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SEROLOGIC EVIDENCE OF LEPTOSPIROSIS IN WOODCHUCKS *(Marmota monax)* IN CENTRAL NEW YORK STATE

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Abstract: Serum samples from 153 woodchucks *(Marmota monax)* from Tompkins County, New York, obtained in 1976 and 1977, were examined by plate agglutination tests for antibodies against five *Leptospira* antigens. Fourteen sera showed significant titers against either *L. hardjo, L. icterohemorrhagiae* and/or *L. pomona.* Reactions against *L. hardjo* were the most frequent. Woodchucks collected from two dairy farms with histories of bovine leptospirosis did not have a greater prevalence of antibodies than woodchucks collected from other locations.

Each of two woodchucks experimentally-inoculated with *L. hardjo* developed titers to *L. hardjo.* Maximum titers occurred approximately 30 days post-inoculation. *L. hardjo* was not observed in urine specimens of these animals.

INTRODUCTION

The woodchuck *(Marmota monax)* is a frequent inhabitant of lawns, gardens, farm fields and pastures. The potential interaction between the woodchuck, man, and domestic animals prompted us to construct a disease profile for the woodchuck in central New York State.[5] The present study was initiated to explore serologic evidence of *Leptospira hardjo* and *L. icterohemorrhagiae* in woodchucks.

Naturally-occurring leptospiral infections in woodchucks have been reported for serotypes *L. icterohemorrhagiae* and *L. pomona,*[1,2,3,8] but data were not sufficient to describe their prevalence at the population level. The specific objectives of our work were to serologically determine the prevalence of *Leptospira* in woodchucks in Tompkins County, New York, and to examine the possible role of woodchucks in the epidemiology of leptospirosis in cattle.

MATERIALS AND METHODS

The study was conducted in and around Tompkins County in the Finger Lakes Region of central New York State. Approximately half of Tompkins County is farmed, with dairy farming the predominant type of agriculture.[9]

One hundred and fifty-three woodchucks were examined for serologic activity against *Leptospira.* Sixteen of these woodchucks were collected from dairy farms with recent herd histories of bovine leptospirosis. Woodchucks were collected by shooting or live-trapping. Collections were made from several physiographic and land-use types.

Serology

Blood was obtained by cardiac puncture immediately following death of the animal or from living animals anesthetized with CO_2. Serum was refrigerated at 2 C or frozen at -16 C until tests were performed. Serum was ex-

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amined by macroscopic plate agglutination (PA)[11] for antibodies against *L. canicola, L. grippotyphosa, L. hardjo, L. icterohemorrhagiae* and *L. pomona*. A commercially-available antigen was used.[3] Plate agglutination at dilutions of 1:40 or greater was considered positive for all serotypes except *L. hardjo*. *L. hardjo* produces a weak host immune response;[12,13] therefore an agglutination at 1:10 dilution was considered positive. PA tests were performed by the Diagnostic Laboratory of the New York State College of Veterinary Medicine. When an adequate amount of sera was available, PA positive sera were re-examined to insure accuracy. Samples of some of the positive sera were forwarded to the National Animal Disease Center (NADC), Ames, Iowa, for microscopic agglutination (MA) tests employing live antigens. In addition to serotypes mentioned above, MA tests were performed for *L. australis, L. autumnalis, L. ballum, L. bataviae, L. pyrogenes, L. tarassovi* and *L. wolffi*.

Experimental Infections

Experimental infections of captive woodchucks were used to assist in interpretation of serologic findings. *L. hardjo* was obtained in culture medium from the NADC. Spirochetes were inoculated into 5 ml of liquid EMJH culture medium and incubated at room temperature for 11 days. Two captive woodchucks were each intraperitoneally injected with half of the 11 day culture. These woodchucks had been in captivity for 9 months and did not show PA titers to *L. canicola, L. grippotyphosa, L. hardjo, L. icterohemorrhagiae* and *L. pomona* prior to exposure. Blood from these two animals was collected by cardiac puncture at 4, 10, 24, 30, and 52 days post-inoculation. Serum from each collection was frozen until all samples were available for analysis. Both PA and MA tests procedures were employed to analyze titer characteristics. Urine was examined for spirochetes by dark field microscopy.

RESULTS

Survey results

Eight of 53 woodchucks collected July through September, 1976, and six of 100 woodchucks collected March through June, 1977, had titers to one or more leptospiral serotypes (Table 1). Titers to *L. hardjo* were encountered more frequently than titers to other serotypes.

Two of 15 young-of-the-year woodchucks collected in May and June, 1977, had positive PA titers. Application of age-weight relationships[10] showed these

TABLE 1. The prevalence of titers to Leptospira in woodchucks *(Marmota monax)* collected near Ithaca, New York, 1976-1977.[a]

Serotype	Number Positive	Number Negative	Percent Positive
L. canicola	0	153	0.0
L. grippotyphosa	1	152	0.6
L. hardjo	13	140	8.5
L. icterohemorrhagiae	5	148	3.3
L. pomona	3	151	1.9

[a]Results are based on plate agglutination tests.

[3] Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

two animals to be 3 and 6 weeks of age. No animals younger than 3 weeks were examined.

Woodchucks with titers by the PA tests were collected from a variety of locations in Tompkins County (Table 2). There was no obvious relationship between PA positive animals and environmental features of the collection sites. Fewer than 40% of the positive reactors were collected within 100 m of a swamp, pond or other type of standing water which might be considered a likely source of infection. Four of the positive reactors were collected from lands pastured by cattle. The remainder were collected from grass meadows or hedgerows bordering meadows. Manure had been applied to some of these meadows, but such fertilization was not correlated with a greater prevalence of *Leptospira*. Positive reactors also were collected within 100 m of human dwellings.

The prevalence of positive titers seemed to increase from spring to summer, but data were insufficient to determine if this trend continued until fall (Table 3). The prevalence of positive titers was greatest in woodchucks collected during July and October. Woodchucks hibernate from November to February in central New York State and thus no samples were available for these months.

Clinical signs and gross necropsy lesions of leptospirosis were not apparent in any of the woodchucks examined. No attempt was made to collect tissues for isolation procedures.

Bovine leptospirosis farms

Farm I was a 35-40 cow dairy farm in Chenango County, New York, on which four abortions at 7 to 9 months gestation occured between September, 1975 and January, 1976. Positive titers to *L. hardjo* were demonstrated in three of 16 cows tested in January, 1976. One cow with antibodies to *L. hardjo* had aborted in October, 1975. The herd was negative for brucellosis.

The farm included a large swampy area which drained from a pastured watershed. Cattle grazed in the swamp and crossed it daily in moving between pastures and the barn. Sera from nine woodchucks collected from this farm in August, 1976, were negative for *Leptospira*.

On farm II, in Tompkins County, New York, two cows of a 35-40 cow dairy herd aborted during the week of 17 March 1977. Both cows were in their third trimester of pregnancy and were negative for brucellosis. Each had positive PA response to *L. icterohemorrhagiae* at a 1:40 dilution.

Seven woodchucks were collected from this farm in May, 1977. Six were negative for *Leptospira* antibodies and one was positive at a 1:40 dilution for *L. pomona*. Cows had not been on pasture since the previous fall.

Experimental infections

Experimentally-inoculated woodchucks developed significant titers to *L. hardjo* at 11 to 23 days post-inoculation (Table 4). Results of MA and PA tests were compatible. The MA tests showed a cross-reaction between *L. hardjo* and *L. wolffi*. MA tests showed titers of 1:100 and 1:200 to *L. wolfii* in serum collected on day 30. *L. wolffi* was not tested by PA. Spirochetes were not observed in the urine of experimentally-infected woodchucks at 7 and 9 weeks post-inoculation.

DISCUSSION

Discrepancies observed between the PA and MA tests during 1976 were not apparent during 1977. During 1977, results of the PA and MA tests were compatible for both natural and experimental infections. A possible explanation for the differences observed in 1976 is that seven samples sent to the NADC that year were in poor condition on arrival, and three were discarded.

Since our study is based solely on serology, results do not positively con-

TABLE 2. Serologic evidence of leptospirosis in 14 woodchucks *(Marmota monax)* from Tompkins County, New York, 1976-1977, with comments on woodchuck collection sites.

Age	Sex	Collection Date	Leptospiral Serotypes	Plate Agglutination		Microscopic Agglutination	Estimate of distance (meters) from collection sites to nearest: Human Dwelling	Farm Animals	Standing Water
Adult	Male	June 1976	hardjo	1/10	1/10	—	100	0	100
Adult	Male	July 1976	hardjo	1/10	1/10	—	400	400	300
Adult	Male	July 1976	hardjo	1/40	1/10	NT	1600	1600	400
Adult	Female	July 1976	hardjo	1/10	1/40	NT	400	400	400
Adult	Male	July 1976	hardjo	1/10	1/40	NT	400	400	400
Adult	Female	Sept. 1976	hardjo	1/160	NT	NT	180	0	100
Adult	Female	Oct. 1976	hardjo	1/10	1/10	—	400	—	—
			ictero*	1/160	NT	—			
Adult	Male	Oct. 1976	hardjo	1/40	1/40	—	400	1600	200
			ictero*	1/160	NT	—			
Adult	Male	May 1977	hardjo	1/40	NT	1/100	800	0	400
			ictero*	1/40	NT	—			
Adult	Male	May 1977	autumnalis	NT	NT	1/1600	180	0	50
			pomona	1/40	NT	1/200			
6 weeks	Male	June 1977	hardjo	1/10	NT	1/100	100	1600	15
Adult	Male	June 1977	hardjo	1/40	NT	1/800	400	1600	20
			ictero*	1/40	NT	—			
			pomona	1/40	NT	—			
Adult	Female	June 1977	hardjo	1/10	NT	1/800	400	1600	200
3 weeks	Male	June 1977	grypo**	1/40	NY	1/200	400	400	200
			hardjo	1/40	NT	—			
			ictero*	1/40	NT	—			
			pomona	1/40	NT	—			
			wolffi	NT	NT	1/100			

NT = No Test *icterohemorrhagiae **grypotyphosa

TABLE 3. Monthly prevalence of anti-leptospiral activity in woodchucks *(Marmota monax)* collected near Ithaca, New York, 1976-1977.[1]

MONTH	March	April	May	June	July	August	September	October
Number Positive	0	0	2	5	4	0	1	2
Number Negative	3	11	32	49	13	13	14	4
Percent Positive	0	0	5.9	9.2	23.5	0	6.7	33.3

[1]Results are based on plate agglutination tests.

firm the identity of these serotypes. Specificity of test antigens and host response vary. For example, *L. hardjo* and *L. miniszwajizak* are serologically indistinct.[6,12] In addition, cross agglutinations also may occur when antibody activity to one serotype is strong.

The limitations of serologic identification of serotypes, coupled with the veterinary and public health importance of the serotypes to which the woodchucks had positive titers, indicate that an attempt should be made to isolate *Leptospira* from woodchucks. *L. pomona* and *L. icterohemorrhagiae* have been isolated from woodchucks[1,2,3,8] and thus our finding of serological activity against these antigens probably denotes exposure to these serotypes. However, *L. hardjo* has not been isolated from woodchucks. Clarke *et al.*[4] attempted to culture leptospires from woodchucks and other free-ranging wild mammals from a Pennsylvania farm with a history of *L. hardjo*, but no members of the Hebdomonas serogroup were isolated.

Although serologic evidence is not sufficient to positively confirm that woodchucks can become naturally-infected with *L. hardjo*, we have demonstrated that the woodchuck is susceptible to experimental infection. However, we did not confirm experimentally that infected animals shed spirochetes. Despite our failure to observe *L. hardjo* in the urine of experimentally-infected animals, it is reasonable to assume, based on extensive knowledge of the leptospiral organisms, that shedding does occur. In retrospect, we should have attempted to culture urine from inoculated animals since darkfield microscopy is not as precise as urine culture for detecting spirochetes.

The finding of serologic activity against *L. hardjo* in woodchucks is im-

TABLE 4. Comparison of plate agglutination (PA) and microscopic agglutination (MA) tests for antibody activity against *L. hardjo* in woodchucks *(Marmota monax)* experimentally-inoculated with *L. hardjo*.

	Woodchuck I		Woodchuck II	
Days post-inoculation	PA	MA	PA	MA
4	—	—	—	—
10	—	—	—	—
24	1/40	1/400	1/10	1/100
30	1/160	1/1600	1/40	1/800
52	1/10	NT	1/10	NT

NT = Not Tested

portant because *L. hardjo* is considered to be the major serotype affecting cattle in North America.[12] Despite its importance nationally, *L. hardjo* rarely had been diagnosed in New York and was of minimal concern in the state prior to 1975. Recently the prevalence in dairy cattle in New York has been increasing. It cannot be determined if this increase is real or is due in part to the acceptance of the significance of lower titers. The possibility of a sylvatic role in the epidemiology of this disease in New York cannot be overlooked, but we failed to demonstrate a direct relationship between the prevalence of *L. hardjo* in woodchucks and in cattle. The prevalence of seropositive woodchucks from two farms with histories of leptospirosis in dairy cattle was no greater than for woodchucks collected elsewhere. Likewise, survey results did not demonstrate an association between cattle and woodchucks with a titer to *L. hardjo*.

The source, time, and frequency of woodchuck exposure to infective leptospires is unknown. Spirochetes require an aqueous environment for survival outside the host. However, woodchucks prefer stony, well-drained soils and apparently do not require drinking water.[7] The prevalence of leptospiral antibodies in our study was not dependent on close juxtaposition of the woodchuck's range (at the time of collection) with free-standing water (Table 2). Transmission to woodchucks likely is associated with transient weather conditions such as rainfall and dew, or with their seasonal or sporadic wanderings.

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