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COMPARISON OF ANTIBODIES TO *LEPTOSPIRA* IN WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) AND CATTLE IN OHIO

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ABSTRACT: A survey was conducted to determine the prevalence of leptospiral antibodies in sera from 248 white-tailed deer (*Odocoileus virginianus*) in Ohio. The sera were collected at check stations during the hunting season in 1983. The microscopic agglutination microtiter test was used to determine the presence of antibodies to *Leptospira interrogans* serovars *pomona*, *icterohemorrhagiae*, *canicola*, *hardjo*, and *grippotyphosa*. Eighteen of 248 (7.3%) serum samples had antibody titers (≥1:100) to at least one of the five serovars tested, with three of these samples reacting to more than one serovar. Prevalence did not differ significantly between sex or age groups. The serovar antigens reacting most frequently with serum antibodies were *grippotyphosa* (10 of 22, 45.5%) and *pomona* (eight of 22, 36.4%). Sera agglutinating with *pomona* antigen had higher titers (ranging from 1:200 to 1:6,400) than did sera agglutinating with the other serovars. These results were compared to results obtained from cattle tested at the Ohio Department of Agriculture Laboratories during 1983. There was a significant relationship between *pomona* infections detected in deer and cattle ($P < 0.05$), but not with *grippotyphosa*.

INTRODUCTION

Leptospirosis affects humans, domestic animals, and wildlife throughout the world. Leptospiral infections are common in white-tailed deer (*Odocoileus virginianus*) and other species of wildlife (Shotts, 1981). Changes in environmental conditions and animal populations cause the prevalence of this infection to vary periodically (Thiermann, 1984). The pathogenic species, *Leptospira interrogans*, is so widely disseminated that eradication may be impossible (Diesch et al., 1976). However, control of leptospirosis in domestic animals and man is possible. Successful control strategies depend on determining the extent of the disease and the reservoir of the organism. Many investigations were conducted in the 1960's and 1970's to determine the existence of wild animal reservoirs for this organism (Shotts, 1981). Even after much study, the relationship between leptospirosis in domestic and wild animals is still uncertain. Many investigators have concluded that deer do not serve as a reservoir of leptospires for other species (Ferris et al., 1961; Trainer et al., 1961; Nixon, 1970). *Leptospira* infection in the population of white-tailed deer in Ohio has not been evaluated recently. In 1969, a prevalence of 8% was found in northeastern Ohio (Rinehart, 1969), whereas in 1958 a prevalence of 20% was reported for the entire state (Goldstein et al., 1958). Therefore, this study was undertaken to determine the prevalence of leptospiral antibodies in deer from selected areas of Ohio, to evaluate risk factors influencing the presence of *Leptospira* in these areas, and to determine the relationship of serological responses to specific leptospiral antigens in deer and cattle from the same area.

MATERIALS AND METHODS

Two hundred forty-eight serum samples were collected from white-tailed deer killed in 13 counties in Ohio. Eleven of these counties were located in the southern and eastern parts of Ohio. The Ohio Department of Health (ODH), working in conjunction with the Ohio Department of Natural Resources (ODNR), collected

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TABLE 1. Proportion of positive cattle to *Leptospira* serovars *pomona* and *grippotyphosa*, in counties with positive and negative white-tailed deer, as determined by the microscopic agglutination microtiter test.

Ohio counties with:	Number of counties	Cattle		
		Total tested	Number positive	Proportion positive
Pomona-positive deer	7	233	49	0.21*
Pomona-negative deer	6	436	62	0.14*
Grippotyphosa-positive deer	7	420	67	0.16
Grippotyphosa-negative deer	6	249	30	0.12

* Proportions are significantly different ($P < 0.05$).

the serum samples during the hunting season in November and December 1983. As hunters brought field-dressed carcasses of white-tailed deer into check stations, sera were collected from the thoracic and abdominal cavities and placed into 10-ml blood collection tubes by personnel from ODH or ODNR. Approximately 0.1 mg of gentamycin sulfate and 0.1 μg of amphotericin B were added to each sample for preservation. The samples were frozen at −20 C until they could be tested. The sex and age (determined by a wildlife biologist) of each deer sampled were recorded.

Results of 1983 leptospirosis diagnostic testing for cattle performed by the Ohio Department of Agriculture (ODA) were reviewed. Samples were submitted generally from cattle showing clinical signs, awaiting export, or suspected of having leptospirosis. All animals, even those with a vaccination history that had titers ≥1:100 were reported as positive reactors. The proportions of positive reactors to *L. pomona* and *grippotyphosa* were used for comparison with deer results.

The microscopic agglutination microtiter test (MAMT), as described by the Leptospirosis Committee of the U.S. Animal Health Association, was used for determining *Leptospira* antibodies in the sera of the deer and cattle (Cole et al., 1979). Sera were screened and titrated using antigens of the following *Leptospira* serovars: *pomona, icterohemorrhagiae, canicola, hardjo,* and *grippotyphosa*. The serovar-specific antigens used in the MAMT were obtained from the National Veterinary Services Laboratory (NVSL) in Ames, Iowa. Live antigen cultures 5 to 7 days of age were adjusted to 62–64% light transmission using a spectrophotometer at 400 nm. Serum samples showing titers ≥1:100 were considered positive; samples showing 50% agglutination in the first well (1:50) of the titrating procedure were recorded also. All deer samples with leptospiral antibody titers ≥1:50 were verified by NVSL utilizing the MAMT.

Chi-square was used to determine if differences in prevalences between sexes and age groups were significant. Deer were grouped as fawns (<1 yr), yearlings (≥1, <2 yr), and adults (≥2 yr) for this comparison. The Z-test for comparison of proportions of independent samples was used to compare results of cattle tested for leptospirosis from counties where deer were sampled. Statistical significance was established at 0.05 probability, Type I error.

RESULTS

Eighteen of 248 (7.3%) sera from white-tailed deer had a titer ≥1:100 to at least one of the five serovars of *L. interrogans* tested. There were 32 of 248 (12.9%) serum samples which reacted at a dilution ≥1:50. Five counties had 25 or more deer serum samples tested, and three of these had prevalences higher than the overall prevalence of 7.3%. Counties with the highest prevalence were Jackson (12%), Guernsey (10.3%), and Tuscarawas (9.4%). Hocking, Knox, and Muskingum counties had no sera reacting at ≥1:100.

Sera positive to *pomona* had the highest titers with four samples reacting at serum titers ≥1:400. Two deer had titers ≥1:400 for *canicola* and *hardjo*. No other sample had titers to any serovar >1:200. The 18 positive sera demonstrated 22 reactions to serovar antigens at a titer of ≥1:100, some sera reacting to more than one serovar. Sera reacted most frequently to *grippotyphosa* ($n = 10$) and *pomona* ($n = 8$) antigens. The MAMT results obtained in

our laboratory were comparable to the MAMT results of NVSL. Of the 32 sera reacting at dilutions ≥1:50, 25 reacted one dilution higher at NVSL, including the 18 positive sera we found.

Fourteen of 143 (9.8%) male deer and four of 105 (3.8%) female deer were positive to at least one serovar. Six of 69 (8.7%) fawns, seven of 93 (7.5%) yearlings, and three of 70 (4.3%) adults were positive to at least one serovar. Occurrence of a positive antibody titer did not differ significantly between males and females, nor between age groups.

Of the 3,536 sera from cattle, 346 (9.8%) were considered positive for *L. pomona* and 293 (8.3%) were considered positive for *L. grippotyphosa*, the two predominant reactors in the deer sera. The proportion (0.21) of *L. pomona* positive cattle from the counties in which *L. pomona* positive deer were found was significantly greater ($P \leq 0.05$) than the proportion (0.14) of positive cattle in counties in which negative deer were found. This relationship was not observed with *L. grippotyphosa* (Table 1). Of the seven counties with deer positive for *L. pomona*, four were found to have deer positive for *L. grippotyphosa*.

DISCUSSION

The prevalence of antibodies to *Leptospira interrogans* in white-tailed deer detected in this study (7.3% at ≥1:100) was less than what has been reported previously. Other studies reported overall *Leptospira* serovar prevalences between 16% and 27% (Wedman and Driver, 1957; Ferris et al., 1961; Abdulla and Fish, 1962; Shotts and Hayes, 1970). The reactor prevalence in this study could have been low for two reasons. The dilution factor of thoracic and abdominal fluids from field-dressed deer is unknown. Some of the fluid collected was serum from damaged peripheral tissues that had flowed into the body cavity after the carcass had been cleaned. Secondly, although the antigen density adjustments on the spectrophotometer were within the acceptable range (60–75%), the densities used in this study (62–64%) would result in relative antigen excess and create a lower antibody titer. The NVSL used a lower antigen density and their results indicated more titers at ≥1:100. Perhaps the greater antigen density used in our study produced an artificially low reactor prevalence. The seven sera which we found negative at 1:100, but suspicious at 1:50, were reported positive by NVSL at one dilution higher (1:100). These differences were consistent with those observations reported by Brown (1978) concerning standardization of tests.

Although the frequency with which *grippotyphosa* (10 of 22 serovar reactions) and *pomona* (eight of 22) reacted was similar, the eight samples agglutinating to *pomona* had titers ≥1:200, whereas the 10 samples agglutinating to *grippotyphosa* had titers ≤1:200. Investigators have suggested that serologic reactions in the 1:100 range represent animals with either early clinical disease or residual titers to a previous infection (Shotts and Hayes, 1970). The high antibody titers to *pomona* suggest that clinical leptospirosis may have been present, although titers as high as 1:10,000 to *pomona* have reportedly persisted in deer for more than 3 mo after infection (Ferris et al., 1960). This difference between the agglutination titers suggests that *pomona* is more likely than *grippotyphosa* to be an active pathogen in deer.

The source of *grippotyphosa* infections for deer and cattle could be other wildlife. Raccoons (*Procyon lotor*) are considered to be a primary carrier for *grippotyphosa* (Hanson, 1982). However, domestic livestock and other deer cannot be excluded as sources of infection. The serovar *hardjo* has not yet been isolated from any wildlife in the United States, whereas *canicola* has only been found occasionally in wild-

life (Hanson, 1981). In our study *icterohemorrhagiae* antibody was not found at a titer >1:50 in any of the deer sera tested, probably owing to a lack of exposure of deer to rats, the primary reservoir of this serovar (Thiermann, 1982).

Areas of risk by county were difficult to determine from the seropositive prevalences, since the sample sizes taken from some counties were small. Also, the counties and samples were not selected randomly. The counties from which larger samples were obtained had variable results; for example, Washington (1/46 or 2.2%) and Vinton (1/45 or 2.2%) counties had relatively low prevalences when compared to Jackson (3/25 or 12.0%), Guernsey (4/39 or 10.3%) or Tuscarawas (3/32 or 9.4%) counties. There appeared to be no pattern to the distribution of positive deer (MAMT ≥1:100) among counties in which sampling occurred.

The results of the comparison of deer and cattle should be interpreted with caution because of certain limiting factors. Of primary concern is biased selection of cattle sera submitted to ODA Laboratories. We recognize many of these samples were submitted for diagnostic confirmation; however, their sources may indicate nidi of leptospirosis within the state. Other considerations that may influence the results include county variation in densities of populations of cattle and wildlife, prevalence of leptospiral infections in cattle and wildlife, agricultural practices, utilization of veterinary expertise and use of leptospiral vaccines. It is possible that some of the positive cattle titers may have been post-vaccinal titers and were not due to natural infections.

Although the potential for statistical error is possible in this analysis, the density of the deer populations in the counties sampled was higher than the remainder of the state. Eleven of the 13 counties where deer were sampled were in southeastern Ohio. This area has more wildlife per unit of area than the other three quadrants of the state. The higher density of wildlife may provide a potential reservoir for maintaining the organism in nature. This area of Ohio is a relatively hilly area in which pasture grazing of cattle is more prevalent than in other areas. This grazing practice would increase the risk of exposure between deer and cattle, and subsequently, possible cross infections.

The role white-tailed deer may have, if any, in the spread of *Leptospira* to cattle is questionable. Deer may be involved in the disease cycle of *pomona* more than *grippotyphosa*, as our results suggest. Also, our results indicated that *pomona* is more likely than *grippotyphosa* to be a pathogen in deer. This observation is consistent with the findings of previous studies (Shotts and Hayes, 1970; Hanson, 1980; Shotts, 1981). The source of *grippotyphosa* for deer and cattle could be other wildlife, in particular raccoons (Hanson, 1980). However, livestock and deer should be considered potential reservoirs for the *Leptospira* in Ohio.

The difficulty with conducting a study of disease prevalence in wildlife is determining the active infection prevalence and the capability of the surveyed population to serve as a reservoir. In our study most of the reactor samples had low titers, indicative of recovered animals or animals in early stages of infection (Shotts and Hayes, 1970). There were only three of the 248 (1.2%) deer serum samples with titers ≥1:1,000 which indicates active infection. Natural shedding of the organism has not been reported; however, investigators have reported that white-tailed deer shed the organism for only 20–35 days after experimental infection (Trainer et al., 1961; Roth, 1964). This is a shorter shedding period than for those animals considered to be reservoirs. More detailed studies would be needed to determine the actual role deer play in the spread of leptospirosis to other animals in Ohio, and

the influence proper vaccination procedures would have on the disease in cattle in counties with infected deer.

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