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LEPTOSPIRES FROM WATER SOURCES AT DIXON SPRINGS AGRICULTURAL CENTER

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Abstract: Fifty-six leptospiral isolations were made from 101 water samples collected from water sources on a 2020 hectares semi-wooded area in southern Illinois. Although four isolates reacted with standard antisera against several pathogenic serotypes, none was able to produce signs in either gerbils or hamsters.

INTRODUCTION

Dixon Springs Agricultural Center of the University of Illinois maintains 2020 hectares of land in southern Illinois. This Center maintains 900 beef cattle including 400 breeding cows, 1200 breeding sheep and a small hog herd of approximately 75 animals. This area has nearly 30 ponds or small lakes. Adjoining forest area provides abundant wildlife which attracts deer hunters. Lake Glendale, the largest water source in this area provides facilities for fishing and swimming.

Antibodies against *Leptospira pomona, L. grippotyphosa, L. ballum, L. canicola, L. icterohaemorrhagiae, L. sejroe, L. hyos, L. hardjo, L. autumnalis,* and *L. illini,* strain 3055 have been detected in sera of deer in southern Illinois.[1,4] Leptospiral agglutinins in sera of reptiles and amphibians were demonstrated in southern Illinois.[2] Hanson (unpublished) isolated *L. illini,* strain 3055 from a bull at Dixon Springs. Majority of Illinois cattle and swine are positive to this serotype. Andrews[3] made a leptospiral isolation from a turtle which was antigenically related to *L. illini,* strain 3055. Killinger *et al.*[7] reported agglutinins against *L. pomona* and *L. hardjo* in sera of cattle at Dixon Springs Agricultural Center.

Surface water has played an important role in the epidemiology of human leptospirosis.[5] Water contaminated with urine of leptospiral shedders, especially rodents and other wild animals, may act as a principal vehicle for transmission of leptospires to domestic animals or vice versa. The present investigation was conducted to examine water sources at Dixon Springs Agricultural Center for the presence of leptospires, especially *L. illini,* strain 3055 and to test the antigenic relationship of the isolates with the pathogenic serotypes.

MATERIALS AND METHODS

(a) Collection of water

Water was collected from 28 sources in sterile tubes from different locations. Where more than one sample was collected at one time from the same pond or lake, different sites were used for collection of water.

(b) Filtration of water

Ten ml of water was filtered through a 0.22μ millipore filter attached to a 10 ml syringe. Five ml of liquid bovine albumin polysorbate 80 medium[6] was flushed twice through the filter through which water had been filtered. The filtrate was collected separately in sterile tubes and was labelled as flush I and flush II respectively. One tube of liquid and one tube of semisolid medium were also inoculated with 0.1 ml of each fil-

tered water sample. The tubes were incubated at 30C and were examined daily for 2 weeks for the presence of leptospires under darkfield microscope. Tubes negative for leptospires were held up to 6 months and were examined at monthly intervals for presence of leptospires. Cultures with visible contamination demonstrated by darkfield examination were purified by combination of dilution, filtration and growth in solid medium.

(c) Growth in solid medium

Leptospiral isolates were grown in solid medium by an agar overlay method.[9]

(d) Animal inoculations

All isolates were inoculated intraperitoneally into two or more hamsters or gerbils. The blood from inoculated animals was cultured on the 3rd or 4th day after inoculation. All animals were killed between 10 and 20 days after inoculation and their kidneys were cultured in liquid and semisolid media. Their sera were tested for microscopic agglutinating (MA) antibody with homologous antigen and in some cases with heterologous antigens.

(e) Antigenic relationshp with known serotypes

All isolates were tested by MA test with specific Difco antisera against serotypes: *autumnalis, canicola, ballum, grippotyphosa, icterohaemorrhagiae, sejroe, pomona, tarassovi (hyos), biflexa, Mini georgia,* LT 117, *australis, andamana, bataviae, pyrogenes, wolffii* and antisera against *Leptospira illini,* strain 3055, prepared in our laboratory. All but three isolates were also tested by the MA test with their homologous antisera produced either in gerbils or in hamsters. Antisera against 30 water isolates which were produced in hamsters or gerbils were tested against *L. autumnalis, L. canicola, L. grippotyphosa, L. icterohaemorrhagiae, L. hardjo, L. pomona* and *L. illini* (3055) using live cultures as antigen.

RESULTS AND DISCUSSION

Fifty-six leptospiral isolations were made from a total of 101 water samples collected at six intervals during summer, fall, and winter from water sources at Dixon Springs Agricultural Center. Leptospiral isolations were made from 18 of the 28 sources. Usually pure leptospiral cultures were obtained from the flushes, i.e., in the liquid medium that had been flushed through the filter through which water had been filtered first. Most of the isolates grew well in the liquid and semisolid media. Cultures showing contamination during primary isolation were purified by dilution or filtration or by growth on solid medium.

Four isolates reacted with sera of several of the pathogenic serotypes (Table 1) to a titer of 1:100 but were not pathogenic for gerbils or hamsters. One isolate was antigenically indistinguishable from *Leptospira andamana.*[8] None of the isolates showed antigenic relationship with *Leptospira illini,* strain 3055. Hamsters or gerbils that had been inoculated with all but one of these isolates developed homologous MA antibody responses. None of the 30 water leptospiral antisera which were produced in hamsters or gerbils reacted with any of the seven serotypes tested.

As water transmission has been associated with leptospiral outbreaks, examination of water is desirable. High rate of leptospiral isolations in the present study indicates that leptospires are widely distributed in natural waters in the area studied. Since gerbils or hamsters were not infected with these isolates, it is presumed that these strains were nonpathogenic at least for the species tested. Cross-reaction of four isolates with pathogenic serotypes in the present study suggests that some of the isolates may perhaps be intermediate forms between pathogenic and nonpathogenic serotypes.

TABLE 1. Cross-Reactions of Leptospiral Water Isolates With Various Serotypes.

	Microscopic Agglutination Titer With Antisera Against Serotypes															
Water Isolate	*L. autumnalis*	*L. canicola*	*L. ballum*	*L. grippotyphosa*	*L. icterohaemorrhagiae*	*L. sejroe*	*L. pomona*	*L. tarassovi (hyos)*	*L. biflexa*	*L. mini georgia* LT 117	*L. australis*	*L. andamana*	*L. bataviae*	*L. pyrogenes*	*L. wolffii*	*L. illini*, str. 3055
DS 3	N	1:100	N	N	1:100	N	1:100	±1:100	1:100	±1:100	1:100	1:100	1:100	N	N	N
DS 18	N	1:100	N	N	1:100	1:100	1:100	N	1:1000	N	1:100	1:100	1:100	N	1:100	N
DS 72	N	1:100	N	N	1:100	1:100	1:100	N	N	N	1:100	N	N	N	N	N
DS 97	N	1:100	N	N	1:100	±1:100	1:100	N	1:100	N	1:100	1:100	N	N	N	N

N = negative; ± = incomplete reaction at a dilution of 1:100

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