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EXPERIMENTAL INFECTION OF GRAY FOXES (*UROCYON CINEREOARGENTEUS*) WITH *BRUCELLA ABORTUS*[1]

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ABSTRACT: Ten gray foxes, eight principals that were fed approximately 4.4×10^{10} colony forming units of *Brucella abortus* strain 2308 and two controls, were examined for serologic responses and tissue distribution of the organisms. Blood sera from each fox were tested on the day of exposure and at seven weekly intervals for antibodies to *B. abortus*, using the brucellosis card, standard tube agglutination, 2-mercaptoethanol and rivanol tests. Control foxes were serologically negative for all tests throughout the study and the principals were negative prior to exposure. On days 14, 21 and 28, the eight principals had positive card reactions and ≥1:100 tube agglutination titers. After 28 days, the titers receded; and by day 49, three principals had negative card reactions and one of these was negative for all tests. *Brucella abortus* was isolated from one or more lymph nodes from seven of eight principals including the one which was seronegative. The bacterium was not isolated from lungs, livers, spleens, kidneys, uteri or testicles.

INTRODUCTION

Serologic surveys and bacteriologic studies have demonstrated that brucellae can infect various species of foxes. Pavlov et al. (1960) examined 550 red foxes (*Vulpes vulpes*) from an area of Bulgaria where swine brucellosis was prevalent and found 22 with standard tube agglutination (STA) titers. *Brucella suis* was isolated from one of the foxes. Davies et al. (1973) tested 87 red foxes from an area of Wales where brucellosis in cattle was endemic. Eight foxes were positive, but seven of these had low titers on the STA and complement fixation (CF) tests. Cultures of three foxes were negative. *Brucella abortus* biotype 1 was recovered from one fox which had high STA (1:320) and CF (1:200) titers. Infection of red foxes and arctic foxes (*Alopex lagopus*) with *B. suis* biotype 4 from Siberian reindeer (*Rangifer tarandus*) has been reported (Petukhova et al., 1970; Petukhova et al., 1971). Susceptibility of red foxes for *Brucella* infection has been further substantiated in Poland and the U.S.S.R. among fox colonies fed contaminated domestic animal viscera, meat and fetuses (Rementsova, 1962). A number of these infected wild or commercially-reared foxes aborted or produced stillborn kits. The infection was shown to persist in some of the 29 foxes examined 1 yr later. Seven foxes were seropositive and brucellae were isolated from one. McCaughey and Fairley (1969) tested nine adult red foxes and 23 kits in Northern Ireland and found four with CF titers of ≥1:32 and two of these had STA titers of 26 and 40 international units. The other 28 foxes were negative to both tests. Cultures for brucellae were negative. Neiland (1970) reported serologic evidence of brucellae in red foxes which was presumed to be caused by *B. suis* biotype 4 from Alaskan caribou. Two of 11 sera tested had CF titers of 1:20 and 1:640 and corresponding negative and 1:320 STA reactions.

Natural infections of wild gray foxes have been reported in Argentina where brucellosis is a common disease of range cattle (Szyfres and Tome, 1966). Of the 728 sera from *Dusicyon gymnocercus antiquus* and *D. griseus griseus*, 173 (24%)

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had STA titers ranging from 1:25 to 1:800, and 82 (11%) of these had titers of ≥1:100. *Brucella abortus* biotype 1 was isolated from five of 31 pooled tissues from 77 foxes (*D. gymnocercus antiquus*) and from three of 34 individually examined foxes.

In this study, we have investigated experimentally the serologic response and bacteriologic distribution of *B. abortus* after ingestion by gray foxes.

MATERIALS AND METHODS

Seven male and three nonpregnant female gray foxes were trapped in eastcentral Alabama. They were vaccinated for canine distemper and canine parvovirus prior to the experiment. The foxes were fed dry dog chow and water ad libitum and were housed individually in cages.

Ten trypticase agar slants with 5% bovine serum were inoculated with *Brucella abortus*, U.S. Department of Agriculture reference strain 2308 (BA 2308) and incubated in humidified air and 8% carbon dioxide at 37 C for 3 days. Each slant was harvested with 2 ml of sterile saline, then all 10 were pooled and quantitated. Serial 10-fold dilutions of the pooled suspension were made in saline. The diluted suspensions (0.1 ml) were inoculated on trypticase agar with 5% bovine serum plates and spread for colony isolation with a sterile bent glass rod. After 3 days incubation at 37 C, the colony forming units (CFU) of brucellae were counted and calculated to be 2.2×10^{10} cells/ml. Eight foxes (numbers 1 through 8) were each fed 2 ml of the undiluted BA 2308 suspension (approximate 4.4×10^{10} cells) mixed in commercial canned dog food; two additional foxes served as controls.

Blood samples were obtained from each fox by jugular venipuncture on days 0 (day of exposure), 7, 14, 21, 28, 35, 42 and 49. Prior to serologic testing, sera were heat inactivated (56 C for 30 min) to eliminate nonspecific agglutinins. All sera were examined for antibodies to *B. abortus*, using the brucellosis card (Brucellosis Card Test, Hynson, Westcott and Dunning, Baltimore, Maryland 21201, USA), standard tube agglutination (STA), 2-mercaptoethanol (2-ME) and rivanol (RIV) tests (Alton et al., 1975). The sera were also evaluated on days 0, 28 and 49 for antibodies to *B. canis*, using the rapid slide agglutination (RSA) test (Canine Brucellosis Diagnostic Test, Pitman-Moore, Inc., Washington Crossing, New Jersey 08560, USA).

The foxes were killed on day 49 with intravenously injected T-61 solution (T-61 Euthanasia Solution, American Hoechst Corp., Somerville, New Jersey 08876, USA) and tissues for culture were collected aseptically. The tissues were briefly dipped in 95% ethanol, seared in an open flame and incised with a sterile surgical blade. The open surface was macerated with the surgical blade and spread on two trypticase agar with 5% bovine serum and antibiotics plates (Alton et al., 1975). The plates were examined for brucellae colonies after 3 and 7 days incubation. *Brucella abortus* isolates were identified, using standard serologic and biochemical criteria (Alton et al., 1975).

RESULTS AND DISCUSSION

Sera from the two controls were negative for all tests during the experimental period. The eight principals were negative for all tests prior to exposure (Table 1). On day 7, three principals had STA titers from 1:25 to 1:50 and one of these had a positive card reaction. On days 14, 21 and 28, the eight principals had positive card reactions and their STA, 2-ME and RIV titers ranged from 1:100 to 1:1,600, negative to 1:200, and negative to 1:200, respectively. From 35 through 49 days, the titers of the STA, 2-ME and RIV tests receded. Five foxes on day 49 had positive card reactions and three were negative. The STA titers ranged from negative to 1:200, the 2-ME titers ranged from negative to 1:25, and all RIV tests were negative. The RSA tests on days 0, 28 and 49 were negative for all foxes.

Tissues from the two controls were culture negative. *Brucella abortus* was isolated from tissues of seven of the eight principals (Table 2). Isolations were made from six retropharyngeal, five mandibular, two parotid, two superficial cervical, one superficial inguinal, one hepatic and three mesenteric lymph nodes. *Brucella abortus* was not isolated from lungs, livers, spleens, kidneys, uteri or testicles.

This study demonstrated that gray foxes can be infected with *B. abortus* follow-

TABLE 1. Serologic test results of gray foxes exposed orally to *Brucella abortus* strain 2308. Titers of STA, 2-ME and RIV tests are expressed as the reciprocal of the final dilution showing positive or incomplete agglutination reactions.

Fox no.	Sex	Serologic test	Time in days							
			0	7	14	21	28	35	42	49
1	F	CARD[a]	−[e]	−	+[e]	+	+	+	+	+
		STA[b]	−	−	400	400	I800[f]	I400	I200	I200
		2-ME[c]	−	−	25	I50	50	25	−	−
		RIV[d]	−	−	−	−	−	−	−	−
2	M	CARD	−	−	+	+	+	+	+	−
		STA	−	−	I400	I400	I200	I100	I100	25
		2-ME	−	−	I50	I25	25	I25	−	−
		RIV	−	−	−	I25	−	−	−	−
3	M	CARD	−	+	+	+	+	+	+	+
		STA	−	50	I1,600	I1,600	I800	I400	I400	200
		2-ME	−	−	100	I100	I100	50	25	25
		RIV	−	−	50	50	−	−	I25	−
4	M	CARD	−	−	+	+	+	+	+	+
		STA	−	−	I800	I800	I400	200	I200	I100
		2-ME	−	−	50	50	I100	25	25	−
		RIV	−	−	25	I100	I50	I50	I25	−
5	M	CARD	−	−	+	+	+	+	+	+
		STA	−	25	1,600	I1,600	I400	I400	I200	100
		2-ME	−	−	50	I200	50	50	I25	25
		RIV	−	−	I50	I200	I50	25	25	−
6	M	CARD	−	−	+	+	+	+	+	−
		STA	−	−	I800	I800	800	I400	I200	I100
		2-ME	−	−	25	25	50	25	I25	I25
		RIV	−	−	−	−	−	−	−	−
7	F	CARD	−	−	+	+	+	+	+	+
		STA	−	25	I800	800	I800	I400	200	I200
		2-ME	−	−	200	200	I100	50	25	25
		RIV	−	−	100	I100	I50	I50	−	−
8	F	CARD	−	−	+	+	+	−	−	−
		STA	−	−	200	I400	100	I100	25	−
		2-ME	−	−	−	I25	I25	−	−	−
		RIV	−	−	−	−	−	−	−	−

[a] Brucellosis card test.
[b] Standard tube agglutination test.
[c] 2-Mercaptoethanol test.
[d] Rivanol test.
[e] − = Negative agglutination reaction. + = Positive agglutination reaction.
[f] I = Incomplete agglutination reaction.

ing ingestion of the organism. The serologic responses (Table 1) were remarkably consistent during the experimental period; however, the card reactions and the titers of the STA, 2-ME and RIV tests were transient and did not always correlate with the isolation of bacteria. *Brucella abortus* was isolated from fox 8 although the serum was negative. Conversely, fox 1 was culture negative and had a positive card reaction. It is apparent that serologic tests used for cattle may be unreliable for epidemiologic studies in gray foxes.

Other studies have demonstrated that

TABLE 2. Bacteriologic results from culturing tissues of foxes 49 days after oral exposure to *Brucella abortus* strain 2308.

Tissue	Principals							
	1[a]	2[b]	3[b]	4[b]	5[b]	6[b]	7[a]	8[a]
Retropharyngeal lymph nodes	−[c]	+[c]	+	−	+	+	+	+
Mandibular lymph nodes	−	+	+	+	−	+	−	+
Parotid lymph nodes	−	+	−	−	−	−	−	+
Superficial cervical lymph nodes	−	−	−	−	+	−	−	+
Superficial inguinal lymph nodes	−	−	−	−	−	−	−	+
Mesenteric lymph nodes	−	+	−	−	−	+	−	+
Hepatic lymph nodes	−	−	−	−	−	+	−	−
Lung	−	−	−	−	−	−	−	−
Liver	−	−	−	−	−	−	−	−
Spleen	−	−	−	−	−	−	−	−
Kidney	−	−	−	−	−	−	−	−
Uterus	−	NA[c]	NA	NA	NA	NA	−	−
Testicle	NA	−	−	−	−	−	NA	NA

[a] Female.
[b] Male.
[c] − = Culture negative for *B. abortus*. + = Culture positive for *B. abortus*. NA = Not applicable.

natural infection of foxes occurs with *B. abortus* and *B. suis* (Pavlov et al., 1960; Petukhova et al., 1970; Petukhova et al., 1971; Davies et al., 1973). On the basis of these data, it is apparent that *Brucella* species endemic in wild and domestic animals can be transmitted to foxes. However, the role of foxes in the epidemiology of ruminant and porcine brucellosis is still not clearly defined.

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