Outbreak of Leptospirosis among Adventure Race Participants in Florida, 2005

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Background. On 21 November 2005, a 32-year-old male resident of New York was hospitalized with suspected leptospirosis. He had participated in an endurance-length swamp race on 4–5 November 2005 outside of Tampa, Florida.

Methods. We interviewed racers to assess illness, medical care, and race activities. A suspected case was defined as fever plus ≥2 signs or symptoms of leptospirosis occurring in a racer after 4 November 2005. Individuals with suspected cases were referred for treatment as needed and were asked to submit serum samples for microscopic agglutination testing (MAT) and for rapid testing by the dot enzyme-linked immunosorbent assay dipstick immunoglobulin M immunoassay.

Results. The Centers for Disease Control and Prevention and participating state health departments interviewed 192 (96%) of 200 racers from 32 states and Canada. Forty-four (23%) of 192 racers met the definition for a suspected case. The median age of the patients was 37 years (range, 19–66 years), and 128 (66.7%) were male. Fourteen (45%) of the 31 patients with suspected cases who were tested had their cases confirmed by serological testing (a single sample with MAT titer ≥400), including the index case patient. Organisms of a potential novel serovar (species Leptospira noguchii) were isolated in culture from 1 case patient. Factors associated with increased risk of leptospirosis included swallowing river water (odds ratio [OR], 3.4; 95% confidence interval [CI], 1.6–7.0), swallowing swamp water (OR, 2.4; 95% CI, 1.1–5.2), and being submerged in any water (OR, 2.3; 95% CI, 1.1–4.7).

Conclusions. This report describes a leptospirosis outbreak that resulted in a high rate of symptomatic infection among adventure racers in Florida. The growing popularity of adventure sports may put more people at risk for leptospirosis, even in areas that have not previously been considered areas of leptospirosis endemcity.

Leptospirosis is the most widespread bacterial zoonosis worldwide. Illness is caused by infection with pathogenic species of the spirochete genus Leptospira. Leptospira isolates are taxonomically categorized into serovars by antibody agglutination with surface antigens, and serovars are grouped into antigenically related serogroups. Over 200 pathogenic serovars have been described [1–3]. Leptospirosis is endemic throughout much of the world, with a higher prevalence in countries with tropical climates [2]. Domestic, peridomestic, and wild animals can act as reservoirs by maintaining chronic infection and shedding leptospires in their urine [2]. Infection in humans is acquired primarily by direct contact with infected animals or their urine or through contact with contaminated environments. Although leptospirosis is classically an occupationally acquired illness, avocational exposures are increasingly associated with outbreaks of leptospirosis, including large outbreaks following a triathlon in Illinois in 1998 and following the 2000 Eco-Challenge adventure race in Borneo [4–10]. Outbreaks may be precipitated by heavy rainfall and flooding and can occur in areas where the disease has not previously been considered to be endemic.
The spectrum of human disease caused by leptospirosis is wide, ranging from subclinical infection to multiorgan involvement associated with a high mortality. Up to 90% of leptospiral infections are subclinical or of mild severity, whereas 5%–10% of infected persons (but the majority of those with recognized cases) present with a severe febrile illness of sudden onset [2]. Even with early diagnosis and aggressive therapy, the severe form of icteric leptospirosis, or Weil’s disease, has a case fatality rate of 5%–15%.

After recovery from leptospirosis, detectable antibody levels may persist for months to years, depending on the infecting serovar and host immune response [11, 12]. Rapid serologic assays are available that detect genus-specific antibodies and aid in clinical diagnosis. These rapid tests are useful screening tools for the diagnosis of leptospirosis, but their results should be confirmed with use of the microscopic agglutination testing (MAT). The MAT is the gold-standard for the diagnosis of leptospirosis and provides epidemiologically useful information by determining concentrations of antibody to serovars representing potentially infecting serogroups.

On 23 November 2005, the Centers for Disease Control and Prevention (CDC) was notified of a suspected case of leptospirosis in a 32-year-old man hospitalized in New York State. He reported sudden onset of fever, headache, and myalgias 11 days after participating in the United States Adventure Racing Association (USARA) National Championship on 4–5 November 2005 in the Hillsborough River State Park near Tampa, Florida. However, anti-leptospiral antibody testing of a serum sample collected 18 days after the race had negative results at a New York hospital. Postings on a Web site sponsored by the race organizers indicated that several other racers were ill with fever and had signs and symptoms that were similar to those of the index patient, including a California racer with illness onset 10 days after the race who had a blood sample tested in a clinical laboratory that yielded an indirect hemagglutination assay result positive for leptospirosis.

The 2005 USARA National Championship took place on 4–5 November 2005 and was an endurance-length swamp race covering a distance of >100 miles and taking ~24 h to complete. A total of 200 racers from the United States and Canada participated in the event (Figure 1). The race involved 4 components: paddling, cycling, trekking, and orienteering. Although these components are consistent in adventure racing, this race was unusual, because it was a swamp race. Two weeks before the race, Hurricane Wilma passed over western Florida, which brought heavy rains and flooding to the Tampa region and to the Hillsborough River State Park (Figure 2). During the event, racers had prolonged exposure to surface water in the Hillsborough River and in creeks and swamps. In response to the reports of possible leptospirosis, CDC and state and local health department partners initiated an investigation. The objectives of the investigation were: (1) to notify racers and race organizers of the outbreak and the need for ill persons to seek medical care, (2) to confirm leptospirosis as the cause of the outbreak by arranging diagnostic testing for ill persons, and (3) to provide recommendations to the adventure race community and to medical professionals by assessing factors associated with increased risk of infection among ill racers. This report summarizes the results of the multistate outbreak investigation.

**PATIENTS AND METHODS**

**Epidemiologic investigation.** CDC and participating state and local health departments contacted race participants and ad-
ministered a questionnaire to assess the presence of acute illness, medical care, and specific activities and behaviors during the race. Ill racers were referred to their health care providers for diagnostic testing and treatment as needed. In accordance with CDC outbreak emergency response provisions, laboratory testing conducted at the CDC was restricted to symptomatic racers. We defined a suspected case of leptospirosis as fever (either subjective fever or temperature ≥38°C) plus at least 2 other classic symptoms of leptospirosis (headache, muscle aches, eye pain, conjunctival suffusion, jaundice, dark urine, or unusual bleeding) in a participant of the USARA National Championship. We defined a confirmed case of leptospirosis as a suspected case of leptospirosis with isolation of leptospires by culture, a Dip-S-Tick (DST; PanBio) result of ≥2 dots (of a maximum of 4 dots) in a single specimen, a MAT result of ≥400 in a single serum specimen, or a 4-fold increase in MAT titers between 2 serum specimens collected at least 2 weeks apart.

Laboratory investigation. Serum specimens for anti-leptospiral antibody testing and blood and urine specimens for culture were requested from all individuals with suspected cases for submission to the CDC through the individuals’ health care providers. Five milliliters of blood were collected from each suspected case patient for detection of anti-leptospiral antibody by DST and MAT. DST was performed according to manufacturer’s instructions [13]. Standard laboratory procedures for MAT were followed [14, 15]. In brief, live leptospiral cell suspensions were incubated with serially diluted serum specimens. Agglutination was examined by dark-field microscopy. The reported titer was the highest dilution of serum that agglutinated at least 50% of the cells for each serovar tested. Serovars included in the MAT screening panel were selected on the basis of prior knowledge of regional epidemiology, geography, potential animal reservoirs, and previous findings from similar settings (Table 1).

Culture of *Leptospira* organisms was performed by inoculating 150 μL of freshly collected blood or urine from ill racers into Ellinghausen, McCullough, Johnson, and Harris (EMJH) semi-solid media enriched with 200 μg/mL of 5-fluorouracil. Culture media were incubated at 30°C and observed weekly for growth [16]. *Leptospira* isolates obtained from blood and urine specimens were identified and characterized by pulsed-field gel electrophoresis (PFGE), cross-agglutination absorption testing (CAAT), and DNA–DNA hybridization, as described elsewhere [17–19].

Environmental investigation. Environmental samples were collected on 17 December 2005, 6 weeks after the race, from areas along the race course that were identified by racers and race organizers as the sites with the most-extreme water exposure or that showed evidence of potential animal reservoirs in the park (eg, muddy areas rooted up by pigs). Samples were filtered using 0.22-micron filters to remove potential environmental contaminants and were cultured for the presence of leptospires. Five hundred microliters of water samples from 10 different environmental collections were cultured in EMJH media enriched with 5-fluorouracil. A 30-mL sample of each water specimen was also centrifuged for 15 min at 4000 rpm. The supernatant was decanted, and the pellet was resuspended in 1 mL of phosphate buffered saline. A total of 250 μL of the resuspended pellet was then cultured in EMJH media enriched with 5-fluorouracil.

Statistical analysis. Questionnaire responses were entered into a Microsoft Access database. Statistical analysis was conducted using SAS, version 9.1 (SAS Institute). Univariate logistic regression was used to explore variables as they related to confirmed cases of leptospirosis. We calculated odds ratios (ORs) for dichotomous variables. Continuous and categorical variables with multiple levels were dichotomized if appropriate. We selected candidate variables from univariate analysis with *P* values <.05 for inclusion in multivariable modeling.

### RESULTS

Epidemiologic investigation. We interviewed 192 (96%) of 200 racers from 32 states and 2 Canadian provinces (Figure 1). The median age of all racers was 37 years (range, 19–66 years), and 128 racers (66.7%) were male. Forty-four (23%) of 192

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Table 1. Panel of Serovars and Their Respective Serogroups Used in Microscopic Agglutination Testing, United States Adventure Racing Association Leptospirosis Outbreak, Florida, November 2005
athletes met the definition for suspected leptospirosis. Three racers, including the index case patient, were hospitalized, and all patients recovered without sequelae. There were no deaths. The most common signs and symptoms reported by the 44 patients with suspected cases were as follows: fever (100%), headache (91%), chills (69%), sweats (68%), muscle or joint pain (68%), and eye pain or photophobia (39%).

Factors associated with increased odds of leptospirosis on univariate analysis included swallowing river water (OR, 3.4; 95% confidence interval [CI], 1.6–7.0); eating wet food (OR, 2.8; 95% CI, 1.3–6.3); swallowing swamp water (OR, 2.4; 95% CI, 1.1–5.2); and being submerged in any water (OR, 2.3; 95% CI, 1.1–4.7) (Table 2). Two variables, swallowing water from the river or creek (OR, 2.5; 95% CI, 1.2–5.4) and eating wet food (OR, 2.5; 95% CI, 1.0–6.4), remained significantly associated with increased odds of leptospirosis on multivariable analysis when controlling for prolonged water exposure. Two suspected risk factors, the presence of severe cuts on the legs (OR, 2.2; 95% CI, 0.6–7.7) and wearing shorts for the majority of the race (OR, 0.8; 95% CI, 0.4–1.7), although highly prevalent among all racers, were not statistically associated with increased odds of infection.

**Laboratory investigation.** Testing performed at the CDC on serum samples collected from the index case patient on 25 November 2005 (21 days after race exposure) revealed a positive DST result (3 of 4 dots) and an elevated anti-leptospiral antibody titer by MAT. Antigen-antibody agglutination by MAT was strongest for serovar Bratislava (1:3200) and also reactive to the leptospiral serovars Australis (1:400), Autumnalis (1:400), Icterohaemorrhagiae (1:400), and Mankarso (1:800). In total, 31 of 44 patients with suspected cases submitted serum samples for anti-leptospiral antibody serologic testing; 14 (45%) of 31 cases were confirmed (Figure 3). All 14 of the patients with confirmed cases were positive by both the DST and MAT, with 13 of 14 racers demonstrating highest titers to serogroup Australis (Table 3). The mean incubation time from the start of the race until onset of illness for the 14 patients with confirmed cases (13.5 days; range, 2–22 days) did not differ significantly from the mean incubation (12.8 days; range, 2–32 days) for the patients with suspected cases without laboratory confirmation. Paired serum samples were obtained from a total of 11 racers (range between acute-phase and convalescent-phase serum samples, 8–115 days), 6 of whom were among the 14 patients with confirmed cases. None of the paired serum samples demonstrated a 4-fold increase in anti-leptospiral antibody titer; however, numerous titers in convalescent serum samples remained elevated (≥400) several months after the race and exhibited similar serovar reactivity to acute serum samples (data not shown). In addition, serum samples were obtained from 9 racers who did not meet the suspected case definition but who were ill and requested testing. All 9 had negative DST and MAT results.

**Leptospira** cultures were performed for 4 case patients, and *Leptospira* organisms were isolated in cultures of both the urine and blood specimens collected on 5 December 2005 (~30 days after the race).

![Figure 3. Confirmed and suspected cases of leptospirosis (n = 44) by date of onset, United States Adventure Racing Association (USARA) leptospirosis outbreak, Florida, November 2005.](image-url)
after exposure) from 1 case patient with onset of symptoms 17 November 2005. Specimens were collected from this racer, inoculated directly into the EMJH semi-solid media in the field, and transported to the CDC Leptospirosis Reference Laboratory on the same day for processing. DNA-DNA hybridization identified the isolate as a member of species *L. noguchii*. PFGE performed on the isolate did not match any of the reference isolates contained in the CDC Leptospirosis Reference Laboratory database. CAAT characterization performed at the CDC preliminarily showed evidence of a potential new serovar within serogroup Autumnalis. Further characterization and confirmation of this isolate is ongoing.

**DISCUSSION**

The epidemiology of human leptospirosis is complex and dynamic, resulting from the interaction of pathogen, host, animal reservoir, and environment. Factors that increase the likelihood of humans and animals coming together in the same environment will continue to increase the risk of transmission of leptospirosis. Increasing urban human and rodent populations, occupational and avocational exposure to surface water, expanding canine leptospirosis, and climatic changes all have been associated with changes in the epidemiology of human leptospirosis [20–24]. Among these factors, climate change holds perhaps the greatest potential to affect the transmission of leptospires to humans. Although leptospirosis is no longer a nationally notifiable disease in the United States, it is critical to document outbreaks of leptospirosis, including microbiologic investigations and the role of climatologic conditions, to understand the changing epidemiology of the disease in the United States.

*L. noguchii* has been reported as a cause of human leptospirosis in the Southern United States, Peru, and Brazil [20]. The species has been isolated from rodents, opossums, toads, sheep, dogs, and cattle [20, 25]. The Hillsborough River State Park and surrounding areas are home to most of these animals. Although MAT titers in patients with confirmed cases were consistently elevated to serovar Bratislava (commonly associated with pigs), titers were also elevated to the leptospiral se-
Djasiman are associated with species, some members of serogroups Autumnalis, Australis, and Mankarso. Molecular and serological testing of the case patient haemorrhagiae, Australis, and Mankarso. Molecular and serovars Djasiman and, to a lesser extent, Autumnalis, Icterohaemorrhagiae, Australis, and Mankarso. Molecular and serological testing of the case patient Leptospira isolate suggest a potential new serovar within serogroup Autumnalis. Although genospecies and serogroups do not exhibit strict correlations, some members of serogroups Autumnalis, Australis, and Djasiman are associated with species L. noguchii [2]. The high MAT titers to serovar Bratislava that were observed in this outbreak may have resulted from cross-reactivity of this new serovar with other serovars [26]. In addition, paradoxical reactions in acute-phase serum samples are common, in which the highest observed titer is to a serovar unrelated to the actual infecting serovar [27]. Often, the interpretation of MAT results becomes more straightforward with later specimens. The observed consistency of antibody reactivity across confirmed cases and in paired serum samples obtained from the outbreak population may indicate infection with the same species and, therefore, may potentially indicate that the same host species is responsible for the outbreak. However, the single culture-confirmed case and failure to identify leptospires in environmental cultures do not allow us to conclude definitively whether this outbreak resulted from large scale exposure to an environment contaminated primarily by 1 species of leptospire with a single host species or from widespread contamination of the environment with pathogenic leptospires from multiple hosts.

The 2005 hurricane season broke records for both the number and the intensity of storms. Hurricane Wilma was the twenty-second named storm and fourth Category 5 hurricane of the 2005 season, as well as the most intense storm ever recorded in the Atlantic basin. On 24 October 2005, Hurricane Wilma made landfall in Florida as a Category 3 storm with sustained winds of 120 miles per hour, which resulted in substantial rainfall and flooding across southern and central Florida. This outbreak and other domestic and international outbreaks with attack rates of 12%-44% have been associated with competitive water sports events following periods of heavy rainfall [4–7, 9, 10]. Although adventure racing events occur in the United States almost weekly and usually include a paddling section, swamp race events are less common but pose an increased risk of substantial and prolonged exposure to surface water. In our investigation, nearly 100% of racers reported cuts and scrapes on the skin, commonly from submerged tree stumps, cypress roots, or saw palmetto plants that were present along the course (Figure 4). Submersion in water was also common, allowing for high levels of exposure to nonintact skin and mucus membranes. Ingestion of surface water resulted in increased odds of acquiring leptospirosis in this study and has previously been demonstrated as a risk factor for outbreak-associated leptospirosis [5, 28, 29]. Recommendations to avoid or mitigate such extreme water exposures in avocational or occupational settings are unlikely to be effective. Primary chemoprophylaxis has been demonstrated to be effective at reducing the risk of leptospirosis during periods of exposure to surface water, and some data suggest that postexposure prophylaxis may protect against the development of asymptomatic leptospirosis but not against seroconversion [30–33]. Furthermore, the potential benefits of short-term primary chemoprophylaxis with doxycycline likely outweigh the risks, because the morbidity associated with leptospirosis can be high. When substantial exposure to surface water is likely or when rainfall or flooding has increased the likelihood of submersion in or ingestion of surface water in an adventure race or similar event, organizers should consider the risk of leptospirosis and should consult with health care providers or public health officials to promote awareness of the disease and of the potential benefits of primary doxycycline chemoprophylaxis.

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Potential conflicts of interest. All authors: no conflicts.

References

3. Levett PN. Leptospira species (leptospirosis). In: Principles and practice