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EXPERIMENTAL *BRUCELLA ABORTUS* INFECTION IN WOLVES

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ABSTRACT: Four juvenile male wolves (*Canis lupus*) each received an oral dose of $1.6–1.7\times10^{12}$ colony-forming units of *Brucella abortus* biovar 1 isolated from a bison (*Bison bison*) in Wood Buffalo National Park (Canada), and two others served as negative controls. Infected wolves did not show clinical signs of disease but did develop high *Brucella* antibody titers. Small numbers of *B. abortus* were excreted sporadically in feces until day 50 postinoculation (PI). Very small numbers of the bacterium were isolated from urine of only one wolf late on the same day that it was infected, and very small numbers of colonies of *B. abortus* were obtained from buccal swabs of three wolves for up to 48 hr PI. Two infected wolves euthanized 6 mo after the start of the experiment had no lesions, and colonies of *B. abortus* were isolated from thymus and most major lymph nodes. The other two infected wolves euthanized 12 mo after the start of the experiment had no lesions, and smaller numbers of brucellae were recovered from fewer lymph nodes compared with the wolves killed 6 mo earlier. The sporadic excretion of very small numbers of brucellae by the wolves was insignificant when compared with the infective dose for cattle.

Key words: *Brucella abortus*, brucellosis, *Canis lupus*, pathogenesis, serology, wolf.

INTRODUCTION

Brucella abortus biovar 1 has been isolated from lymph nodes of four of 13 wolves (*Canis lupus*) taken by hunters and trappers in Wood Buffalo National Park (WBNP) and the adjacent Slave River Lowlands, Canada (Tessaro, 1988). This biovar was the same as that found in the local bison (*Bison bison*) population (Tessaro et al., 1990), which the wolves prey and scavenge on, and was the first reported occurrence of *B. abortus* in wolves. This raised the question of whether or not wolves might be a significant maintenance host and reservoir of the bacterium. Efforts to control or eradicate brucellosis in bison and to prevent it from spreading to neighboring herds of disease-free bison (Tessaro et al., 1993) and livestock around WBNP could be compromised by the presence of other reservoir hosts. This would also apply to the recent introduction of wolves into the greater Yellowstone ecosystem in the United States, where bison and elk (*Cervus elaphus*) are infected with *B. abortus* and cattle and other livestock are present. The purpose of this study was to evaluate the significance of *B. abortus* infection in wolves and whether or not they might pose a risk of transmitting brucellosis to other wildlife and livestock species.

MATERIALS AND METHODS

Six 31-day-old, captive-reared male wolf pups from the same litter were obtained in Saskatchewan (Canada) under a provincial wildlife permit. They were vaccinated on arrival and again at 52 days of age against distemper, infectious canine hepatitis, adenovirus type II, parainfluenza virus, and parvovirus enteritis. Fecal samples from all pups were examined for parasites. Clinical hematology and chemistry were done at the beginning and end of the experiment. The animals were maintained in compliance with the guidelines of the Canadian Council on Animal Care (1984). The pups were started on a diet of commercial canned dog food and gradually switched to a balanced dry commercial kibble (Purina® Puppy Chow, Purina Dog Chow, Mississauga, Ontario, Canada). Drinking water was provided ad libitum. The pups were reared together until they were 60 days old. They were then randomly assigned to three groups of two wolves each. The two wolves in group 1, individually identified as 1A and 1B, served as negative controls. The four wolves in groups 2 and 3 were individually identified as 2A, 2B and 3A, 3B, respectively. Each group was housed in a 10-m^2 floor run, each in a separate room. The runs were cleaned daily. The wolves were weighed periodically during the experiment. All six wolves

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were serologically negative for brucellosis prior to the start of the experiment. They were 78 days old when the experiment began on 14 July 1987.

The four wolves in experimental groups 2 and 3 were each given 100 g of canned dog food containing a final measured concentration of $1.6–1.7\times10^{10}$ colony-forming units (CFU) of *B. abortus* biovar 1 per gram for a total dose of $1.6–1.7\times10^{12}$ CFU. The isolate was from a bison in WBNP. The negative control wolves were each fed sterile saline in 100 g of canned dog food. Buccal swabs were collected daily for culture from day 0 to day 3 postinoculation (PI). Urine samples were also collected for culture daily via bladder catheterization from day 0 to day 9 PI. All feces were collected from each wolf from day 0 to day 10 PI, and once daily thereafter, for bacteriologic culture. Fecal samples were not collected from the control wolves after day 49 PI. Blood samples were collected from the cephalic vein of each wolf twice weekly for 2 wk and every 1–2 wk thereafter. Sera were tested for anti-*Brucella* antibodies with the buffered plate antigen test (BPAT), Brewer's card test (BCT), standard tube agglutination test (STAT), and complement fixation test (CFT) as previously described (Stemshorn et al., 1985). A STAT result of ≥125 international units (IU) of anti-*Brucella* antibody and a CFT result of ≥1/10 are considered significant for cattle and were used as the positive cutoff for this study. Buccal swabs, urine, feces, and blood clots were bacteriologically examined for the presence of *Brucella* sp. as previously described (Forbes et al., 1996), except that the blood clots were homogenized in an equal volume of phosphate-buffered saline.

One negative control wolf (1B) and experimental wolves 3A and 3B were euthanized on day 184 or 185 PI, and a complete necropsy was performed. The remaining three wolves were killed and necropsied on day 365 or 366 PI. Lymph nodes (mandibular, retropharyngeal, parotid, suprascapular, prefemoral, superficial inguinal, internal iliac, bronchial, mesenteric, popliteal, and axillary), palatine tonsils, liver, spleen, left and right kidney, lung, thymus, brain, left and right testicle, and urinary bladder were collected for bacteriology. Swabs from the left and right stifle joint and left and right coxofemoral joint were also collected for culture. A portion of each tissue collected for bacteriology was fixed in 10% neutral buffered formalin, processed to paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin for light microscopy.

RESULTS

All six wolves gained weight at a uniform rate during the study, and no clinical signs of disease were seen in any of them throughout the study period. Clinical chemistry and hematology values for all six wolves were unremarkable at the start and end of the experiment. Twenty colonies of *B. abortus* biovar 1 were recovered at 12 hr PI from the buccal swab of experimental wolf 2A, and one and three colonies were recovered, respectively, from buccal swabs of experimental wolves 3B and 3A on day 2 PI. No isolates were obtained from any other buccal swabs. *Brucella abortus* biovar 1 was only recovered from a single urine sample: 12 CFU/ml were recovered from the urine of experimental wolf 2A at 12 hr PI.

Small numbers of *B. abortus* biovar 1 were recovered sporadically in feces of the four experimental wolves for up to 50 days PI: experimental wolf 2B shed the bacterium (6.7 CFU/g of feces) on day 2 PI only; experimental wolf 3B shed the bacterium on day 5 PI (36.0 CFU/g of feces) and day 16 PI (1.3 CFU/g of feces) only; experimental wolf 3A shed the bacterium on days 5, 37, and 50 PI (1.3 CFU/g of feces on each occasion); and experimental wolf 2A shed 2.7 CFU/g of feces on days 0, 37, and 40 PI and 1.3 CFU/g of feces on day 46 PI.

Brucella abortus biovar 1 was recovered from the blood clots of wolf 2B on day 7 PI (8 CFU/g of blood clot) and day 10 PI (2 CFU/g of blood clot). Wolf 3B had 2 CFU/g of blood clot on each of days 7 and 10 PI. On day 13 PI, all four experimental wolves had 4 CFU/g of blood clot. Wolves 3A and 3B had 2 and 4 CFU/g of blood clot, respectively, on day 21 PI. Thereafter, *B. abortus* was not recovered from blood clots of any of the wolves.

All four experimental wolves seroconverted, and they were positive on all four serological tests by day 10 PI. On the BPAT, wolf 2A and 3A were positive on day 7 PI, wolf 2B and 3B were positive on day 10 PI, and all four wolves remained positive for the remainder of the experiment. Wolf 2A, 3A, and 3B became positive on the BCT on days 7, 7, and 10 PI,

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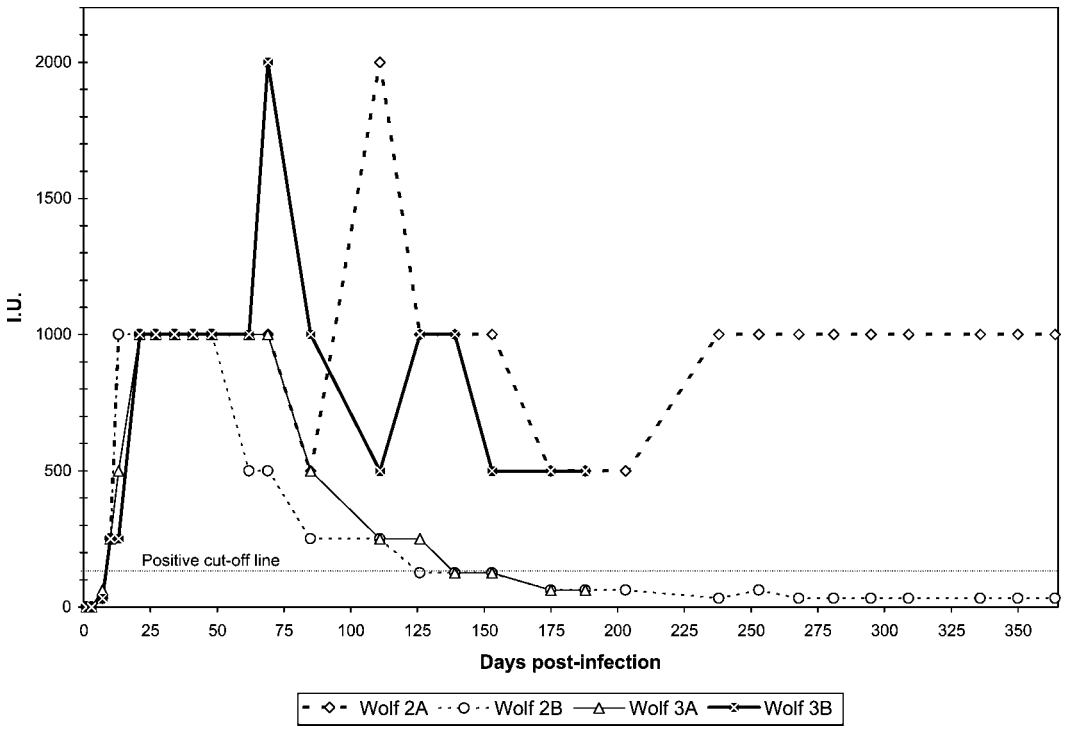

FIGURE 1. Standard tube agglutination test titers expressed in international units over time for four wolves infected with *Brucella abortus* biovar 1.

respectively, and remained positive for the remainder of the experiment. Wolf 2B became positive on the BCT on day 10 PI but reverted to negative status on day 203 PI and thereafter. All four experimental wolves showed seroconversion on the STAT and CFT by day 7 PI and developed high antibody titers (Figs. 1, 2). All four experimental wolves were positive on STAT and CFT throughout the experiment, but 2A and 3B retained high antibody titers, whereas the titers of 2B and 3A began to decrease at approximately 3 mo PI.

No gross lesion was seen at necropsy in any of the wolves, and no histologic lesions were evident. *Brucella abortus* biovar 1 was recovered from the thymus, spleen, liver, and lymph nodes from one or more of the four experimental wolves (Table 1), but there was no evidence of *B. abortus* in the kidneys, lungs, brain, testicles, urinary bladder, or synovial fluid from any of these animals. The palatine tonsils could not be assessed because the culture plates were overgrown with contaminant microorganisms. Colony counts and the number of positive tissues were higher in the two infected wolves euthanized on days 184–185 PI than in the two euthanized on days 365–366 PI. Both negative control wolves remained seronegative throughout the study and were culture-negative at necropsy.

DISCUSSION

This experiment indicates that male wolves infected with a single dose of *B. abortus* become bacteremic for a short period, develop disseminated infection, and maintain the bacterium in lymphoreticular tissues for at least 1 yr. There was no evidence of localization of *B. abortus* in the reproductive tract of juvenile male wolves. Epididymitis and polyarthritis attributed to *B. abortus* have been reported in some infected dogs (Whitby et al., 1936; Clegg and Rorrison, 1968), but under our exper-

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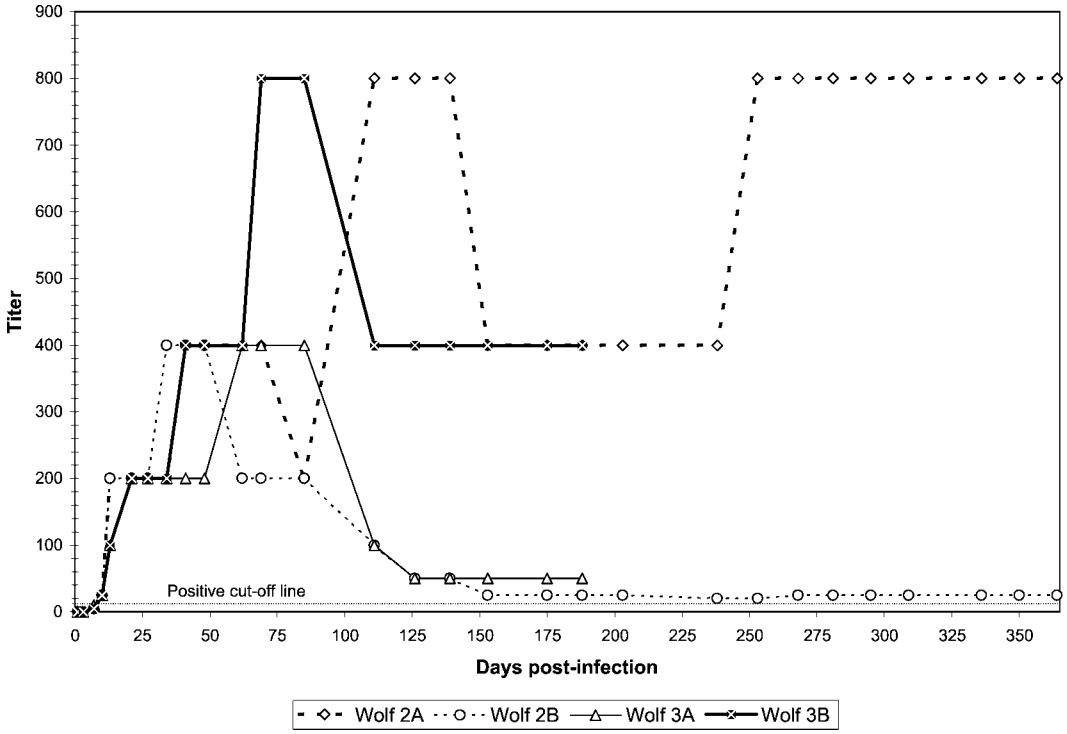

FIGURE 2. Complement fixation test titers over time for four wolves infected with *Brucella abortus* biovar 1.

TABLE 1. Tissues that were culture-positive for *Brucella abortus* biovar 1 from one or more of four wolves fed *B. abortus* biovar 1 and sampled 6 mo or 1 yr postinoculation.

	B. abortus biovar 1 (CFU/g tissue)[a]			
	6 mo postinoculation		1 yr postinoculation	
Tissue	3A	3B	2A	2B
Lymph nodes				
Medial retropharyngeal	0	120	1,016	72
Parotid	464	544	ND[b]	0
Mandibular	>800	C[c]	32	0
Suprascapular	208	640	0	C
Prefemoral	48	296	C	0
Superficial inguinal	104	640	80	ND
Internal iliac	ND	ND	728	ND
Bronchial	120	560	112	0
Mesenteric	32	320	88	64
Popliteal	>800	>800	8	C
Axillary	136	296	0	4
Other tissues				
Spleen	4	32	0	0
Liver	0	0	1.3	0
Thymus	360	>800	32	104

[a] CFU = colony-forming units; ND = not done; C = overgrown with contaminant microorganisms.

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imental conditions, we did not observe any clinical signs or lesions in wolves. Previous studies found no lesions in naturally infected, free-ranging wolves or foxes (*Vulpes vulpes*) in WBNP (Tessaro, 1988) or in naturally or experimentally infected coyotes (*Canis latrans*; Davis et al., 1979, 1988), and it would appear that *B. abortus* has little effect on the health of these wild canids.

Conventional serologic tests for brucellosis in cattle were effective in detecting infected wolves by 10 days PI. Similar results have been observed in both field-infected and experimentally infected dogs and coyotes (Davis et al., 1979, 1988; Scanlan et al., 1989; Forbes, 1990), indicating that there is a consistent response to the bacterium, although the actual sensitivity and specificity of these tests in canids is unknown. The declining serologic titers observed in two of the wolves and the declining numbers and distribution of bacteria between wolves euthanized at 6 versus 12 mo suggests that wolves can clear the infection after a single exposure.

Excretion of *B. abortus* in feces from the infected wolves was infrequent, and the numbers of *B. abortus* recovered were always far below the reported infective dose for cattle of approximately 10^3–10^8 CFU of the bacterium via the conjunctiva (McEwen et al., 1939; Manthei, 1950; Payne, 1959, 1960; Crawford et al., 1987). Bite transmission of brucellosis by wolves would appear unlikely given the low numbers and short duration of recovery of residual *B. abortus* from the oral cavity. The recovery of a small number of *B. abortus* from the urine sample of only one wolf (2A), and just 12 hr PI, likely reflected oral contamination of the penis and prepuce during grooming by this animal rather than bacterial colonization of, and shedding from, the urinary or genital tracts so soon after infection. This was supported by lack of detectable *B. abortus* bacteremia in any of the wolves prior to day 7 PI.

Numerous cases of *B. abortus* infection have been reported in dogs, but there is no confirmed case of natural transmission from dogs to cattle (Forbes, 1990). Experimental transmission of *B. abortus* from dogs and coyotes to cattle has been documented under conditions of close confinement (Kiok et al., 1978; Davis et al., 1988), but these unnatural conditions are not likely relevant in assessing the risk of transmission of *B. abortus* from free-ranging wolves to ungulates. Excretion of *B. abortus* from the female reproductive tract of dogs and coyotes has been reported (Morse et al., 1953; Taylor et al., 1975; Bicknell et al., 1976; Bicknell and Bell, 1979; Davis et al., 1979; Forbes, 1990), but again, programs to eradicate brucellosis from cattle populations have not been impeded by the presence of dogs and coyotes. Female wolves were not available for the present experiment, but given the similarities between brucellosis in dogs, coyotes, and male wolves, the effect of brucellosis in female wolves is likely similar to that in female dogs and coyotes and inconsequential in transmission of the disease.

A variety of species can become "spillover" hosts of *B. abortus* in areas where brucellosis is enzootic, but these species are sentinels rather than vectors of the bacterium. Programs to control or eradicate brucellosis by managing the primary reservoir hosts, such as cattle and bison, have succeeded under these conditions. Although wolves can harbor *B. abortus* in circumstances where prey species are infected, this experiment would suggest that wolves do not play a significant role in maintenance and dissemination of *B. abortus* and should not pose an obstacle to eradication of the disease.

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