Isolation of Pathogenic Leptospires From Waters Used for Recreation

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OUTBREAKS of leptospirosis have been reported in man and animals following immersion in water. In 1951, Schaeffer (*1*) reported 50 cases of leptospirosis in persons who became ill after swimming in a slow-moving stream alongside a field where cattle and swine were pastured. These cases represented the first major outbreak of waterborne leptospirosis due to *Leptospira pomona* in man in the United States. Several recent outbreaks of leptospirosis in man after swimming have been summarized by Galton and associates (*2*). In 1958, Alexander and associates (*3*) isolated a new pathogenic *Leptospira* from a water sample collected from a river in South Dakota. In 1956, the same area of the river was the locus of a small outbreak of leptospirosis in man after swimming. Gillespie and co-workers (*4*) in the State of Washington isolated pathogenic leptospires from surface waters frequented by infected cattle shedding leptospires in urine. Clark and co-workers (*5*) isolated *Leptospira grippotyphosa* from a stream in Pennsylvania where similar serotype infections were also noted in cattle and voles.

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During the past two decades research workers have compiled substantial evidence that discharges of some domestic and wild animals constitute a hazard to the health of man. These newly recognized hazards are of mounting significance because of expanding populations of both man and animals. The age of automation has brought man more leisure. As each year participation in outdoor recreation grows, so does man's potential exposure to waterborne infections. Most people seeking outdoor recreation want to be near the water. Swimming, it is predicted, will be the most common form of outdoor recreation by the year 2000 (*6*). The recreational activities that people seek, combined with an outdoor environment altered by the effects of biological, chemical, and physical changes, are increasing man's opportunity to contract waterborne diseases.

During an investigation of 40 human cases of leptospirosis in Iowa in 1959 (*7*, *8*), *L. pomona* was isolated from urine of two persons and five cattle. The persons had been swimming in a stream to which the cattle had direct access. Another leptospirosis outbreak in man in Iowa occurred in the summer of 1964. The 15 persons affected had swum in a creek in the area where in 1959 two persons had contracted the disease. This paper describes attempts to isolate leptospires from this creek. The body of water was located less than 3 miles from an urban center of 100,000 population.

Study Area

The Iowa creek where persons involved in the 1964 outbreak had swum enters a large river approximately ½ mile below two swimming

areas. The creek drains 111 square miles of land surface (*9*). Land is primarily agricultural. Some of the land adjacent to the stream floods following excessive precipitation but, between the time of exposure of the people and isolation attempts, the land had not flooded. Unfortunately the pH of the water was not determined at the time of isolation. In repeated tests of the water following isolation attempts, however, the pH varied between 7.0 and 8.6. Much of the lowland surrounding the stream and its tributaries is used for pasturing cattle and swine. Many other pastures drain into the stream. Cattle were pastured in direct contact with the creek and ponds during the interval of human exposure. At the time of the isolation attempts the cattle had been removed to another pasture 100 yards upstream but again had direct access to the creek. Swine were pastured approximately 200 yards from the swimming area on land which drained into the stream. Signs of raccoons, opossums, foxes, muskrats, and mice were noted in the vicinity of the swimming areas.

Materials and Methods

Water isolation procedures were complicated by major highway construction activities which bisected the area between the two swimming sites where the ill persons had reported swimming. These sites were 1,600 feet apart and were designated as upstream and downstream. Water samples were collected on August 19, 1964, from both sites. Each collection from the creek and pond areas of the two sites contained approximately 1 inch of mud, sand, and sediment with 500 ml. of water.

Upstream, five collections were pooled from a stagnant pool, separated at the time from the mainstream by land but joined to the mainstream during high water. One sample was obtained from a nearby stream, from shallow, quiescent water adjacent to some brush and refuse. Downstream, by identical procedures, five water samples were pooled from collections made in a stagnant pond, which was separated from the mainstream by a sandbar but joined to the mainstream at high water levels. All water samples were pooled for return to the Institute of Agricultural Medicine laboratories at Iowa City.

In attempts to isolate leptospires, the two separately pooled water samples and heart blood, urine, and kidney samples obtained from guinea pigs after they had been inoculated with water samples were examined by darkfield microscopy. Six normal guinea pigs were each inoculated intraperitoneally with 5 cc. of turbid water from the upstream sample ("upstream guinea pigs"); six other guinea pigs were each inoculated with downstream water ("downstream guinea pigs"). Each animal weighed 350–400 grams. Samples of each animal's heart blood obtained on the 7th and 14th day after inoculation were diluted with saline and examined for leptospires. Also, from 1 to 2 drops of each blood sample was inoculated into Fletcher's semisolid medium containing 11 percent rabbit serum (*2*). The tubes of media were incubated at 28–30° C. for 6 weeks and examined periodically. Thirty days after inoculation, the guinea pigs were anesthetized, exsanguinated, and their urine and kidneys aseptically removed. The urine was examined for leptospires. A kidney from each animal was ground, Stuart's liquid medium (*2*) was added to make a 10 percent suspension, and the suspension was examined.

Blood serums of all 12 animals were tested for leptospiral agglutinins by the rapid macroscopic slide test.

A second attempt to isolate leptospires from water from the upstream area was made on September 28. At that time, six 500–600 gram guinea pigs were each inoculated intraperitoneally with 5 cc. of very turbid water.

Results

A leptospiral-like organism was observed when the upstream water sample was examined by darkfield microscopy.

No leptospires were observed by darkfield microscopy in heart-blood samples of any of the 12 animals. Fletcher's semisolid medium, inoculated with 7th-day heart blood from three of the six upstream guinea pigs, contained leptospires. Leptospires were also present in the kidneys of five of the six upstream guinea pigs and of these five, leptospires were observed in the urine of four; no urine was available from the fifth guinea pig. No leptospires were ob-

Swimming pool for children and water source for animals

served in either the kidney tissue or urine of the sixth guinea pig.

Examination of the blood serums of the upstream animals by the rapid macroscopic slide test (*2*), using 12 single antigens, indicated presumptively that the serotype was *L. pomona.* Blood serums of five of these upstream animals contained agglutinins against leptospires. Blood serum of the guinea pig in which leptospires were not observed in the kidneys or urine did not contain agglutinins.

The isolate was subcultured in Fletcher's semisolid medium and in guinea pigs.

Attempts to isolate leptospires from the guinea pigs inoculated with downstream water were negative. Serum specimens from these animals were also negative for leptospiral agglutinins. Following the second attempt, on September 28, to isolate leptospires from the upstream water, five of the six upstream guinea pigs died from peritonitis. The sixth animal was killed at 30 days. Serologic tests and isolation attempts on all six were negative.

The water isolate was definitively identified as *L. pomona* by M. M. Galton, chief, Veterinary Public Health Laboratory, Communicable Disease Center, Public Health Service, Atlanta, Ga. This report documents the first isolation or pathogenic lepospires from natural waters in Iowa.

Discussion

The isolation of pathogenic leptospires from waters following infection in man is significant in Iowa, the United States, and other areas of the world. Domestic animals and wildlife infected with leptospires are potential carriers and shedders of leptospires in the urine (*2*, *10–18*). Roth and associates (*18*) have investigated the

duration of leptospiruria in striped skunks, naturally infected. *L. pomona* and *Leptospira hyos* were recovered from urine 321 and 774 days after the animals were originally trapped. According to Gillespie and associates (*4*), urine of cattle may contain as many as 100 million leptospires per milliliter.

Animals using streams or ponds as a source of water and also for relief from heat and insects discharge feces and urine into these waters. Some wild animals such as raccoons, opossums, and mink feed on fishes, amphibians, reptiles, and aquatic invertebrates.

In Iowa, warm summer temperatures are ideal for maintaining leptospiral organisms in the water. Heavy rains may transport the organisms downstream. During periods of low precipitation and high temperature, water becomes stagnant, and conditions are suitable for maintaining the organisms for varying lengths of time. Recently, leptospires were isolated from a stream with enough flow to maintain a high degree of dilution (*19*). Therefore, both rapid flowing and stagnant streams which contain pathogenic leptospires constitute a health hazard.

The pH of water affects the maintenance of leptospires (*20*). These organisms survive longer in water that is neutral to slightly basic pH, as opposed to strongly acidic or basic water. In Iowa, the vast majority of surface waters are slightly alkaline in pH (personal communication in 1964 from R. L. Morris, assistant director, Iowa State Hygienic Laboratories, Iowa City, Iowa). Therefore, the water is suitable for survival and maintenance of leptospires.

An increased urban population and its greater participation in outdoor activities have expanded the need for recreational areas located close to U.S. population centers. Demands too excessive for Federal, State, or local recreational facilities to meet are being created. However, many of our public forests, parks, shorelands, and water areas are distant from population centers. Private lands nearer these centers need to be developed for outdoor recreation. In rural areas, the U.S. Department of Agriculture has promoted and aided in the development of recreational facilities on private land in order to increase farm income, promote orderly land use as a benefit to the owner, and provide outdoor recreational facilities for the general public (*21*).

Farm ponds in the United States accounted for 2 million surface acres of water in 1960; an increase of one-half million acres by 1976 and an increase of another million acres by the year 2000 have been predicted (*6*). Farm ponds, streams, and rivers are widely used in Iowa for recreational purposes, since natural lakes are few. In 1964, there were approximately 26,470 farm ponds in the State, and 135 recreational farm facilities were operating for income.

Many of the waters in rural areas developed for recreation provide a habitat for wildlife. Some of them are also used as a water supply for livestock. The operator of new recreational facilities may initially receive only a small monetary return from this business venture and may need to maintain livestock and farming operations to provide the major portion of his income.

Such dual or multiple use of water in recreational areas will create future health problems. The increasing demands for recreation need to be met. One must consider, however, the effect on the health of millions of persons increasingly in contact with water upon which millions of animals and lower forms of life are also dependent. Contaminated water can be hazardous, and its use for recreation must be severely curtailed.

Man, domestic and wild animals, and lower forms of life may discharge, into water, organisms that will cause disease in man and other animals. During the summer and other warm periods, both people and animals increase their activities in or near water. The clinical symptoms of leptospirosis in man are often similar to other diseases. Therefore, a diagnosis of leptospirosis must be considered when a patient gives a history of recent exposure to potentially contaminated water.

Must water used for agriculture and industry compete with water used for esthetic purposes, preservation of fish and wildlife, public supplies, and recreation? Some overlapping of use is necessary and justified. A growing population with expanding needs will continue to exert greater and greater demands on our limited water supplies. Preventive measures must be taken to control water contamination and protect man against exposure to the agents of in-

fectious diseases. Those whose work requires exposure should wear protective boots, gloves, and clothing. Rodent control in all human habitations is necessary, but especially in those located in rural areas and in those set up for recreational use. People must be taught the modes of transmission of disease. The public must either avoid swimming or wading in waters contaminated by infected domestic or wild animals or animals must be banned from these waters. Such segregation of domestic animals is, however, often economically unrealistic; the prevention of use of natural waters by wild animals is nearly impossible. Disease control in domestic animals is possible but is extremely difficult in the wild animal population. Only careful planning, along with education, research, and cooperation, will help solve the problems of multiple uses of water.

Summary

Recreational use of water can become a hazard to man's health, as seen in 1959 and 1964 outbreaks of leptospirosis in one area in Iowa. The infected persons had swum in water to which cattle, swine, and wild animals had direct access or water into which pastureland drained.

When water samples from the bathing sites were examined by darkfield microscopy, a leptospiral-like organism was observed in one sample. Further studies indicated that the serotype was *Leptospira pomona*. The first isolation of pathogenic leptospires from natural waters in Iowa had been accomplished. Samples of heart blood, urine, and kidneys from five of six guinea pigs inoculated with the water sample in which the organism had been observed also evidenced leptospires when examined by darkfield microscopy. Blood serums of five of the six animals contained agglutinins against leptospires.

Our growing population is demanding more water for recreational purposes. Supplementing recreational facilities established by Federal, State, and local governments, many privately operated recreational facilities are being established near centers of population. Ponds and other waters on farms are being used for recreation. Sometimes domestic animals share these waters; wild animals and lower forms of life also depend on the same waters. Preventive measures are needed to protect man against exposure to the agents of infectious disease that may be present in such recreational waters.

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Education Notes

Environmental Health and Food Protection. The University of North Carolina has expanded to provide comprehensive education in environmental health and in food protection. The master of science degree in public health is offered to students preparing for professional careers in these areas, and the doctor of philosophy degree is intended for applicants interested primarily in research.

Also being offered is an advanced curriculum in planning, administering, and managing environmental health programs. This course of study is designed for persons with an extensive background in environmental health and leads to a master of public health degree.

Fellowships are available.

For information write to Dr. Daniel A. Okun, Head, Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina, Chapel Hill 27515.

Environmental Health Fellowships. Under an interdepartmental program to give students broad training for careers in research, teaching, and practice in environmental health, the Consolidated University of North Carolina (Chapel Hill and Raleigh campuses) is offering environmental health fellowships for graduate study during 1966–67.

Fellowships, which provide tuition, fees, and a stipend, are sponsored by the departments of environmental sciences and engineering, biostatistics, epidemiology, city and regional planning, zoology, botany, chemistry, and food science. Students may enroll in the department of their basic specialty and select courses in other departments to obtain a broad understanding of environmental health problems and the application of their specialized knowledge to solving them.

Additional information is available from the chairmen of the departments mentioned, located at Chapel Hill 27515, except for the Department of Food Science at Raleigh 27607.

Hospital Administrators Development Program. A short course of lectures, readings, and discussions dealing with the important developments and trends which affect the professional responsibilities of the medical care administrator will be held at Cornell University from June 26 to July 22, 1966. Twenty-five $500 scholarships are available for experienced administrators. Information can be obtained from the director, Hospital Administrators' Development Program, Sloan Institute of Hospital Administration, Malott Hall, Cornell University, Ithaca, N.Y. 14850.

Clinical Psychology. Predoctoral internships and postdoctoral fellowships are available for 12 months of intensive training at the Devereux Schools in suburban Philadelphia, a group of residential treatment, remedial education, and rehabilitation centers. The training program, partly supported by a grant from the National Institute of Mental Health, Public Health Service, emphasizes work with mentally retarded or emotionally disturbed children, adolescents, and young adults with problems of learning and personal adjustment.

For information write to Dr. Henry Platt, Director of Training, Devereux Foundation Institute for Research and Training, Devon, Pa. 19333.