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Rosalie T. Trevejo

José G. Rigau-Pérez

David A. Ashford

Emily M. McClure

Carlos Jarquín-González

*See next page for additional authors*

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## Authors

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*Centers for Disease Control and Prevention, Epidemiology Program Office, Epidemic Intelligence Service, and National Center for Infectious Diseases, Division of Bacterial and Mycotic Diseases, Childhood and Respiratory Diseases Branch and Emerging Bacterial and Mycotic Diseases Branch, and Division of Viral and Rickettsial Diseases, Molecular Pathology and Ultrastructure Activity, Atlanta, Georgia; Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases, Bacterial Zoonoses Branch and Arbovirus Diseases Branch, and United States Department of Agriculture, National Wildlife Research Center, Fort Collins, Colorado; United States Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, Iowa; Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Vector-Borne Infectious Diseases, Dengue Branch, San Juan, Puerto Rico; Asociación Pro-Bienestar de la Familia Nicaragüense and Ministerio de Salud, Complejo Nacional de Salud, Managua, Nicaragua*

In October 1995, epidemic "hemorrhagic fever," without jaundice or renal manifestations, was reported in rural Nicaragua following heavy flooding; 2259 residents were evaluated for nonmalarial febrile illnesses (cumulative incidence, 6.1%) and 15 (0.7%) died with pulmonary hemorrhage. A case-control study found that case-patients were more likely than controls to have ever walked in creeks (matched odds ratio [MOR], 15.0; 95% confidence interval [CI], 1.7–132.3), have household rodents (MOR, 10.4; 95% CI, 1.1–97.1), or own dogs with titers ≥400 to *Leptospira* species (MOR, 23.4; 95% CI, 3.6–∞). Twenty-six of 51 case-patients had serologic or postmortem evidence of acute leptospirosis. *Leptospira* species were isolated from case-patients and potential animal reservoirs. This leptospirosis epidemic likely resulted from exposure to flood waters contaminated by urine from infected animals, particularly dogs. Leptospirosis should be included in the differential diagnosis for nonmalarial febrile illness, particularly during periods of flooding or when pulmonary hemorrhage occurs.

Leptospirosis is a zoonosis of worldwide distribution with many wild and domestic animal reservoirs [1]. Human infection typically results from exposure to infected animal urine, by either direct contact or indirect exposure through water or soil [2]. The early clinical presentation is often nonspecific, with fever, headache, chills, myalgia, and abdominal pain. Two classic forms of leptospirosis have been described: the anicteric (most common and mildest) and the icteric (Weil's syndrome), which causes severe renal, hepatic, and vascular dysfunction [3]. Mild pulmonary involvement has been reported in 20%–70% of leptospirosis patients but is often overshadowed by other manifestations [4]. Except for case reports from Brazil, leptospirosis with severe pulmonary involvement has not been reported previously in the Western Hemisphere [5, 6]. Leptospirosis epidemics with pulmonary hemorrhage and few or no jaundiced patients have been reported in Korea and China [7, 8].

## Methods

### Background

During October and November 1995, two health centers in the neighboring rural jurisdictions of Achuapa and El Sauce in western Nicaragua (total population, 37,030) reported an increased number of patients with an illness characterized by fever, headache, chills, and musculoskeletal pain; some died with hemorrhagic manifestations and shock [9]. No jaundice or renal manifestations were reported. These reports immediately followed a series of tropical storms, with rainfall reaching 5000 mm by October 1995, compared with an annual average of 1300 mm during 1992–1994. During

9–29 October 1995, 100 patients from Achuapa were diagnosed with "hemorrhagic fever" and hospitalized; males and females were equally affected, and the majority were between 5 and 14 years of age.

In October 1995, the Nicaraguan Ministry of Health (MOH) initiated epidemiologic and laboratory investigations in collaboration with the United States Centers for Disease Control and Prevention (CDC). The study objectives were the following: to identify and characterize the etiologic agent through laboratory studies, to describe the epidemic and clinical illness, and to identify possible risk factors and prevention strategies. The study site consisted of the neighboring jurisdictions of Achuapa and El Sauce, including their towns and associated outlying areas (5107 km$^2$).

### Descriptive Epidemiology

The cumulative incidence of nonmalarial febrile illness in the study site for October and November 1995 was estimated by using a register of patient visits to the two health centers. National hospital surveillance data collected by the Nicaraguan MOH for fatal hemorrhagic fever cases occurring in October and November 1995 were used to estimate the case-fatality rate for the study site.

### Case-Control Study (CCS)

Study site health centers' medical records from October 1995 were reviewed to identify potential case-patients for enrollment in the CCS. Case-patients were defined as having the following: fever with onset during October 1995; headache, musculoskeletal pain, or chills; abdominal pain, respiratory distress, hemorrhagic manifestations, or hypotension; negative results for malaria by blood smear; and an illness severe enough to require hospitalization. Case-patients who died with pulmonary hemorrhage in October or November 1995 were interviewed by proxy. A standardized questionnaire collected information on daily activities and travel during October 1995, food and water sources, environment, and pre-existing medical conditions. The illness history of case-patients and other family members was also determined.

One control subject was randomly selected for each case-patient from the same town or outlying area and matched by age group (0–4 years, 5–14 years, and ≥15 years). For the selection of controls from towns, town maps were consulted, and one block was randomly selected for each case-patient, from which interviewers were instructed to randomly select one household. For the selection of controls from outlying areas, a line listing of all persons by household in the district with the highest incidence was consulted, and one household was randomly selected for each case-patient. For controls from towns and outlying areas, if an appropriate age-matched control was unavailable at a household, the next house to the right or the next household on the list was selected, respectively. Potential controls with a history of fever during the month of October were excluded.

### Specimens Collected

*Non-CCS specimens.* The Nicaraguan MOH initially submitted 53 serum specimens and 4 postmortem tissue specimens from patients with outbreak-associated illness to CDC to rule out dengue and other infectious agents associated with febrile hemorrhagic illness. Entomologic surveys of the study site were conducted by CDC and the Nicaraguan MOH to identify potential vectors of mosquitoborne illnesses. During the field investigation, all available postmortem tissues from patients diagnosed with fever and hemorrhage were obtained for pathologic and immunohistochemical evaluation at CDC.

*Active surveillance specimens.* From 14 through 30 November 1995, active surveillance was conducted at the two health centers and the regional referral hospital by requesting health care workers at these sites to collect whole blood and urine for culture from patients presenting with febrile illnesses not diagnosed as malaria (nonmalarial febrile illnesses). An investigator would visit each site at least once daily to retrieve collected specimens and to consult with health care workers about which patients should provide specimens for culture.

*CCS specimens.* For the CCS, acute-phase serum specimens were collected from case-patients by the MOH; control and convalescent-phase case-patient serum specimens were collected during CCS interviews conducted from 14 through 22 November 1995. Serum specimens were collected from all available household contacts of case-patients and all available domestic animals at CCS households, regardless of illness history. In addition, febrile case-patients and household members who had not received antibiotics were asked to provide whole blood and urine for culture. Whole blood and urine for culture were obtained at CCS households from domestic animals that had been ill during October 1995. Rodents were trapped at CCS households and surrounding community sites, serum specimens were collected, and kidneys were harvested for culture and fluorescent antibody (FA) testing.

In December 1995, a follow-up survey was conducted of dogs from households of patients identified through the CCS and active surveillance to collect urine for culture, regardless of the dogs' illness histories.

### Laboratory Studies

Mosquitoes were pooled, triturated in BA-1 medium, and tested for viruses by Vero cell plaque assay by a double agar overlay system. Immunohistochemical and silver staining for *Leptospira* species were done on postmortem tissue specimens [10].

The microscopic agglutination test (MAT) was used to determine antibody titers to pathogenic *Leptospira* species [11]. Human and animal serum specimens were tested against multiple serovars at CDC and the Agricultural Research Service, US Department of Agriculture (USDA), respectively. Inclusion of an antigen panel of local isolates is recommended but was unavailable for this investigation [12].

FA testing was used to detect *Leptospira* antigen in rodent kidney specimens [13].

Whole blood and urine specimens from humans and domestic animals and rodent kidney tissues were cultured for *Leptospira* species in polysorbate liquid medium (Introgen, Purchase, NY), supplemented with 1.5% agar (Difco Laboratories, Detroit) and neomycin (10 $\mu$g/mL) [14]. Cultures were shipped at room temperature to CDC, where they were incubated at 30°C for a minimum of 3 months. Cultures were inspected by darkfield micros-
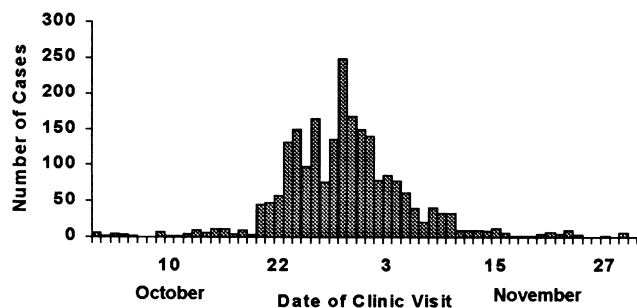
**Figure 1.** Nonmalarial febrile illnesses, Achuapa and El Sauce, Nicaragua, 1 October to 30 November 1995 (*n* = 2259).

copy on arrival and weekly thereafter. Identification of *Leptospira* isolates was done by CDC and USDA by use of serogrouping, restriction endonuclease digestion, polymerase chain reaction, restriction fragment length polymorphism, and 16s rRNA sequencing.

The following laboratory case definitions were used for human leptospirosis: confirmed, *Leptospira* species isolated from whole blood or urine, ≥4-fold rise in titer, or demonstration of *Leptospira* species in postmortem tissue by immunohistochemistry (IHC); probable, a titer ≥400 to one or more serovars; and seronegative, a titer <400 to all serovars. The following laboratory case definitions were used for leptospirosis in animals: confirmed, *Leptospira* species isolated from whole blood or kidney tissue, or kidney tissue positive for *Leptospira* species by FA; probable, a titer ≥400 to one or more serovars; and seronegative, a titer <400 to all serovars.

### Statistical Analysis

For the CCS, Epi Info 6.02 (CDC, Atlanta) was used to determine descriptive statistics and $\chi^2$ analyses, including univariate matched analyses. Variables identified as potential risk factors ($P \le .20$) were analyzed by conditional logistic regression if they were biologically plausible or had been suggested as important risk factors in previous studies. Conditional logistic regression was done with LogXact 1.3 (Cytel Software, Cambridge, MA) to obtain exact and asymptotic estimates of the matched odds ratios (MOR) and 95% confidence intervals (CI); all variables were analyzed as dichotomous variables. For conditional logistic regression, variables with associated $P \le .05$ when adjusted for sex were considered to be significant.

### Results

*Descriptive epidemiology.* During October and November 1995, 2259 patients were evaluated for nonmalarial febrile illnesses at the two study site clinics (figure 1). The estimated cumulative incidence of nonmalarial febrile illness was 2259/37,030 (6.1%), with males and females equally affected. The estimated minimum case-fatality rate from pulmonary hemorrhage was 15/2259 (0.7%). The age-specific incidence for El Sauce patients between the ages of 1 and 14 years was signif-

icantly higher ($P < .05$) than the rates for the other age groups (figure 2).

*CCS.* The CCS questionnaire was administered to 51 (85.0%) of 61 eligible case-patients (or their proxy) and 51 matched controls. This included 25 (80.6%) of 31 recovered case-patients from towns, all 15 (100.0%) recovered case-patients from the outlying areas, and (by proxy) 11 (73.3%) of 15 patients who died.

The case-patient group had a significantly higher percentage of males (54.9%) than did the control group (31.4%) ($P < .05$) and had a slightly lower mean age (14.9 vs. 17.7 years). Signs and symptoms reported by case-patients are listed in table 1.

A matched, univariate analysis found that case-patients were significantly more likely than controls to have reported walking through creeks or swimming in rivers; on the other hand, having rodents in household food storage areas, walking through mud, or living in homes with dirt floors were not significantly associated with illness (table 2). Conditional logistic regression, adjusting for sex, found that case-patients were significantly more likely than controls to have reported walking through creeks or to have rodents in household food storage areas; living in homes with dirt floors, walking through mud, or swimming in rivers were not significantly associated with illness (table 2).

Exact estimates of the MOR and 95% CI found that case-patients were significantly more likely than controls to own seropositive dogs, but not more likely to own seropositive pigs or cows, or to have rodents with laboratory evidence of leptospirosis on their property (table 3).

*Laboratory studies.* Dengue virus, New World arenaviruses, lymphocytic choriomeningitis virus, bunyaviruses, filoviruses, flaviviruses, alphaviruses, spotted fever and typhus-group rickettsiae, *Ehrlichia chaffeensis,* and *Coxiella burnetii* were ruled out by serologic testing and polymerase chain reaction of serum specimens from the 53 patients with outbreak-associated illness and IHC studies of postmortem tissue specimens from the 4 patients with fever and pulmonary hemorrhage [9]. No *Aedes aegypti* mosquitoes (the major dengue virus vector) were collected by entomologic surveys, and
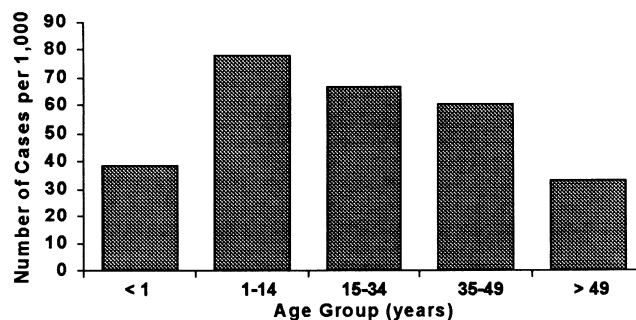


**Figure 2.** Age-specific incidence of nonmalarial febrile illnesses, El Sauce, Nicaragua, 1 October to 30 November 1995.

**Table 1.** Case-control study: reported symptoms or signs among case-patients (n = 51).

| Symptom or sign | No. of case-patients affected (%) |
|---|---|
| Headache | 46 (90) |
| Chills | 45 (88) |
| Musculoskeletal pain | 41 (80) |
| Vomiting | 35 (69) |
| Dyspnea | 31 (61) |
| Hemorrhage[a] | 20 (39) |

[a] Includes pulmonary hemorrhage in fatal cases (11), and among recovered case-patients, hemoptysis (4), gingival and/or vaginal bleeding (2), epistaxis (1), ecchymosis (1), and hematuria (1).

no viruses were isolated from the other mosquito species cultured.

*Leptospira* species were initially identified by IHC and silver staining in postmortem tissue specimens from the 4 patients with fever and pulmonary hemorrhage [9]. Postmortem tissue specimens were eventually obtained from 14 hemorrhagic fever patients; 13 were positive for *Leptospira* species by IHC, and 5 of the 13 were enrolled in the CCS. Subsequent serologic testing of the 53 outbreak-associated patients revealed titers ≥400 to one or more *Leptospira* serovars among 23 (43.4%).

Laboratory specimens were available from 39 (76.5%) of 51 case-patients from the CCS: serum specimens from 34 (17 paired and 17 single) and postmortem tissue specimens from 5. The mean collection time after onset was 7 days for acute-phase serum specimens (range, 1–17) and 28 days for convalescent-phase serum specimens (range, 17–40). Fourteen (27.5%) case-patients were laboratory-confirmed for leptospirosis, 12 (23.5%) were probable, and 13 (25.5%) were seronegative.

Of serum specimens obtained from 47 (92.2%) of 51 controls, 36 (70.6%) were seronegative, and 11 (21.6%) had titers ≥400 to one or more *Leptospira* serovars. Serovars detected among case-patients and controls are listed in table 4.

Of 200 household contacts of case-patients, 72 (36%) had titers ≥400 to one or more *Leptospira* serovars. Household contacts were not significantly more likely than controls to have titers ≥400 to *Leptospira* species (OR, 1.8; 95% CI, 0.8–4.1). A history of febrile illness during October 1995 was reported by 67 (33.5%) household contacts but was not associated with having a titer ≥400 to *Leptospira* species (OR, 1.2; 95% CI, 0.6–2.3).

Of 199 domestic animals from CCS households, 39 (56.5%)

of 69 pigs, 29 (44.6%) of 65 dogs, 4 (33.3%) of 12 horses, and 16 (30.2%) of 53 cows had titers ≥400 to one or more *Leptospira* serovars (table 4).

Serum or kidney specimens, or both, were collected from 190 rodents (134 mice [*Mus musculus*] and 56 rats [*Rattus rattus*]): 69 (36.3%) from CCS households and 121 (63.9%) from other sites in the community. Three (1.8%) of 165 available rodent serum specimens had titers ≥400 to one or more *Leptospira* serovars (table 4). Of 59 available rodent serum specimens from CCS households, 1 (3.0%) of 33 from case-patient households and 1 (3.8%) of 26 from control households had titers ≥400 to one or more *Leptospira* serovars.

Kidney specimens were available from 185 (97.4%) rodents for FA testing; 81 (43.8%) were positive for *Leptospira* species, including 67 (51.1%) of 131 from mice and 14 (25.9%) of 54 from rats. Mice were significantly more likely to have kidney tissue positive for *Leptospira* species by FA testing than were rats (OR, 3.0; 95% CI, 1.4–6.4). Of 67 available rodent kidney specimens from CCS households, 16 (38.1%) of 42 from case-patient households and 12 (48%) of 25 from control households were positive by FA.

Of whole blood and urine specimens from 4 CCS household animals that had been ill during October 1995, *Leptospira interrogans* serovar pomona type kennewicki was isolated from the urine of a pig. A case-patient household mouse had kidney tissue positive by culture for *L. interrogans* serovar canicola and positive for *Leptospira* species by FA and a serum specimen with a titer of 200 to serovar canicola. The follow-up survey in December 1995 collected urine from 10 dogs from households of patients identified through the CCS and active surveillance, of which 6 (60%) were culture-positive for *L. interrogans* serovar canicola.

Through active surveillance, *L. interrogans* isolates were obtained from blood of 3 acutely ill patients and from urine of 1 convalescent patient: 2 serovar canicola isolates and 2 serogroup Pyrogenes isolates for which no serovar classification currently exists.

## Discussion

A large epidemic of human leptospirosis resulted in high morbidity and mortality in Nicaragua in October and Novem-

**Table 2.** Potential risk factors among case-control study participants with associated matched odds ratios (MORs) and 95% confidence intervals (95% CIs) (n = 51 matched pairs).

| Risk factor | Exposed case-patients (n = 51) | Exposed controls (n = 51) | Univariate MOR (95% CI) | Multivariate MOR[a] (95% CI) |
|---|---|---|---|---|
| Walking in creeks | 30 | 18 | 13.0 (2.0–552.5) | 15.0 (1.7–132.3) |
| Rodents in food storage areas | 14 | 7 | 4.5 (0.9–42.8) | 10.4 (1.1–97.1) |
| Dirt floor in home | 43 | 37 | 3.0 (0.8–17.2) | 3.4 (0.8–13.6) |
| Walking in mud | 38 | 32 | 4.0 (0.8–38.7) | 3.2 (0.7–15.5) |
| Swimming in river/creek | 23 | 13 | 3.0 (1.04–10.6) | 2.5 (0.9–7.3) |

[a] Adjusted for sex.

**Table 3.** Animals from case-patient and control households with laboratory evidence of leptospirosis, with associated matched odds ratios (MORs) and 95% confidence intervals (95% CIs).

| | Case-patients | Controls | Multivariate MOR (95% CI) |
|---|---|---|---|
| Canine | 21/28 | 8/37 | 23.4 (3.6–∞) |
| Bovine | 9/28 | 7/25 | 1.5 (0.1–14.4) |
| Porcine | 21/40 | 18/29 | 0.5 (0.1–4.1) |
| Equine | 2/6 | 2/6 | Insufficient data |
| Rodents | 16/43 | 12/26 | 0.9 (0.2–4.0) |

NOTE. Data include results from serology, fluorescent antibody testing (rodents only), and culture and are no. positive/total tested.

ber 1995. The most likely explanation for this epidemic was increased exposure to flood waters that had become contaminated by urine from animals infected with *Leptospira* species [15]. The recovery of multiple serovars from humans and animals during this epidemic implies involvement of more then one serovar in the epidemic and is consistent with flood contamination of groundwater and the epidemiologic association between illness and creek exposure. Temporal associations between heavy rainfall and human leptospirosis have been reported in Brazil, Puerto Rico, and Barbados [5, 16, 17]. A recent outbreak found an association between illness and ingesting or being submersed in river water during a rafting trip in Costa Rica early in the rainy season [18].

A unique feature of this investigation is the combined, systematic sampling of humans and animals in the study site for epidemiologic and laboratory studies. A 1962 study of the epidemiology of leptospirosis in Nicaraguan domestic and wild animals recovered 9 *L. interrogans* serovar canicola isolates from mammals trapped in the western region but did not examine for any associations with human leptospirosis [19]. Previous leptospirosis outbreaks have included sampling of potential animal reservoirs, but not in the context of a CCS [7, 8, 20–22]. The approach of the current study allowed identification of potential animal reservoirs and assessment of epidemiologic risk associated with animal contact.

The selection of recently or currently symptomatic domestic animals from CCS households for culture was a limitation of the CCS, since leptospirosis in animals is often asymptomatic and *Leptospira* species can be shed in the urine for prolonged periods [15, 23]. However, serology conducted for all available domestic animals from CCS households, regardless of illness history, found a high seroprevalence (56.5%). In addition, urine from 6 (60%) of 10 dogs sampled during the follow-up survey was culture-positive for *L. interrogans* serovar canicola.

The high infection rate among dogs, the predominance of serovar canicola isolates, the high seroprevalence to serovar canicola among case-patients, and the epidemiologic risk associated with ownership of dogs with titers ≥400 to *Leptospira* species suggest that urine shedding by infected dogs played a major role in amplifying the level of environmental contamination near households (peridomestic amplification) during the epidemic. It is possible that disease transmission resulted from

direct or indirect exposure to urine from infected dogs, a common-source exposure (i.e., creeks or rivers) for dogs and humans, or a combination of both. Given the propensity of young children for outdoor activities and animal contact, exposure to contaminated water or infected dogs, or both, would also account for the high incidence among children and the equal distribution among male and female case-patients. Flooding would presumably result in contamination of water sources by multiple animal species, but of domestic animal exposures, only ownership of seropositive dogs was found to be associated with illness. In a retrospective CCS, it is only possible to hypothesize about potential scenarios for disease transmission, since the timing of each exposure in relation to the development of disease cannot be distinguished. This study raises questions about the role of dogs and different environmental exposures that need to be explored in future studies.

Seroprevalence to *Leptospira* serovars was much higher among domestic animals (56.5%) than among rodents (1.8%). A high rate of carriage among rodents was shown by the high percentage of FA results that were positive for *Leptospira* species (43.5%). The low seroprevalence among rodents suggests a maintenance host infection with a resultant poor immunologic response or possible carriage of new or previously unrecognized serovars or serovars not represented on the MAT panel [24, 25]. There was a slight epidemiologic risk of illness associated with having rodents in household food storage areas after adjusting for sex in the multivariate model. The doubling of the odds ratio for having rodents in household food storage areas after adjusting for sex is due to the higher proportion of men in the case-patient group. Men reported seeing rodents in household food storage areas much less frequently than women, likely because they tended to work outside the home and not be involved with food preparation. However, the role of rodents as reservoirs in this epidemic remains unclear, since only 1 rodent isolate was recovered.

Some case-patients may have been seronegative because of timing of specimen collection, the antigen panel used for the MAT, or case-patient misclassification. For instance, serum specimens may have been collected before seroconversion for some case-patients. It is estimated that up to 10% of patients may not seroconvert within 30 days of clinical onset, a cutoff that some case-patients fell short of in this study [14]. It is possible that inclusion of an antigen panel of local isolates, which was unavailable for this investigation, may have increased the sensitivity of the MAT. Misclassification of case-patients could have occurred if other illnesses, such as malaria or dengue, were responsible for the observed clinical symptoms. For instance, someone with a blood smear that was falsely negative for malaria agents, but who met the clinical criteria of the CCS, would have been misclassified as a case-patient. Dengue as a cause of misclassification bias is less likely, since there was no evidence of dengue in the study site.

Controls with titers ≥400 to *Leptospira* species may have been

**Table 4.** Microscopic agglutination test-positive specimens by *Leptospira* serovar.

| Serovar (strain) | Case-patients | Controls | Canine | Porcine | Bovine | Equine | Rodent |
|---|---|---|---|---|---|---|---|
| alexi | 4/33 (12.1) | 0/47 | ND | 1/69 (1.4) | ND | ND | ND |
| australis | 1/32 (3.1) | 2/47 (4.3) | 1/65 (1.5) | 0/69 | 0/53 | 1/12 (8.3) | 0/165 |
| autumnalis | 3/33 (9.1) | 2/47 (4.3) | ND | 1/69 (1.4) | ND | ND | ND |
| ballum (Mus 127) | 12/33 (36.4) | 2/47 (4.3) | ND | ND | ND | ND | ND |
| ballum (S102) | ND | ND | 20/65 (30.8) | 27/69 (39.1) | 8/53 (15.1) | 1/12 (8.3) | 2/165 (1.2) |
| bratislava | 5/33 (15.2) | 6/47 (12.8) | 8/65 (12.3) | 6/69 (8.7) | 3/53 (5.7) | 2/12 (16.7) | 0/165 |
| canicola (Hond-Utrecht IV) | 18/33 (54.5) | 7/47 (14.9) | 22/65 (33.8) | 24/69 (34.8) | 7/53 (13.2) | 0/12 | 2/165 (1.2) |
| celledoni | 1/30 (3.3) | 0/47 | ND | ND | ND | ND | ND |
| copenhageni | 7/32 (21.9) | 1/41 (2.4) | ND | ND | ND | ND | ND |
| grippotyphosa | 0/33 | 0/47 | ND | 1/63 (1.6) | ND | 0/12 | ND |
| hardjobovis | ND | ND | 3/65 (4.6) | 0/69 | 1/53 (1.9) | 0/12 | 0/165 |
| icterohaemorrhagiae | 6/33 (18.2) | 4/47 (8.5) | 4/65 (6.2) | 24/69 (34.8) | 2/53 (3.8) | 2/12 (16.7) | 3/165 (1.8) |
| mankarso | 11/33 (33.3) | 0/47 | ND | ND | ND | ND | ND |
| pomona (Pomona) | 2/33 (6.1) | 1/47 (2.1) | ND | ND | ND | ND | ND |
| pomona type kennewicki | ND | ND | 1/65 (1.5) | 5/69 (7.2) | 3/53 (5.7) | 1/12 (8.3) | 0/165 |
| pyrogenes | 12/33 (36.4) | 5/47 (10.6) | 12/65 (18.5) | 5/69 (7.2) | 3/53 (5.7) | 2/12 (16.7) | 0/165 |
| shermani | ND | ND | 4/55 (7.3) | 18/69 (26.1) | 1/43 (2.3) | 1/2 (50.0) | 3/165 (1.8) |
| wolffi | 1/33 (3.0) | 1/47 (2.1) | 0/64 | ND | 4/52 (7.5) | 1/12 (8.3) | 0/165 |

NOTE. Data are no. positive/total tested (%). ND = not done. No reactivity was demonstrated to serovars bataviae, borincana, cynopteri, georgia, and tarassovi in human serum specimens; to serovar djasiman in human or rodent serum specimens; or to serovar javanica in human, canine, bovine, or equine serum specimens. Other species were not tested for these serovars.

misclassified as a result of subclinical infection with *Leptospira* species. It is also possible that some of these controls had serologic evidence of a previous infection with *Leptospira* species.

Laboratory criteria were not used in the CCS to exclude case-patients or controls because of the limitations of laboratory testing discussed above and the lack of availability of clinical specimens for some CCS participants who otherwise met the clinical criteria. However, the results of a separate analysis, which included only case-patients with laboratory evidence of infection and seronegative controls, were similar to those obtained when only the clinical criteria were used.

Leptospirosis was first diagnosed in patients who had died with pulmonary hemorrhage. In addition to the 15 reports from the study site, 33 hemorrhagic fever deaths were reported from other areas of Nicaragua. Possible reasons for the high observed number of cases with pulmonary hemorrhage are that the magnitude of the epidemic was such that this number of cases would not be unexpected or that there was a new or previously unrecognized serovar more likely to cause pulmonary hemorrhage. The former explanation seems more likely, given the estimated cumulative incidence (6.1%) in the study site during October and November 1995. Since clinical specimens were unavailable for culture from the 13 patients who were confirmed by IHC, the possibility of a new or previously unrecognized serovar associated with pulmonary hemorrhage cannot be ruled out. Efforts are currently underway to identify *Leptospira* species serovars in postmortem tissue sections by polymerase chain reaction.

The absence of jaundice is inconsistent with the classic description of the severe form of leptospirosis (Weil's syndrome) [1–3, 26]. The perception persists that jaundice characterizes severe leptospirosis, despite reports of leptospirosis with pulmonary hemorrhage from Korea, China, and Brazil in which few or no patients developed jaundice [5–8]. In these epidemics, fatality rates ranging from 2.4% to 5% were reported for patients with hemoptysis, respiratory distress, and pulmonary hemorrhage. Although no single serovar was implicated in these epidemics, serovar icterohaemorrhagiae isolates were recovered from the Chinese epidemic, and 1 serovar lai isolate was recovered from the Korean epidemic.

Several measures could be taken to prevent and control morbidity and mortality in future epidemics. Providing community education to residents in at-risk areas on the need for exclusion of domestic animals and rodents from households and food and water sources, removal of potential rodent harborage, institution of appropriate food storage practices, and avoidance of contact with potentially contaminated water can reduce the risk of exposure. Increased awareness of leptospirosis and its respiratory manifestations among physicians in at-risk areas should increase the likelihood that patients will be diagnosed and treated appropriately. The early detection of leptospirosis would be enhanced by the local availability of rapid, easily performed, sensitive diagnostic tests [27, 28]. Inclusion of leptospirosis in the differential diagnosis for febrile illness in areas at high risk for malaria and dengue, particularly during periods of flooding, is essential. Prompt administration of penicillin, based on clinical suspicion of leptospirosis, could be lifesaving, or at least reduce the severity of illness, in circumstances when rapid laboratory diagnostics are not available. While antibiotic prophylaxis is indicated in certain situations, such as military training or travel in areas in which leptospirosis is highly endemic, an evaluation of the effectiveness, feasibility, and safety of community-wide prophylaxis in the face of a leptospirosis epidemic is still needed.

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