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## SEROLOGIC EVIDENCE OF LEPTOSPIROSIS IN A SOUTHERN ARIZONA COYOTE POPULATION<sup>□</sup>

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**Abstract:** Histologic examination of kidney tissue from a morbid coyote (*Canis latrans*) suggested a leptospiral infection. Sera from nine wild coyotes captured subsequently in the same general area were tested by the rapid plate agglutination method. Four of nine sera contained antibodies to *Leptospira canicola*, while one serum also contained antibodies for *L. icterohaemorrhagiae*. Epidemiology and morbidity are discussed.

### INTRODUCTION

Leptospirosis is known to occur in a wide variety of wild mammals.<sup>1\*</sup> Among wild Canidae, evidence of the disease has been reported most often among foxes,<sup>2,3,5,6,9,12,17</sup> but it has also been reported in wolves<sup>20</sup> and in jackals.<sup>22</sup> Various serotypes have been implicated.

The disease is not unexpected in coyotes. Trainer and Knowlton<sup>21</sup> reported serologic evidence of *L. canicola* in 1 of 33 coyotes (*Canis latrans*) that they tested in Texas. Cirone, *et al.*<sup>1</sup> while evaluating detection techniques, obtained seropositive results from all of 12 coyotes in California; nine different serotypes were involved. The purpose of this report is to present and discuss serologic evidence of leptospirosis in coyotes in Arizona.

### CASE REPORT

During a field investigation related to a coyote ecology study in March, 1973, an extremely ill, weak coyote was encountered on the Santa Rita Experimental Range (SRER) south of Tucson. The animal was shot and its head submitted

to the Arizona State Department of Health Laboratory, where a fluorescent antibody test for rabies yielded negative results.

Necropsy examination revealed no gross abnormalities that could account for the weakened condition of the animal (University of Arizona Department of Veterinary Science Accession No. 73-529). However, the renal cortex appeared unusually pale and histologic examination revealed an interstitial nephritis characterized by multiple foci of mononuclear cells consisting of lymphocytes and occasional plasma cells. Also evident was tubular degeneration in scattered areas along with proteinaceous material in tubular lumens. These changes have been shown to be associated with leptospiral infection in domestic dogs (*C. familiaris*) and coyotes.<sup>1,16</sup> Additionally, glomerulitis characterized by increased cellularity of glomerular tufts was noted in association with thickening of Bowman's capsule.

Although the interstitial and renal tubular changes noted suggested the possibility of leptospiral infection,

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definitive changes associated with acute leptospirosis in other organs, notably the liver, were lacking. No leptospirae were seen in sections of kidney or liver stained by the Warthin-Starry silver impregnation method.

Since coyotes were being live-trapped on the SRER in connection with the behavioral phase of the study, blood samples were drawn from subsequently captured animals and tested for the presence of leptospiral antibodies.

#### MATERIALS AND METHODS

Using monoject <sup>□</sup> blood collection tubes, blood samples were drawn by cephalic venipuncture from 9 coyotes captured alive on the SRER during May and June, 1973. These animals represented both sexes and a variety of age classes (see Table 1). Aging was based upon incisor wear.<sup>11</sup>

Blood samples were allowed to clot and were then centrifuged at 1500 × g in an International CM-1 centrifuge. Serum was drawn off and the rapid plate agglutination test was performed. Commercial antigens prepared by Difco Laboratories were used following the procedure supplied by the manufacturer. Antigen serotypes used were *Leptospira canicola*, *L. icterohaemorrhagiae*, and *L.*

*pomona*, the three most common serotypes found in North America.<sup>18</sup>

Since stagnant ponds may be a source of leptospiral infections, water samples were taken from eight different stock tanks and ponds scattered across the study area in June, 1973. Portions of these samples were spun at 3020 × g for 5 min in a Servall RC-2 centrifuge. Wet mounts of sediment were examined for leptospirae utilizing dark-field microscopy.

#### RESULTS

Results of serologic screening are presented in Table 1 for 9 coyotes collected during May and June, 1973. In four cases positive reactions were obtained for *L. canicola*, and one of these also proved positive for *L. icterohaemorrhagiae*. No reaction was obtained for *L. pomona* in any case.

Endpoint reactions corresponding to the highest serum dilution showing any agglutination during the plate test were then transposed into titers according to the Difco procedure. Antibody levels are indicated in parentheses in Table 1. Reactions were obtained for dilutions ranging from 1:16 to 1:512.

No spirochetes were observed in water samples collected from the study area.

TABLE 1. Results of rapid plate agglutination testing of Arizona coyote sera for leptospiral antibodies, May-June, 1973.

Coyote Accession Number	Sex	Est. Age (yrs.)	Agglutinating Reaction		
			<i>L. canicola</i>	<i>L. icterohaemorrhagiae</i>	<i>L. pomona</i>
73-820	F	1	N	N	N
73-821	M	2	N	N	N
73-853	F	1	P(1:512)	P (1:512)	N
73-862	F	3	P (1:128)	N	N
73-909	F	1	P (1:512)	N	N
73-933	F	1	N	N	N
73-949	M	2	N	N	N
73-1012	M	5	P (1:16)	N	N
73-1013	M	1	N	N	N

<sup>□</sup> Sherwood Medical Industries, St. Louis, Missouri 63103, USA.

## DISCUSSION

An increase in the prevalence of leptospirosis appeared to have occurred among the SRER coyote population during or prior to spring, 1973, as 4 of 9 coyotes showed serologic evidence of infection during May and June of 1973. Three of the four had high titers and one (75-853) apparently cross-reacted or was simultaneously infected with *L. icterohemorrhagiae*. Acute-phase canine sera from dogs infected with *L. canicola* occasionally cross-react with and show an equal or higher titer with *L. icterohemorrhagiae*.<sup>10</sup>

Leptospirae are passed in the urine of infected animals and may survive for weeks in moist soils or stagnant ponds.<sup>16, 18</sup> While none were observed in samples of water sources from the study area, it should be pointed out that these samples were gathered in early June, by which time the occurrence of leptospirae in the environment may have declined. The unusually wet spring of 1973, with precipitation accumulations of as high as 10 cm above normal during March alone,<sup>8</sup> provided humid conditions favoring survival of the disease organisms in an otherwise arid environment. The coyote's use of urine in scent marking<sup>14</sup> may have provided a convenient means of transmission. Marler, Cook and Kerr<sup>16</sup> demonstrated that leptospirosis occurred in coyotes experimentally infected with *L. canicola*. A more likely means of transmission, however, was direct physical contact during social interactions about common feeding sites, or during the mating season which reaches its peak in February in southern Arizona. Acquisition of leptospirosis by coyotes from rodent prey also was a possibility as rodents have been shown to have antibodies to *L. canicola*.<sup>4</sup>

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Because little is known about leptospirosis in wildlife generally, its effect upon wild populations remains largely a matter of conjecture. Roth<sup>18</sup> stated that most leptospiral infections among wildlife are probably self-limiting in that the animals recover from the initial stages of the disease and remain carriers for only a short time. The relatively high prevalence of infection during the spring of 1973 and the apparent drop in the infection rate among SRER coyotes trapped subsequently<sup>7</sup> seemed to support this contention.

The occurrence of acute infections leading to fatalities are a possibility. Smith, Jones and Hunt<sup>19</sup> note that acute leptospirosis often results in death among domestic dogs, although Marler *et al.*<sup>16</sup> found that no mortality resulted in coyotes experimentally infected with hamster-virulent laboratory strains of *L. canicola*. Jubb and Kennedy<sup>13</sup> note that the acute disease occurs most often among dogs in the 1 to 3 year age range. If the disease acts similarly among coyotes, morbidity possibly could be substantial, particularly since a large proportion, often over 70%<sup>11, 15</sup> of wild coyote populations fall in the age classes below the 3 year level.

Animals that survive the acute disease may later succumb to uremia resulting from subsequent nephritis.<sup>19</sup> Evidence of a declining coyote population on the SRER is presented by Danner,<sup>7</sup> and he mentions chronic leptospirosis as a possible contributing factor. However, in his succeeding study, a series of 16 animals were captured in the SRER from December, 1973, through April, 1975, and only one animal, captured on 28 November, 1974, gave positive serologic results for *L. canicola*.<sup>7</sup>

Veterinary Science. The Arizona State Department of Health Laboratory, Phoenix, performed a fluorescent antibody test for rabies on the coyote head.

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