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## PREVALENCE OF SELECTED PATHOGENIC MICROBIAL AGENTS IN THE RED FOX (*Vulpes fulva*) AND GRAY FOX (*Urocyon cinereoargenteus*) OF SOUTHWESTERN WISCONSIN

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**Abstract:** Free-ranging red foxes (*Vulpes fulva*) and gray foxes (*Urocyon cinereoargenteus*) were trapped in southwestern Wisconsin. Fox sera were tested to determine the prevalence of antibody for five different *Leptospira interrogans* serovars, canine distemper virus (CDV), infectious canine hepatitis virus (ICHV), and *Franciscella tularensis* infections. *Grippytyphosa* was the most prevalent leptospiral serovar antibody observed. Twenty-five of 53 (47%) red foxes and 11 of 36 (31%) gray foxes had specific antibodies to *grippytyphosa*. Juvenile foxes had geometric mean antibody titers to *grippytyphosa* significantly higher ( $P < 0.05$ ) than those of the adults of both species. CDV antibody was detected in sera of red foxes only. Six of 57 (11%) red foxes had CDV antibody. ICHV antibody was detected in 2 of 57 (3%) red foxes and 3 of 32 (9%) gray foxes. Antibody to *F. tularensis* was not detected in any fox sera.

### INTRODUCTION

Red fox (*Vulpes fulva*) and gray fox (*Urocyon cinereoargenteus*) populations have become economically important in the past few years. Fur prices of red fox have increased nearly 30-fold since 1964.<sup>34</sup> In many states, the red and gray fox are no longer classified as predators, but have gained the status of game species. The state game managers now must regulate the fox harvest to avoid decimating the populations. Game managers base fox fur harvests on studies of population dynamics, movements, food habits and fur returns, all of which are well documented. However, little is known of morbidity and mortality rates in free-ranging fox populations and of the epizootiology of infectious and parasitic disease agents in these populations. There is evidence to suggest that rabies,<sup>17,33</sup> sarcoptic mange,<sup>30,31</sup> canine distemper,<sup>20</sup> and infectious canine hepatitis<sup>7</sup> may be important mortality factors in free-ranging fox populations. Leptospirosis also could have profound effects on the health of the fox population, however, the

pathogenesis and pathologic features of leptospirosis have not been documented in the fox as they have in domestic canid species.

Naturally-occurring canine distemper virus (CDV),<sup>1,13,14,20,23</sup> *Leptospira*,<sup>1,10,11</sup> infectious canine hepatitis virus (ICHV),<sup>1,6,7,23</sup> and *Franciscella tularensis*<sup>1,5,12,19,25,26</sup> have been reported in free-ranging fox populations. However data were insufficient to describe their long-term prevalence and effects on the populations.

The objective of this study was to determine by serology the prevalence of CDV, ICHV, *Leptospira*, and *F. tularensis* infections in free-ranging red and gray fox populations.

### MATERIALS AND METHODS

**Animals Sampled.** Fifty-seven red foxes and 32 gray foxes were live-trapped in Dane, Iowa, LaCrosse, Lafayette, Monroe, and Vernon Counties in southwestern Wisconsin from November, 1978 to January, 1979 by

members of the Wisconsin Trapper's Association.

Fifty-six percent (32/57) of the red foxes and 70% (22/32) of the gray foxes were older than young of the year as determined by overall body size, tooth wear examination, and measurement of mean enamel line.<sup>2</sup> The sex distribution for red foxes was 49% (28/57) males and 51% females and for the gray foxes it was 60% (19/32) males and 40% females.

**Test Sera.** Blood was collected by cardiac puncture. Sera were separated from blood by centrifugation and were stored at -20 C until time of testing.

**Viruses, Bacterial Antigens and Reference Antisera.** The viruses used in the serological tests were the Rockborn strain of CDV, passage level 45-50, and the Miranda strain of ICHV, passage level 35. Viruses were stored at -70 C until the time of testing. Antisera against the Snyder Hill strain of CDV and Miranda strain of ICHV were obtained from the National Veterinary Services Laboratory, USDA, Ames, Iowa. Viruses were originally obtained from the Veterinary Virus Research Institute, Cornell University, New York. A stained *F. tularensis* antigen and reference antisera were obtained commercially from Difco.<sup>1</sup> Live *Leptospira* antigens and reference antisera were furnished by the Central Animal Health Diagnostic Laboratory of the Wisconsin Department of Agriculture.

**Cell Culture.** The Vero line of African green monkey kidney cells was used in the CDV test. Cell culture media for Vero cells was the same as described by Zarnke.<sup>35</sup> Primary canine kidney cells were used for the ICHV test. Cell culture medium for canine kidney cells consisted of: Eagles MEM,<sup>4</sup> 5% heat-inactivated

fetal calf serum,<sup>5</sup> 0.1% gentomycin sulfate,<sup>3</sup> 0.5% lactalbumin hydrolysate, 1.0% sodium pyruvate,<sup>2</sup> 0.22% sodium bicarbonate, and 1.0% L-glutamine.<sup>2</sup>

**Serological Tests.** The presence of serum-neutralizing antibodies to CDV and ICHV was tested by the constant virus-serum dilution neutralization test (NT) method<sup>22</sup> in 96 well microtiter plates.<sup>23</sup> Duplicate serial two-fold dilutions of sera were made, beginning at 1:5. A serum was considered positive for specific antibody present if cytopathic effect (CPE) was inhibited in test wells at a serum dilution of 1:5 or greater. Sera positive to ICHV were forwarded to the National Veterinary Services Laboratory, Ames, Iowa for confirmation also using NT. Sera were tested at a 1:40 dilution for *F. tularensis* agglutinating antibodies by the rapid slide agglutination test.<sup>27</sup> Sera were examined by the leptospiral microscopic agglutination test (MAT)<sup>6</sup> for antibodies against *pomona*, *icterohemorrhagiae*, *hardjo*, *grippotyphosa*, and *canicola*. Sera were initially tested at a dilution of 1:100. Sera that agglutinated the antigen were retested in 96 well microtiter plates at higher dilutions; those that were positive at dilutions of 1:100 or greater were indicative of past infection. The MAT were performed at the Central Animal Health Diagnostic Laboratory of the Wisconsin Department of Agriculture.

## RESULTS

Serological tests showed a high prevalence of leptospiral antibody in both red and gray foxes. Twenty-five of 53 (47%) red foxes and 12 of 31 (39%) gray foxes had antibody titers to one or more leptospiral serovars. Two red foxes had antibody titers to two serovars and one

<sup>1</sup> Difco Laboratories, Detroit, Michigan 49232, USA.

<sup>2</sup> International Scientific Industries, Inc., Cary, Illinois 60013, USA.

<sup>3</sup> KC Biological Inc., Lenexa, Kansas.

<sup>4</sup> Schering Corp., Kenilworth, New Jersey 07033, USA.

<sup>5</sup> Linbro Chemical Company, Hamden, Connecticut 06517, USA.

gray fox had antibody titers to three serovars. *Grippytyphosa* was the most prevalent serovar in red and gray foxes (Table 1). Juvenile red and gray foxes had geometric mean (GM) antibody titers significantly higher ( $P < 0.05$ ) than those of the adults of both species (Table 2).

CDV antibody occurred only in sera from red foxes. Six of 57 (11%) red foxes had antibody titers ranging from 1:10 to  $\geq 1:640$ .<sup>[a]</sup> Antibody to ICHV was found in 2 of 57 (3%) red foxes and 3 of 32 (9%) gray foxes with antibody titers ranging from 1:5 to  $\geq 1:540$ .<sup>[a]</sup> None of the sera from either species of fox had antibody to *F. tularensis*.

## DISCUSSION

This study demonstrates that leptospiral infections are very prevalent in

red and gray foxes of southwestern Wisconsin. The occurrence of antibodies to *grippytyphosa*, the most prevalent serovar detected, is not surprising. In North Carolina,<sup>11</sup> *grippytyphosa* was isolated from a gray fox. A study conducted in Wisconsin from 1963-64<sup>1</sup> reports the antibody prevalence to *grippytyphosa* of 7% in red foxes and 11% in gray foxes. Other studies have also reported the presence of *grippytyphosa* antibodies in red foxes.<sup>10,11</sup> However, we found a much higher prevalence to *grippytyphosa* in Wisconsin in red and gray foxes (Table 1) than was previously reported.

The apparent serovar affinity within red and gray fox populations examined indicates a definite relationship between *grippytyphosa* and the fox populations of

TABLE 1. Antibody prevalence in red and gray foxes of southwestern Wisconsin, 1978-1979 to 5 *Leptospira interrogans* serovars.

Serovars	Red fox		Gray fox	
	No. sero-positive (%)	Titer range	No. sero-positive (%)	Titer range
<i>grippytyphosa</i>	25 (47) <sup>a</sup>	100- $\geq 6400$ <sup>b</sup>	11 (36)	100- $\geq 1600$
<i>pomona</i>	2 (4)	100	1 (3)	100
<i>icterohemorrhagiae</i>	0	—	1 (3)	100
<i>canicola</i>	0	—	1 (3)	100
<i>hardjo</i>	0	—	0	—

<sup>a</sup>% prevalence (number positive/number tested)  $\times 100$ .

<sup>b</sup>Titer = reciprocal of serum dilution which agglutinated leptospiral antigens.

TABLE 2. Comparison of GM titers for sera positive to *Leptospira interrogans* serovar *grippytyphosa* from red and gray foxes.

Subgroup	No. positive	GM titers*
Red fox		
Adult	14	297+
Juvenile	11	492
Gray fox		
Adult	7	148
Juvenile	4	800

\*GM titers = geometric mean titer of the reciprocal of serum dilution which agglutinated leptospiral antigens.

+Significant at ( $P \leq 0.05$ ) by two-tailed student t-test with comparison of GM titers between adult and juvenile red fox and between adult and juvenile gray fox.

<sup>[a]</sup> Reciprocal of serum dilution neutralizing 100 TCID<sub>50</sub> (Median tissue culture infectious doses) of virus.

southwestern Wisconsin. This type of relationship agrees with the observation made by Shotts *et al.*<sup>28</sup> that a leptospiral serovar frequently is associated with a particular host or group of host species within a given geographic region.

We did find antibody to *pomona* in 3 foxes, and to *icterohemorrhagiae* and *canicola* in 1 animal each, but the prevalence was so low we were unable to say anything about the relationship of these serovars to fox populations.

Higher GM titers observed in juvenile red and gray foxes are suggestive of more recent *grippytypyphosa* infections. Possible explanations for the relatively higher proportion of juveniles infected may be two-fold, either decreased susceptibility to leptospiral infection with age or increased contact with infected individuals by juveniles. We have no indication that juvenile foxes are more susceptible to leptospiral infection; however due to the nature of juvenile rearing at den sites and the dispersal patterns of subadults, we believe increased contact to be the most plausible explanation. Juvenile foxes may become infected at or near the den site in spring or summer, but more likely during their fall dispersal. The potential for leptospiral contact increases with increased direct and indirect intraspecies contact. As juvenile foxes begin fall dispersal to establish new territories, they come in contact with more scent-posts along existing territory boundaries than do their established adult counterparts.

This study would indicate the CDV and ICHV infections in red and gray fox populations are not uncommon. Previous studies have reported antibody prevalence of 18% in New York<sup>23</sup> and 10% in Wisconsin<sup>1</sup> to CDV in red foxes, and 10%<sup>6</sup> and 11%<sup>23</sup> in New York and 10% in Wisconsin<sup>1</sup> for ICHV in red and gray foxes. The 11% antibody prevalence for CDV in red foxes and the ICHV prevalence of 3% in red foxes and 9% in gray foxes in this study agrees with the results of these previous surveys.

Although we detected no antibodies to CDV in gray foxes, other studies have confirmed that CDV infections do indeed occur in this species.<sup>1,13,14,20,23</sup> While no CDV antibodies were found in gray foxes, we suspect that infection occurs within populations of this species in Wisconsin because of the presence of CDV antibodies in red foxes which inhabit areas in close juxtaposition with the gray fox. However, due to the relatively low number of gray foxes sampled within any single area, the true prevalence to CDV in gray foxes cannot be determined.

Antibody prevalence in diseases such as ICHV or CDV which may have high mortality rates, may not reflect accurately the frequency of infection in the population. In order to determine the occurrence of infection, one must know the case fatality rate of the infectious agent involved. Case fatality rates may vary from area to area, depending on the virulence of the strain of the disease causing agent in question and age structure of the susceptible population.

It is difficult to determine what effect if any CDV or ICHV had on the population of red and gray foxes in southwestern Wisconsin. There have been no reports of widespread decimation of red or gray foxes over the past few years in Wisconsin. (Berquist, John. 1979. Pers. commun., Wisconsin Department of Natural Resources). Therefore, CDV and ICHV are probably maintained at low levels in the fox populations surveyed, with only scattered cases or widespread mortality.

Agglutinating antibodies to *F. tularensis* were not detected in the fox populations surveyed. Although tularemia has been associated with red foxes<sup>1,18,19</sup> and gray foxes<sup>1,5,12,19,25,26</sup> in the past, there has been a general decline in the occurrence of this disease in the United States in recent years.<sup>21</sup> Jellison<sup>16</sup> has reported there were few recorded instances of tularemia in red or gray foxes in North America from 1930-1974.

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