AN OUTBREAK OF LEPTOSPIROSIS AMONG PERUVIAN MILITARY RECRUITS

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Abstract. Acute undifferentiated febrile illnesses are common in tropical developing countries but are difficult to diagnose on clinical grounds alone. Leptospirosis is rarely diagnosed, despite evidence that sporadic cases and epidemics continue to occur worldwide. The purpose of this study was to diagnose an outbreak of acute undifferentiated febrile illness among Peruvian military recruits that developed after a training exercise in the high jungle rainforest of Peru. Of 193 military recruits, 78 developed an acute febrile illness with varied manifestations. Of these, 72 were found to have acute leptospirosis by a microscopic agglutination test (MAT). An enzyme-linked immunosorbent assay using \textit{Leptospira biflexa} antigen was insensitive for the detection of anti-leptospiral IgM antibodies compared with the MAT (20 of 72, 28%). This outbreak of acute undifferentiated febrile illness among Peruvian military recruits was due to leptospirosis. High clinical suspicion, initiation of preventative measures, and performance of appropriate diagnostic testing is warranted in similar settings to identify, treat, and prevent leptospirosis.

INTRODUCTION

Several outbreaks of leptospirosis in recent years have highlighted the global importance of this widespread zoonotic disease.\textsuperscript{1−4} The spirochetes that cause leptospirosis are transmitted to humans from a variety of chronically infected peridomestic mammalian reservoir hosts such as rodents, cattle, pigs, and dogs, as well as potentially from wild mammals such as rodents, marsupials, and bats.\textsuperscript{5,6} Humans become infected either through percutaneous or mucosal exposure to infected urine or ground waters containing leptospires. Infected mammals shed leptospires from renal tubules where the organisms can persist for months to years.\textsuperscript{5} Pathogenic leptospires are enzootic throughout tropical regions including Peru.\textsuperscript{6,7}

Risk factors for leptospirosis include living in rural and tropical settings with exposure to leptospire-contaminated fresh water and agriculture, sewer, and sanitation work; military personnel are also regarded as at-risk because of field activities that bring them into close contact with zoonotic reservoirs.\textsuperscript{8−10} However, these same patient populations are also frequently at risk for a variety of other infections that can manifest as acute, undifferentiated febrile or “influenza-like” illnesses. On clinical grounds alone, it is often difficult to distinguish among the many causes of acute undifferentiated febrile illness in the tropics, which can include malaria, arboviruses such as dengue, rickettsioses such as scrub typhus, or ehrlichiosis. Here we show how a systematic, comprehensive approach led to the identification of leptospirosis as the cause of an outbreak of acute undifferentiated febrile illness that occurred among military recruits participating in training exercises in a high jungle region of Peru. The performance of a commercially available enzyme-linked immunosorbent assay (ELISA) for the diagnosis of leptospirosis was also evaluated in this outbreak.

MATERIALS AND METHODS

From March to May 1999, 193 Peruvian military recruits accompanied by 25 officers and support personnel attended a training exercise in the high Amazonian jungle near the city of Pichanaqui (Chancharayo Province, Junín Department), Peru (Figure 1). This is a farming region where a variety of agricultural and animal products are produced. Although not the rainy season, occasional rainstorms occurred during this hot, humid summer period with an average temperature of 22°C. No flooding was experienced during this period. Recruits received a yellow fever vaccination three days prior to arrival at the training site; officers and support personnel had been vaccinated previously. The recruits spent two weeks in the area. During a period of approximately four weeks after the training period, 78 of the 193 recruits were hospitalized in Lima, Peru with an acute undifferentiated febrile illness. Some cases were probably missed since recruits that did not report to the hospital were unavailable for questioning.

A standard questionnaire was used to obtain demographic information, exposure history to animals and various sources of ground water, and clinical data from all 78 of the hospitalized recruits and from 14 of the 25 healthy support staff. A case was defined as any individual present during the training exercises who reported to the hospital with an acute febrile illness during the month following the completion of the exercise. Acute blood samples were obtained from the 78 hospitalized recruits and from 14 healthy support staff; convalescent samples were drawn three weeks later. None of the healthy recruits were available for sampling.

An IgM ELISA was used to test all acute and convalescent sera for evidence of acute infection to the following arboviruses: dengue, Oropouche, Venezuelan equine encephalitis (VEE), Mayaro, and yellow fever.\textsuperscript{11−13} Acute sera were tested for virus in cultures of Vero and C6/36 cells by standard procedures. Immunofluorescence was performed on all cell cultures using polyclonal antibodies against dengue, yellow fever, Mayaro, VEE, Oropouche, and group C bunyaviridae viruses.\textsuperscript{12} The sera were also examine for antibodies against rickettsial and ehrlichial species using an immunofluorescence test previously described.\textsuperscript{14}

Convalescent serum samples were screened for anti-leptospiral IgM antibodies using a \textit{Leptospira biflexa} antigen in a commercial ELISA kit (Leptospira IgM ELISA Test, catalogue no. LPM-200; Pan Bio, Gaithersburg, MD). Acute samples were also tested from patients that tested positive for IgM antibodies in their convalescent sera. The microscopic agglutination test (MAT) was used as the gold standard, and included a standard panel of 23 leptospiral serovars repre-
senting the known pathogenic serogroups. The MAT results were interpreted as “definitely positive” with a four-fold or higher increase in antibody titer, a single titer $\geq 1/800$, or conversion from negative to positive between the acute and convalescent samples; “probably positive” was defined as a titer $\geq 1/400$ with either no change or less than a four-fold increase in titer between the paired samples. These criteria are consistent with those in the published literature.

RESULTS
Potential exposures to infectious pathogens were similar for recruits and the support staff with the exception of the
recruit’s sleeping location, common latrine location, and certain training exercises. Each day the recruits swam in a small pond located on site; in addition, they were exposed to a drainage ravine contaminated with excreta from local farm animals two weeks and again three days prior to departure. No support staff took part in these exercises.

The epidemic curve is presented in Figure 2. On detailed questioning, none of the officers or support personnel became ill (0 of 25). The age of the cases ranged from 19 to 26 years (median = 23). Apart from fever, myalgia and headache were the most common manifestations. Jaundice occurred in only 8% of the patients (Table 1). Symptom duration ranged from 1 to 34 days (median = 9.1). All patients fully recovered.

Acute serum samples were obtained a median of 9.1 days after the onset of illness (range = 1–34, SD = 5.8) and convalescent samples were obtained a median of 30.1 days after the onset of illness. All acute samples were obtained on May 24, 1999; all convalescent samples were obtained on June 16, 1999. Serologic and culture results for sera from the 78 symptomatic recruits and 14 sampled support personnel revealed no evidence of acute infection with dengue, Oropouche, VEE, Mayaro, yellow fever, group C bunyaviridae viruses, or with rickettsial or ehrlichial species. Malaria smears were also negative for all patients. Of the symptomatic patients, anti-leptospiral IgM antibodies were demonstrated in 26% (20 of 78) of the convalescent serum samples by commercial ELISA. Among these positive samples, IgM antibodies were found in 18% (n = 14) of the acute sera. Confirmatory testing by MAT was definitely positive for 59 (76%) and probably positive for 13 (16%) of the 78 patients. Combined, 72 (92%) of 78 were definitely or probably positive by the MAT. Six of the 78 hospitalized recruits (8%) demonstrated non-diagnostic results or no evidence of anti-leptospiral antibodies in paired sera. The serovars in the MAT panel that were most frequently detected were cynopteri (n = 11), bataviae (n = 9), and djasiman (n = 7). Most patients had multiple serovars detected by the MAT but only the highest titer is reported. Anti-leptospiral antibodies were not detected in any of the 14 asymptomatic officers tested.

Using the MAT as the gold standard, the sensitivity and specificity of the Pan Bio ELISA were 26% and 60%, respectively, with a positive predictive value of 95% and a negative predictive value of 9.0%.

**DISCUSSION**

The diagnosis of acute undifferentiated febrile illnesses is difficult in tropical settings where many possible agents can be responsible for infectious disease outbreaks. Such was the case with the outbreak of leptospirosis in Nicaragua in 1995.
when thousands of patients developed acute undifferentiated febrile illnesses, and several dozen died of pulmonary hemorrhage. Not until spirochetes were seen by special staining of necropsied kidney tissue was leptospirosis seriously entertained as the cause of this epidemic. Similarly, after extensive investigation for viral, rickettsial, and ehrlichial causes of this outbreak of acute febrile illness among Peruvian military recruits, we found that leptospirosis was the cause. Since asymptomatic recruits were not available for questioning, a case-control comparison of exposures was not possible. However, we were able to find evidence that this outbreak was most likely due to extensive exposure of a large group of military recruits to a drainage ravine contaminated with urine from infected animals. An exposure of this type is consistent with risk previously associated with outbreaks of leptospirosis. No support personnel took part in these exercises, and no support personnel became ill or seroconverted.

By their nature, military operations often require exposure to environments at high risk for contracting leptospirosis. As illustrated in this study, an acute febrile illness occurred in nearly half of the military trainees with a median duration of more than a week. The high attack rate that we observed is similar to the 50% attack rate that occurred in the 2000 Eco-Challenge-Sabah athletic event, in which an estimated half of 305 participants developed a syndrome compatible with leptospirosis. The presumed exposure in that outbreak was swimming in the Segama River in Borneo, Malaysia. A two-year study of fever among servicemen in South Vietnam revealed that 20% (159 of 793) was caused by leptospires. Among military personnel in Okinawa, Japan, two case-clusters in 1987 affecting 18% and 47% of exposed was reported. Many additional studies report high incidence of leptospirosis in military personnel abroad, presumably because of their exposure to contaminated water during field exercises. Early diagnostic testing is important for expeditious use of appropriate antibiotics, thus minimizing the mission-compromising impact of this illness.

The Centers for Disease Control and Prevention has reported that commercial assays (one of which was used in our study) using L. biflexa antigen for detecting genus-specific anti-leptospiral IgM antibodies were useful in identifying leptospirosis as the cause of the Eco-Challenge-Sabah outbreak focused in Malaysian Borneo. These investigators found that such tests had a sensitivity of approximately 27% at three days following the onset of fever, increasing to 84% at 7–9 days, and nearly 100% by 10–12 days. A recent report from Hawaii, the state with the highest reported incidence of leptospirosis in the United States, also found that similar tests were relatively insensitive in the first week of illness, with maximal sensitivities of 80–90% after 2–4 weeks of illness. Other investigators have found that a genus-specific antigen is useful for the detection of anti-leptospiral antibodies from diverse geographic locations, including Thailand, Hawaii, Puerto Rico, and some of the continental states in the United States. We found that in the Peruvian rainforest high jungle setting, where a high biodiversity of Leptospira might be expected (and thus possibly less cross-reactivity with a genus-level leptospiiral antigen), a L. biflexa-based serologic assay was relatively insensitive for supporting the diagnosis of leptospirosis in any given patient. However, when using this test on an outbreak population, the test was useful for suggesting leptospirosis as the cause of the outbreak, which was then confirmed by gold standard serologic testing (MAT). The reason for the discordance of these results is likely the presence of different leptospiral serovars in the different regions; however, as recently shown, it is impossible based on serologic data to identify the causative leptospiral serovar or the precise mammalian source of infection. Leptospirosis isolates from patients would have been necessary to definitively identify the zoonotic source(s) of infection in this outbreak.

Identifying leptospirosis as the cause of an outbreak of undifferentiated febrile illness among military recruits in Peru reminds us of the epidemic potential of this disease, and the association of this disease with particular epidemiologic scenarios. With the widespread global distribution of pathogenic leptospires and their mammalian hosts, humans will always live side-by-side with animal reservoirs of these spirochetes. When possible, avoidance of high-risk behaviors should be encouraged. Uniquely, the military and other select professions cannot always avoid exposure, and infection will be demonstrated by mission-crippling epidemics. When a high risk of contracting leptospirosis can be identified, for example in jungle training, it may be useful to provide weekly doxycycline as prophylaxis. An L. biflexa-based screening ELISA can be useful for suggesting leptospirosis as the cause in populations of patients; however, improved, affordable, simple diagnostic testing is needed for early diagnosis of leptospirosis in individual patients.

Received February 12, 2003. Accepted for publication April 26, 2003.

Acknowledgments: We sincerely thank Carolina Guevara and Vidal Felices for their extensive laboratory processing of samples from this outbreak. We also thank Juan Perez for data analysis and Monica Barrera for expertise and patience with the graphic arts.

Financial support: This work was supported by the U.S. Department of Defense, the Naval Medical Research and Development Command (Work Unit No. 62787 A870 1612), the Texas Higher Education Coordinating Board, Advanced Technology Program, and the National Institutes of Health, Fogarty International Center (grant 1R01TW005860-01).

Disclaimer: The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or as reflecting the views of the Navy Department or the naval service at large.

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