Outbreak of leptospirosis associated with swimming

LISA A. JACKSON, MD, ARNOLD F. KAUFMANN, DVM, WILLIAM G. ADAMS, MD, MARY BETH PHELPS, RN, BSN, CINDY ANDREASEN, RS, MS, CARL W. LANGKOP, MPH, BYRON J. FRANCIS, MD AND JAY D. WENGER, MD

Between July 7 and 18, 1991, five boys from a small town in rural Illinois experienced the onset of an acute febrile illness subsequently confirmed as leptospirosis by serologic tests. A cohort study found that swimming in a small swimming hole, Steel Tunnel Pond, was associated with disease \( (P < 0.01) \), the attack rate being 28%. *Leptospira interrogans* serovar grippotyphosa was isolated from urine cultures from two of the case patients and from a culture of Steel Tunnel Pond water. A high seroprevalence for grippotyphosa was found in animals near the pond. Drought conditions had been present in the month before the outbreak, creating an environment in the pond which probably facilitated transmission of the organism from area animals to humans. Although leptospirosis is infrequently reported in humans in the United States, it is endemic in animals and the potential for outbreaks exists, especially when environmental conditions are favorable.

INTRODUCTION

Leptospirosis is an acute systemic infection which may be caused by any of the more than 240 pathogenic
The organism can be isolated from the blood or urine of affected humans or animals or from water sources. However, because leptospires are fastidious and require special culture methods, the clinical diagnosis of leptospirosis is usually confirmed only by serologic tests. Leptospires were isolated from affected persons in 3 of the 15 previously reported waterborne outbreaks and from the implicated water source in only 2\textsuperscript{2,4}; in none of those outbreaks were leptospires isolated from both the water source and the affected humans. We report an outbreak of disease caused by \textit{Leptospira interrogans} serovar grippotyphosa associated with swimming in a rural swimming hole. It is the second waterborne outbreak of leptospirosis caused by the grippotyphosa serovar reported in the United States\textsuperscript{3} and the only waterborne outbreak of leptospirosis in which the organism was isolated from both the patients and the implicated water source.

**METHODS**

**Case finding.** A possible case was defined as fever (maximum temperature $\geq 101.4^\circ$F) and at least three other symptoms compatible with leptospirosis (headache, chills, myalgia, nausea and/or vomiting and stiff neck) in a person between the ages of 5 and 60 years who did not have another proved etiology of illness. In addition to these criteria the definition of a confirmed case included a leptospiral microscopic agglutination (MA) titer of $>1:100$.

Hospital records from May 15 through August 1 were reviewed for the four area hospitals serving the majority of LaSalle and Bureau Counties. In addition physicians in LaSalle and Bureau Counties were contacted and asked to report patients seen in the past month with a syndrome similar to the case patients. Patients found by either method who met the definition of a possible case were then contacted, interviewed and asked to submit a serum sample for confirmatory serologic testing.

**Cohort study.** Because five of the six case patients were residents of Dalzell, a cohort study of children in Dalzell was conducted to identify the source of the outbreak and risk factors for disease. The cohort was defined as all residents of Dalzell who would be enrolled in Grades 7 through 11 (corresponding to the grades of the case-patients) in the 1991 to 1992 school year. A questionnaire was administered to cohort members and one of their parents. The parents were asked questions concerning the child's employment, recent illnesses, pets, visits to physicians and antibiotic use. The child was then asked about places where he or she had been swimming in June or July, swimming behaviors, animal contacts, outdoor activities and sports.

Preliminary analysis of the cohort study implicated two swimming areas, Steel Tunnel Pond (STP) and Black Bridge Pond (BBP). STP was a small swimming hole fed by Spring Creek and by a drainage creek which ran through two pastures with cattle. BBP was an area a few hundred meters south of STP where Spring Creek widened and deepened to form an area suitable for swimming (Fig. 1). At a second interview children that had been swimming in either location were shown a calendar and asked to mark down the dates of swimming in STP or BBP.

**Human studies.** Acute and convalescent serum samples were obtained on all case patients. In addition 43 of the 50 well cohort members had serum samples obtained for leptospiral serologic testing on August 12, 1991, 5 weeks after the onset of the outbreak. Urine samples were obtained from all case patients at least 48 hours after the last dose of antibiotics.

**Animal and environmental studies.** The drainage creek emptying into STP ran through two pastures with cattle, Farms A and B (Fig. 1). On August 6, 1991, an investigation was initiated to (1) detect additional cases, (2) determine the source of the outbreak, (3) identify risk factors for disease and (4) identify possible animal sources of infection. Null
1991, serum was obtained for leptospiral serologic testing from four of the six cattle on Farm A and 10 of the 12 cattle on Farm B. Urine samples for leptospiral culture were obtained in a nonsterile manner from the six female animals from Farm B that urinated while penned for the blood drawing. No other livestock in the Dalzell area with access to the drainage creek or Spring Creek were identified.

In an effort to obtain wild animals for testing which had access to the pond, live animal traps and glue boards were placed around STP for 4 days. A raccoon and an opossum were trapped and then euthanized by a licensed veterinarian. After euthanasia the animals were necropsied and brain and kidney samples were obtained. The tissue was macerated and added to 5 ml of semisolid PLM medium for culture. Serum was obtained for leptospiral serologic testing, and urine was obtained for culture by bladder aspiration.

Water samples were collected between July 31 and August 7 for leptospiral culture from six of the ponds, creeks and rivers in Dalzell and Oglesby.

Weather data were obtained for the Peru, IL, weather station, located approximately 2 miles from Dalzell, from the National Climatic Data Center.

**Laboratory methods.** Serum samples were sent to the Leptospirosis Laboratory, Meningitis and Special Pathogens Branch, Centers for Disease Control. MA was used to test for the following serovars: ballum, canicola, icterohaemorrhagiae, bataviae, grippotyphosa, pyrogenes, autumnalis, pomona, wolfii, australis, tarassovi, georgia, mankarso, panama, boriniana, alexi, djasiman, cynopteri, celledoni and andamaniana.

Urine samples were processed within 1 hour of collection by adding 2 drops of undiluted urine, 2 drops of urine diluted 1:10 with sterile buffered saline and 2 drops of urine diluted 1:100 with saline to vials with 5 ml of PLM semisolid medium. A 30-μg neomycin antibiotic disk was added to each vial after inoculation. The vials were kept at room temperature before being delivered the next day to the Leptospirosis Laboratory at Centers for Disease Control for further processing.

Water samples were processed by injecting 3 ml of the sample intraperitoneally into a weanling guinea pig within 72 hours after collection. Heart blood was obtained for culture 4 hours after inoculation. The animal was killed 4 weeks later, at which time blood and tissue samples were obtained for serologic tests and culture.

All leptospiral cultures were examined by dark field microscopy once a week for 5 weeks and then twice a month for 4 months. Isolates were serotyped by agglutinin absorption with specific antisera.

Epi-Info® (Version 5.01b) was used for data analysis. Results of the cohort study were analyzed by Fisher's exact test.

**RESULTS**

**Case ascertainment and description.** Only the six boys initially identified met the definition of a confirmed case. Four other individuals detected by case finding methods met the definition of a possible case; however, all were seronegative.

Onset of illness in the six patients with confirmed leptospirosis, five of whom were Dalzell residents and all of whom were between the ages of 12 to 16 years, was between July 7 and July 18. Duration of symptoms ranged from 6 to 25 days with a mean of 13.8 days. All patients had high fever, with a mean maximum temperature of 104.2°F. Other common symptoms were myalgia, nausea and/or vomiting, headache, chills and lethargy (Table 1). The mean duration of hospital stay for the four patients requiring hospitalization was 2.8 days.

Results of MA testing are shown in Table 2; only
the titers to the grippotyphosa and australis serovars are recorded. Persons infected with grippotyphosa may have detectable titers to the australis antigen by MA testing because of cross-agglutination reactions and may even have higher titers to australis than to grippotyphosa. Two patients had a positive urine culture; L. interrogans serovar grippotyphosa was isolated from both.

The case patient from Oglesby, one of the four hospitalized case patients, had high titers to grippotyphosa and australis serovars. He and his mother were questioned extensively regarding his activities in the month preceding his illness. There was a small stream behind his house which he occasionally swam in and in a large lake in a separate county. He did not, however, have any exposures, including swimming areas, summer jobs, participation on sports teams or attendance at social events, in common with the Dalzell case patients.

Cohort study. Questionnaires were administered to 55 of 58 (95%) Dalzell cohort members. All 5 Dalzell case patients and 56% of well cohort members were male. The mean age was 14.0 years for patients and 13.6 years for well cohort members (P = 0.57 by Mann-Whitney U test). Swimming in 2 areas, STP and BBP, in June or July was associated with disease. Five of 5 patients and 14 of 50 well cohort members swam in STP (relative risk (RR) undefined, P < 0.01), whereas 4 of 5 case patients and 11 of 50 well cohort members swam in BBP during that period (RR = 10.7; 95% confidence interval (CI) 1.3 to 87.9). No other swimming area, occupational or animal exposures were associated with disease.

To define more accurately the time period during which the patients may have acquired infection, the incubation period for leptospirosis and the dates of onset of illness were used to determine a period of possible exposure to the organism. The incubation period of leptospirosis ranges from 2 to 20 days. Because the onset of illness for case patients was between July 7 and July 18, the exposure resulting in illness for these patients presumably occurred between June 17 and July 16.

Further analysis of the cohort showed that swimming in STP between June 17 and July 16 was associated with disease (RR undefined, P < 0.01) but that swimming in BBP during that time period was not associated with disease (RR = 2.0; 95% CI 0.27 to 15.4). The dates of swimming in STP and the incubation period of disease suggest that for all five patients to be infected, the organism must have been present in the pond on June 30 and July 12 at a minimum (Fig. 2).

The attack rate of leptospirosis was 27.8% (5 of 18) for the cohort members who swam in STP between June 17 and July 16. For that group there was no significant difference in the number of days case patients went swimming compared with the number of days well cohort members went swimming (P = 0.36 by Mann-Whitney U test). No swimming behavior variables, such as duration or frequency of swimming, swallowing water or immersing the head, were associated with disease in the cohort of children who swam in STP during the exposure period.

Forty-three of the 50 (86%) well cohort members interviewed had blood drawn for leptospiral serologic testing on August 12. Six had a titer of 1:100 to one or more serovars; none had a titer >1:100 to any serovar. Two of the 6 children with positive titers had been swimming in STP between June 17 and July 16; the other 4 had not been in STP in June or July. Swimming in STP between June 17 and July 16 was not associated with a positive serologic result in the well cohort members tested (RR = 1.27; 95% CI 0.3 to 6.1). None of the persons with positive titers reported being ill in July.

Animal and environmental study results. Four of the 14 (28.6%) cattle tested had detectable titers to
Minimum period of leptospiral contamination of pond

* Swimming in steel tunnel pond (STP) during incubation period (2-20 days before onset of illness)

○ Swimming in STP outside of incubation period

▲ Date of onset of illness

FIG. 2. Dates of swimming in Steel Tunnel Pond between June 17 and July 19 for five case-patients, Dalzell, IL, 1991.

grippotyphosa; 2, with titers of 1:100 and 1:1600, were from Farm A and 2, both with titers of 1:400, were from Farm B. Leptospires were not isolated from any of the urine samples obtained from the cattle. No cattle had been recently added to either stock, and no animals were known to have been vaccinated for leptospirosis. No illness, stillbirths or abortions were reported by either owner. The raccoon had a titer of >1:100 to the following serovars: icterohaemorrhagiae (1:800); grippotyphosa (1:1600); australis (1:800); mankarso (1:400); alexi (1:400); and djasiman (1:200).

The opossum had no titers >1:50 to any serovar tested.

Urine and organ cultures from the raccoon were contaminated with bacterial overgrowth and leptospires were not isolated from these cultures. However, a guinea pig injected intraperitoneally with urine from the raccoon had a titer of 1:800 to grippotyphosa on subsequent testing.

*L. interrogans* serovar grippotyphosa was isolated from the blood of a guinea pig inoculated with water from STP. Leptospires were not isolated from guinea pigs inoculated with water from BBP, other ponds and creeks in Dalzell or water from Oglesby sources.

Drought conditions were present in the area during the period preceding the outbreak. From June 15 to June 30, 1991, there was a total of 1 inch of rain compared with an average total of 2.3 inches (range 0.2 to 4.8; 95% CI 1.7, 2.8) for that same time period in the years 1964 to 1990. The daily maximum temperature for June 15 to 30, 1991, ranged between 69 and 94°F, with a mean daily maximum temperature of 86.4°F for that period. The daily maximum temperature for June 15 through June 30 averaged for the years 1964 to 1990 varied between 80 and 85°F; the mean of the averaged daily maximum temperatures for that period was 83.7°F (95% CI 83.0, 84.4).

DISCUSSION

Although still considered a significant public health problem in parts of the developing world, leptospirosis is infrequently recognized in the United States. An average of only 70 cases/year were reported from the 50 states from 1978 to 1987. The disease is undoubtedly underreported because it is not routinely considered by clinicians and requires diagnostic confirmation with a serologic test performed in only a few laboratories. This outbreak emphasizes that leptospirosis does occur in the United States and has the potential to cause significant morbidity in an epidemic situation. In addition it provided an opportunity to evaluate several aspects of the ecology and epidemiology of the disease that highlight important issues in its transmission to humans.

STP was identified as the source of disease in this outbreak by both epidemiologic and laboratory methods. A cohort study determined that swimming in STP was a risk factor for disease. The patients' serologic results suggested that grippotyphosa was the infecting serovar; this was confirmed by isolation of this organism from urine cultures from two of the patients. *L. interrogans* serovar grippotyphosa was then isolated from STP water, providing further evidence linking exposure to STP with disease.

Leptospires were isolated from the implicated water source in only 2 of the 15 previously reported swimming-associated outbreaks in the United States.

Isolation of the organism from water in outbreak investigations is rarely successful because of the fastidious nature of the organism and because several weeks have usually elapsed between the outbreak and the investigation, and leptospires are believed to have a survival time of less than 20 days in water. Interestingly grippotyphosa has been noted by many inves-
tigators to be especially difficult to culture.\textsuperscript{6-8} Our success in this investigation, despite the fact that a month had elapsed since the onset of illness in the last patient, indicates either that the pond was repeatedly contaminated or that the organism can survive longer in certain environments than previously estimated.

Drought conditions were present during this outbreak and have been noted in at least 14 of the 15 previously described outbreaks, including the one previous grippotyphosa outbreak, which was associated with swimming in a creek in Tennessee.\textsuperscript{2-4} There are several possible explanations for the association of leptospirosis outbreaks with drought. In this case the high temperature and subnormal rainfall for the month preceding this outbreak changed the conditions in STP substantially. The pond normally received flowing water from Spring Creek and from a drainage creek (Fig. 1); however, by the end of June water flow had decreased such that creek water no longer flowed into the pond. Since water temperature and pH are known to influence survival of leptospires,\textsuperscript{1} the resulting stagnation may have caused one or both of these factors to become more suitable for the survival of the organisms. The concentration of leptospires in the pond resulting from a given inoculum would also be higher with lower water levels, which may lead to an increased likelihood of infection, although the “infectious dose” for leptospirosis is unclear.\textsuperscript{5} Hot, dry conditions would also be likely to increase the utilization of ponds and streams for swimming by area residents.

There was serologic evidence that area animals had been exposed to grippotyphosa. One (the raccoon) of the two wild animals trapped at the pond and 29% of the cattle tested were seropositive. Leptospires were not isolated from urine cultures from any of the animals; however, a guinea pig injected with an aliquot of the raccoon urine culture was seropositive for grippotyphosa, indicating that the organism was present in the raccoon’s urine and could therefore be transmitted to other animals and humans. Because only two wild animals were trapped and not all of the cattle pastured near the pond were tested, these results do not indicate the specific animal source of contamination of the pond but do indicate which area animal species were likely to have contaminated the pond.

Information from other studies also indicates that cattle and raccoons are likely sources of grippotyphosa contamination. Many mammalian species, including cattle, raccoons, skunks, opossums, voles, squirrels and foxes, are susceptible to infection with grippotyphosa.\textsuperscript{6,9-11} However, raccoons and cattle may be more likely than other mammals to acquire infection. Repeated serologic testing of animals on a large farm in Illinois, done to study the prevalence of leptospiral serovars in animals, showed that of the numerous wild and domestic animal species tested, only raccoons and cattle had positive serologic results for grippotyphosa.\textsuperscript{9} In another study raccoons were found to be more likely than the four other species of wild animals tested to develop infection after experimental inoculation with grippotyphosa.\textsuperscript{12} These data suggest that raccoons and cattle were likely animal sources for this outbreak of grippotyphosa infections in humans, although the possibility that other animals contaminated the pond cannot be excluded.

A high attack rate, 27.8%, was found for those persons who swam in STP between June 17 and July 16. However, many were exposed to the source during this interval but did not develop disease or show serologic evidence of asymptomatic infection. Factors influencing individual susceptibility to disease are unclear although one study of an outbreak of leptospirosis among military personnel in Okinawa, Japan, suggested that swallowing water was a risk factor for disease.\textsuperscript{13} In this investigation various swimming behaviors, including swallowing water, were not found to be associated with disease, nor was there a dose-response relationship between the number of days of swimming and the risk of developing disease.

Grippotyphosa was first isolated in 1928 but is believed to have caused disease in Europe since the 18th century.\textsuperscript{14} Particular serovars were once thought to cause specific clinical syndromes, and grippotyphosa was named for the purported resemblance of its clinical syndrome to typhoid fever. Current knowledge indicates that each serovar can cause a broad spectrum of clinical syndromes; however, in Europe grippotyphosa has been more commonly associated with gastrointestinal symptoms than other serovars.\textsuperscript{1} It is therefore noteworthy that nausea, vomiting and abdominal pain were reported more frequently by case patients in this outbreak than in other series of patients with leptospirosis\textsuperscript{1} and that the percentage of these symptoms was similar to that reported in the Tennessee grippotyphosa outbreak.\textsuperscript{3}

Despite the temporal association of onset of symptoms with the Dalzell case patients, the case patient from Oglesby was not part of this common source outbreak. He did not swim in STP and extensive investigation did not reveal any exposures in common with the Dalzell case patients, although he had been swimming in other area rivers and lakes. His serologic results suggest that he was also infected with grippotyphosa, a serotype which is relatively rare, accounting for only 6% of all cases of leptospirosis in the United States from 1978 to 1987.\textsuperscript{3} This information suggests that an epizootic of grippotyphosa was present in area animals and that exposure to urine from these affected animals caused both the disease outbreak and this sporadic case.

Based on the results of this investigation, it was recommended that STP be closed to swimming. Other local swimming areas were not implicated; however,
because it is likely that disease could result from exposure to similar bodies of water, it was also recommended that individuals avoid swimming in other bodies of water, especially in the setting of drought, presents with a febrile illness.

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