

## Tularemia in a Park, Philadelphia, Pennsylvania

**To the Editor:** Tularemia is a bacterial zoonosis caused by the gram-negative, nonmotile coccobacillus *Francisella tularensis*, which is endemic in lagomorphs in North America (1,2). Tularemia is considered a possible biological weapon of terrorism (Centers for Disease Control and Prevention [CDC] category A) because of its high infectivity, ease of dissemination, and considerable ability to cause illness and death in humans (3). The BioWatch Program monitors the environment in urban areas throughout the United States for *F. tularensis* and other potential bioterrorism agents. The epidemiology of many of these pathogens in urban ecosystems is not well understood; reservoirs may not be known or suspected, which leads to an inability to differentiate natural infection from a bioterrorism event. We describe a cluster of tularemia infections (in the absence of identified human illness or environmental detection) in feral rabbits found in a 0.5-km<sup>2</sup> area of a large city park in Philadelphia, Pennsylvania, USA.

During the spring and summer of 2006, a total of 14 eastern cottontail rabbits (*Sylvilagus floridanus*) and 2 woodchucks (*Marmota monax*) were found dead or trapped and euthanized (2 rabbits only) at a zoological park. The animals were necropsied, and specimens of liver and spleen were sent to the Pennsylvania Bureau of Laboratories (BOL) for *F. tularensis* culture and PCR. Two years earlier, in the spring of 2004, a single rabbit found dead at this same location had tested positive for *F. tularensis*; PCR and culture identified the organism in liver and spleen. Of the 14 rabbits submitted in 2006 for *F. tularensis* testing, 6 were positive (collection dates ranged from March through August). Five of these were positive

by PCR and culture, and 1 was positive by PCR alone; *F. tularensis* was identified only in animals found dead. The 2 woodchucks tested negative by PCR and culture. The 2004 isolate and 2006 isolates were identified by CDC as type A *F. tularensis* and were found genetically identical by pulsed-field gel electrophoresis.

These additional 2006 positive findings triggered efforts to use available resources to identify other tularemia sources: the Philadelphia Department of Public Health (PDPH) heightened surveillance for tularemia by requesting that other city agencies and wildlife rehabilitation centers report and submit for testing any mammals found dead from unknown causes. (City agencies reported a few larger mammals, e.g., groundhogs and raccoons, dead from trauma; these animals were not tested.) The zoological park continued routine illness monitoring of collection animals, animals on grounds, and staff. In addition, during October 2006 and March 2007, the PDPH collected ticks on the outskirts of a heavily wooded area with frequent foot traffic  $\approx$ 1.5 miles from the site where the rabbits were found dead. (The specific tick collection method involved dragging a white cotton bath towel along the edge of a wooded area; this activity took place during the hours of 10:00 AM–2:00 PM. Other tick-dragging attempts during August 2007, on the outskirts of a heavily wooded area  $\approx$ 0.5 miles away that was accessible to foot traffic but across the river from the zoological park, yielded no results.) A total of  $\approx$ 30 deer ticks (*Ixodes scapularis*, which are not a known vector for tularemia) were collected each month; no other species were identified. These tick specimens were submitted to BOL for *F. tularensis* testing by PCR and culture. During November and December 2006, 5 crayfish (*Procambarus acutus*, cited as a possible reservoir for type B tularemia by Anda et al.) (4), were trapped from a pond near the site where the rabbits were found dead and

submitted to BOL for *F. tularensis* testing by PCR and culture. None of these readily available surveillance activities resulted in identifying tularemia except in the rabbits found dead in the zoological park. Additionally, no cases of human tularemia were reported to PDPH during this period, despite distribution of a health alert to medical providers to heighten clinical suspicion for the disease. Furthermore, the organism was not detected by routine environmental monitoring of air samples by the city's BioWatch sensors.

Even though this limited investigation failed to identify additional *F. tularensis* infections in humans and in any of the animals and ticks tested, the cluster of infections in rabbits in Philadelphia indicates that *F. tularensis* is present in the environment in sufficient numbers to cause a noteworthy die-off of animals (i.e., 6 rabbits in a 0.5-square-mile area over a 5-month period). Environmental biomonitors in other metropolitan areas have been triggered by reported detection of tularemia on at least 2 occasions in the past 5 years—Houston in 2003 and the Washington, DC, National Mall in 2005 (5).

This investigation underscores that *F. tularensis* identification in the environment requires a systematic approach beyond environmental biomonitoring, random convenience sampling, and increased passive surveillance for human cases. Standard methods such as serologic studies of wildlife may not be available to resource-limited urban institutions. Possible strategies such as the collection of ticks, specifically the American dog tick, *Dermacentor variabilis* (a known vector for tularemia), from animals upon entry into urban animal shelters and mapping of areas where the animals were found need to be considered if resources are limited. Additional research is necessary to understand the occurrence of disease caused by *F. tularensis* in humans and animals, especially in urban environments (6).

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## Genotyping of *Orientia* *tsutsugamushi* from Humans with Scrub Typhus, Laos

**To the Editor:** Rickettsial diseases have been only recently identified as underrecognized but important causes of fever of unknown origin in Laos. In 2006, 63 (14.8%) of 427 adults with negative blood cultures admitted to Mahosot Hospital in Vientiane had scrub typhus, an infection caused by *Orientia tsutsugamushi* and transmitted by the bite of larval trombiculid mites (1). *O. tsutsugamushi* is characterized by a wide antigenic diversity, and isolates are conventionally classified on the basis of reactivity with hyperimmune serum against prototype strains (e.g., Karp, Kato, Gilliam, Kawasaki, Kuroki, or Shimo-goshi). The 4 hypervariable regions within the 56-kDa type-specific antigen of *O. tsutsugamushi*, which is located on the outer membrane surface, are considered to play an essential role in type strain assignment (2).

In the Lao study (1), in addition to acute-phase serum samples, a 5-mL blood sample anticoagulated with EDTA was collected at admission from all patients. After centrifugation, buffy coat of the serum sample was removed and stored at  $-80^{\circ}\text{C}$  (1). DNA was extracted from buffy coat samples of 63 patients whose conditions were diagnosed by immunofluorescence assay as

scrub typhus (3). Two amplification reactions were performed, a real-time quantitative PCR with a probe targeting the *O. tsutsugamushi* 47-kDa outer membrane protein gene with appropriate primers and probes (4) and a standard PCR targeting a 372-nt fragment of the 56-kDa protein gene (3).

Buffy coat samples from 11 (17.5%) patients were positive for *O. tsutsugamushi* in the real-time quantitative PCR and 56-kDa antigen gene PCR (Table). All 11 patients were from Vientiane or Vientiane Province. PCR products for the 56-kDa gene fragments were purified and sequenced as described (3). Comparison (3,5) of amplicons for the 11 patients with each other and with GenBank sequences identified 6 genotypes. Percentages of nucleotide sequence similarity with other sequences available in GenBank ranged from 95.9% to 100% (Table). Interpretation of our results was also supported by recent phylogenetic studies that compared sequences of the entire 56-kDa type-specific antigen gene of isolates from Thailand (6). LaoUF238 and LaoUF220 genotypes clustered with those of strains related to the Karp serotype, and LaoUF136 and LaoUF187 clustered with genotypes of strains related to the Gilliam serotype (2). Other genotypes found in this study were grouped in 2 clusters that contained genotypes identified in Thailand (5) and Taiwan (7) that have not been linked to a reference serotype (Table).

Detection of *O. tsutsugamushi* in humans in Laos provides useful information on genotypes prevalent in this country. Our results were confirmed by using 2 target genes in 2 PCRs. No differences were found between the number of days of fever in 11 PCR-positive patients and number of days of fever in 52 PCR-negative patients. However, the PCR-negative patients may not have had bacteremia at the time of sample collection.

Diversity of *O. tsutsugamushi* genotypes found in Laos includes