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## ***Brucella abortus* IN COYOTES. I. A SEROLOGIC AND BACTERIOLOGIC SURVEY IN EASTERN TEXAS** □

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**Abstract:** Prevalence of *Brucella abortus* serum antibodies in coyotes from east central Texas was determined by the buffered *Brucella* antigen (card test), rivanol, standard agglutination tube, and cold complement fixation tube tests. Eighteen percent (9 of 51) of the coyotes were positive serologically. *B. abortus* biotype 1 was isolated from various tissues from 7 of 43 coyotes by bacteriologic culture. Congenital transmission was found.

### INTRODUCTION

Brucellosis has been recognized as a contagious disease of man and animals since 1887 when Bruce discovered the cause of Malta fever (*Brucella melitensis*).<sup>2</sup> Brucellosis has a worldwide distribution and occurs in a variety of wild and domestic species.<sup>23</sup> Because of public health implications and a potential economic threat to various livestock industries, the epizootiology of brucellosis has been studied extensively.

Brucellosis is primarily a disease of domestic livestock and wild ruminant species; however, several species of *Brucella* have been reported to infect carnivores. *Brucella suis*, the etiologic agent for swine brucellosis, was found to occur naturally in foxes (*Vulpes vulpes*) in Bulgaria<sup>15</sup> and in Russia.<sup>17</sup> Serologic evidence of *Brucella abortus* was reported in the spotted hyena (*Crocuta crocuta*), wild dog (*Lycaon pictus*) and black-backed jackal (*Canis mesomelas*) of Tanzania.<sup>19</sup> Two species of Argentine wild foxes (*Dusicyon gymnocercus antiquus* and *D. griseus griseus*) were shown by serologic and bacteriologic methods to be naturally infected with *B.*

*abortus* biotype 1.<sup>20</sup> In Northern Ireland, foxes (*V. vulpes*) were found to be serologically positive to *B. abortus*.<sup>9</sup>

In North America, *B. suis* biotype 4 was isolated from an Alaskan sled dog.<sup>13</sup> Serologic evidence of natural infections of *B. suis* biotype 4 was also found in Alaskan wolves (*Canis lupus*), grizzly bears (*Ursus arctos horribilus*), red foxes (*V. fulva*), and sled dogs from native villages.<sup>14</sup> In other parts of the United States, a serologic survey for *B. canis* conducted on seven species of wild carnivores from five states, detected positive reactions in a raccoon (*Procyon lotor*) from Florida, a red fox (*V. fulva*) from New York, and two coyotes (*Canis latrans*) from Texas.<sup>5</sup> More recently sera from 11 of 198 (5.6%) coyotes collected in Southern Texas were shown to have *Brucella* antibodies as determined by the card test.<sup>16</sup> Of 1,028 wild mammals of 38 different species collected in California, only six carnivore species (1/16 raccoons, 2/6 badgers, 3/49 skunks, 9/148 coyotes, 5/75 bobcats) had a standard rapid plate agglutination test titer  $\geq$  1:25.<sup>6</sup> If a titer of  $\geq$  1:100 is considered to be significant, 3.4% (5/148) of the coyotes

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would qualify as reactors by the standard rapid plate agglutination test as per the bovine standard.

The present serologic and bacteriologic survey was conducted to determine the prevalence of *B. abortus* infections in coyotes to better assess the possible role of this species in the epizootiology of bovine brucellosis.

## MATERIALS AND METHODS

### Animals

Fifty-one adult ( $\geq 1$  year) coyotes were live-trapped in Madison Co. in east-central Texas from March to May, 1978 by personnel of the Rodent and Predatory Animal Control Service (RPCS) of the U.S. Fish and Wildlife Service. The coyotes were transported alive to Texas A&M University by RPCS personnel.

Seventy-five percent (38/51) of the coyotes utilized in this study were two years of age or less as determined by tooth wear examination.<sup>4</sup> The sex distribution was 55% (28/51) males and 45% (23/51) females.

### Blood and Tissue Samples

Upon arrival the coyotes were euthanized (with the exception of two pregnant bitches 822 and 829 which were held for 120 days) and blood samples were collected by cardiac puncture. Blood samples were allowed to clot at room temperature and centrifuged at 600 xg for 10 min. Sera were decanted and stored at -20 C.

Tissue samples (medial retropharyngeal lymph nodes, palatine tonsils, spleen, superficial inguinal lymph nodes and the testes or uterus) were collected aseptically at necropsy and stored at -20 C. Pups and post-partum vaginal exudates from one coyote (829) were cultured for 14 days following parturition.

Sex, general condition, and age (as determined by tooth wear)<sup>4</sup> were noted at necropsy.

### Serology

The prevalence of serum antibodies to *B. abortus* was determined at the Brucellosis Laboratory, Texas A&M University, by the buffered *Brucella* antigen (BBA) test,<sup>12</sup> the rivanol precipitation test (RIV),<sup>12</sup> the standard agglutination tube test (SAT),<sup>12</sup> and the cold complement fixation tube test (CFT).<sup>7</sup> At least three of the four tests were conducted on all sera. Sera serologically reactive by two or more methods were considered positive. Criteria for positive reactions were BBA positive, RIV  $\geq 50$ , SAT  $\geq 50$ , and CFT  $\geq 40$ .

### Bacteriology

For bacteriologic culture, the tissue was thawed and a 2-3 cm<sup>2</sup> section was macerated and streaked on agar media (Bacto *Brucella* Agar<sup>®</sup> or BBI Formula Agar<sup>®</sup>). Inoculated plates were incubated at 37 C in 10% CO<sub>2</sub>. After five days the cultures were examined and bacteria from *Brucella*-like colonies were characterized by a rapid slide agglutination test.<sup>12</sup> Those reacting to the slide agglutination test were inoculated onto agar slants, and these subcultures were submitted to the National Veterinary Services Laboratory, Ames, Iowa to be biotyped.

## RESULTS

### Serology

Eighteen percent (9/51) of the sera were positive by two or more of the serologic tests at the criteria indicated (Table 1).

Twenty percent (10/51) of the sera were positive by the BBA test. The RIV test was conducted on 34 sera, four (12%) of

<sup>®</sup> Difco, Detroit, Michigan.

<sup>®</sup> National Veterinary Services Laboratory, Ames, Iowa 50010, USA.

TABLE 1. Summary of *Brucella abortus* serologic results and bacteriologic culture isolations from coyotes (*Canis latrans*) trapped in east central Texas, 1978.\*

ID#	Age (Years)	Sex	Serology				Bacteriology	
			BBA (Card)	RIV	SAT (Tube)	CFT	Biotype of isolation	Tissue location of culture
802	1	F	P	0	1:50	1+,1:20	(-)	...
811	1	M	N	(Not Done)	0	1+,1:20	(-)	...
815	1	M	P	0	1:50	1+,1:20	1	Spleen
819	3	M	P	0	1:50	4+,1:10	(-)	...
821	3	M	P	0	0	3+,1:10	(-)	...
822	2	F	N	0	1:25	0	(-)	...
823	2	F	N	0	0	0	1	Retrophar.L.N.
825	2	F	P	0	1:50	1+,1:20	(-)	...
829	2	F	P	1:25	1:50	1+,1:40	1	3 pups; 2 vaginal swabs
833	1	F	P	1:200	1:200	4+,1:80	1	Retrophar.L.N.; Tonsil
835	2	M	P	1:200	1:100	4+,1:80	(-)	...
836	1	M	N	0	0	1+,1:20	(-)	...
839	1	M	N	0	0	1+,1:10	(-)	...
840	1	F	N	0	0	1+,1:10	(-)	...
843	5	M	N	0	0	0	1	Tonsil
848	2	F	P	0	1:50	2+,1:20	(-)	...
849	1	F	P	1:100	1:50	3+,1:80	1	Retrophar.L.N.
850	7	F	N	0	0	0	1	Retrophar.L.N.

\*The results of the other 33 coyotes tested serologically and the other 25 bacteriologically cultured were negative.

which reacted at a  $\geq 1:25$  dilution. The SAT was conducted on all 51 sera, with 10 (20%) reacting to a  $\geq 1:25$  dilution. The CFT was conducted on 49 sera of which ten (19%) reacted at  $\geq 1:20$  dilution and four (8%) at  $\geq 1:40$  dilution (Table 1).

### Bacteriology

Twelve separate isolations of *B. abortus* biotype 1 were made from tissues collected from seven (16.3%) of the coyotes. Four isolates were from medial retropharyngeal lymph nodes, and two isolates were from palatine tonsils. One female coyote accounted for five isolates. The gastric contents of three newborn full term pups, and 2 swabs of vaginal discharges (6 and 11 days postpartum) from this bitch contained *B. abortus* biotype 1. The remaining isolate was cultured from the spleen (Table 1).

*B. abortus* was not isolated from the tissues of five coyotes (802, 819, 825, 835, and 848) which were serologically positive. Three isolates of *B. abortus* (823, 843, and 850) were cultured from coyotes showing no reaction to the serologic tests used (Table 1).

### DISCUSSION

The role of carnivores in the epizootiology of bovine brucellosis remains unclear. Certain facts are known concerning the inter- and intra-specific transmission of *B. abortus*. Carnivores are known to be "more readily infected than herbivorous animals in enzootic brucellosis areas, probably through ingestion of aborted fetuses and membranes."<sup>24</sup> Madison Co., the area from which the coyotes were collected for the present study, and the contiguous six counties have a high prevalence of *B. abortus* in cattle ( $> 2.5$  times the quarantined herd/county average rate for the state).<sup>21</sup> Ingestion of contaminated bovine tissue by coyotes is presumed to be the primary route of infection. No data are present to support this hypothesis. Data from other studies indicate that a positive correlation exists "between BBA

test-positive results in cattle herds and coyotes within the same county."<sup>16</sup>

The resistance of *Brucella*, the chronicity of brucellosis in mammals, and the mobility of the coyote may present special problems to the eradication of *B. abortus* in some areas. *Brucella* organisms possess "extraordinary powers of resistance" and survive "in liquid manure, in numbers of 100-1000/ml for four months after the last date of contamination."<sup>24</sup> The dispersion of coyotes has been studied. In Iowa the overall mean straight-line distance from tagging site to recovery was 35.5 km for 63 animals.<sup>1</sup> Instances of coyote movements in excess of 160 km have been recorded.<sup>1,3,18</sup>

Domestic canines can become infected and subsequently shed the *Brucella* organisms in discharges, urine, and feces.<sup>8,10,11,22</sup> Human infections have been contracted from *Brucella* infected dogs.<sup>10</sup> Excretion of viable *B. abortus* in post-partum vaginal discharges for as long as 11 days and congenital transmission in coyotes allows for contamination of the environment. The probability of transmission from coyotes to other individuals or species is beyond the scope of this investigation.

The isolation of *B. abortus* from 16% of the coyotes in the present study, and the detection of significant levels of serum antibodies to *B. abortus* in 18% of the sera indicate that the organism is commonly disseminated in certain coyote populations. Serologically reactive coyotes from other areas of Texas<sup>5,16</sup> suggest that this is not a localized phenomena.

Current knowledge supports the view that *B. abortus* in coyotes may be a potential public health threat. The overall risk posed by all wildlife in the spread of brucellosis in Texas is unknown. Trappers, wildlife researchers, fur buyers, veterinarians and others who handle coyotes may have a high exposure risk. These personnel should be informed of the precautionary measures necessary to minimize this risk.

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