. The organism requires a source of sulfhydryl groups, which are present in media such as glucose cysteine blood agar, thiglycollate broth, chocolate agar for gonococcal growth, modified Thayer-Martin medium, buffered charcoal-yeast agar, or cysteine heart agar with blood. Laboratory staff should be alerted when tularemia is suspected so that suitable media are used and appropriate safety precautions are taken; \( F.\) \( \text{tularensis} \) becomes aerosolized readily, and it is highly infective when inhaled.
Francisella tularensis subsp holarctica caused life-threatening disease in the squirrel monkey of this report, consistent with 4 other cases at this facility over a period of 15 years. In those other instances, the squirrel monkeys were either found dead, died shortly after evaluation, or were euthanized because of their grave condition; definitive diagnosis was made on the basis of results of bacterial cultures of specimens collected at necropsy. Necropsy findings included lesions of necrosis, hemorrhage, and microthrombosis in multiple organs, consistent with sepsis and disseminated intravascular coagulation. These experiences with F. tularensis–infected squirrel monkeys suggest that in Saimiri spp, tularemia is usually an acute to peracute febrile illness with wide dissemination of organisms throughout the body. It is rapidly fatal in the absence of early and aggressive antimicrobial treatment.

Among the scant reports of tularemia in squirrel monkeys, including a prior report from this institution, only 1 describes a different clinical course of disease. The squirrel monkey of that report had an illness (2 weeks' duration) that was complicated by other conditions and disease agents before it was euthanized. Relevant findings included extensive ulcerative stomatitis and lymphadenitis of the head and neck, possibly suggestive of ingestion of F. tularensis. Variations in signs of disease in squirrel monkeys is consistent with wide clinical variations in tularemia-affected humans; such variations appear to depend on the subspecies of F. tularensis, dose and route of exposure, and host factors. All 5 F. tularensis isolates detected at this research institution have been biotyped, and each has been identified as F. tularensis subsp holarctica. There is evidence that animal species differ in their susceptibility to F. tularensis. Squirrel monkeys, which are native to South America where tularemia in humans and other animals has not been reported, appear to be particularly susceptible to F. tularensis, even to the less virulent F. tularensis subsp holarctica.

Assessment of acute and convalescent sera via microagglutination procedures revealed that the squirrel monkey of this report seroconverted only after it developed fever and bacteremia. This provides evidence for recent infection acquired at this research facility because the monkey had been a resident for many months. Although a strong humoral response was mounted, the monkey was unable to eliminate the organisms and relapsed despite appropriate antimicrobial treatment. This is consistent with findings in tularemia-affected humans; cell-mediated immunity is reportedly required for complete recovery. In humans, recovery typically confers lifelong protection against reinfection.

At this research facility, incidents of tularemia have always involved a single animal and cagemates have been unaffected. Paired samples of sera from 1 cage-mate of the monkey of this report were assessed, but the former did not seroconvert. That cage-mate was treated for another condition and was returned to the colony without complication. In a report by Waggie et al, 4 cagemates of a squirrel monkey with fatal tularemia remained seronegative. The absence of evidence for monkey-to-monkey transmission mirrors findings for the disease in humans. The experience with squirrel monkeys at this research facility is similar to a report in a common marmoset (Callithrix jacchus); that animal was found moribund and died the same day. Postmortem lesions were consistent with septicemia, and F. tularensis was grown in cultures of liver, spleen, kidney, and lung. Via DNA sequencing, that organism was identified as F. tularensis subsp holarctica (referred to by its former name F. tularensis biovar palaearctica). No other marmosets at that site became ill or seroconverted. Infections with F. tularensis among squirrel monkeys at this institution differ from 2 tularemia epizootics affecting other species of nonhuman primates in 2005. In those reports, multiple animals died or became sick over several weeks. In one of those instances, it was believed that rodent urine or feces contaminated the monkeys' food during a tularemia epizootic in mice, and in the other, it was believed that fleas from infected ground squirrels transmitted the infection to the primates.

The specific mode of transmission to the squirrel monkeys at this research facility has not been determined. Biting flies, ticks, small rodents, small reptiles, and birds can enter gaps between the walls, floor, and roof of the shelter. Potentially infected animals of larger size cannot gain entry to the pen, and multiple monkeys would likely become sick if the food or water was contaminated. A bite from an arthropod vector or ingestion of a small rodent captured after entering the pen is considered the most likely source of infection. In the squirrel monkeys, the absence of dermal or pharyngeal lesions that often develop following infection via the cutaneous or oral routes in humans is consistent with the impression that F. tularensis is capable of rapid systemic spread in this species. Without prompt treatment, the monkeys typically die as a result of sepsis before localized lesions have an opportunity to develop at the point of entry. The few individual cases identified at our institution are consistent with an enzootic source of tularemia in the area. A possible way of reducing exposure of the monkeys to vectors of tularemia would be to install screens at the shelter.

Humans and other animals become infected with F. tularensis through the same fundamental mechanisms. Exposure to a contaminated environment or activities that increase exposure to arthropod vectors, infected wildlife or their carcasses, and tick-infested domestic animals can lead to infection. Most F. tularensis infections in people are of the ulceroglandular form and develop when the organism penetrates damaged skin. Recent clusters of pneumonic tularemia in humans are theorized to have developed when aerosolized particles were inhaled. Hunters and trappers, farmers and livestock workers, meat handlers and cooks, veterinarians, laboratory workers, and landscapers are at increased risk for occupational exposure.

Risk of exposure to personnel handling squirrel monkeys with tularemia is believed to be low because direct human-to-human transmission has not been reported. In another report of a squirrel monkey with tularemia, serum anti-F. tularensis antibody titers in 7 humans who had direct contact with the affected monkey were assessed and results indicated that no one sero-
converted. Transmission to personnel could occur while handling samples of blood or other clinical specimens or via a bite wound. The remaining risk to personnel is the potential for exposure to arthropod vectors on entering and leaving the squirrel monkey facility.

Tularemia is enzootic throughout the Northern Hemisphere and should be considered in the differential diagnoses for febrile illness in animals of any species. Even limited or indirect exposure to the outdoor environment in which reservoir wildlife hosts and arthropod vectors are present can result in transmission of *F. tularensis*. Because of its highly infectious nature, ease of dissemination, and potential to cause severe disease and death in humans, *F. tularensis* is classified as a class A agent of bioterrorism.21 All cases of tularemia in humans or other animals should be reported to public health officials.4

b. BBL, Cockeysville, Md.
c. Provided by Dr. Marty Schrieffer, CDC, Fort Collins, Colo.
d. BIOLOG GN Dangerous Pathogen system, BIOLOG Inc, Hayward, Calif.

References