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Experimental Infection of White-tailed Deer with Rangiferine Brucellosis

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ABSTRACT: Experimental infections of white-tailed deer (*Odocoileus virginianus*) with *Brucella suis* biovar 4 were evaluated over a period of 6 wk. Five adult male hand-raised white-tailed deer were inoculated with 1×10^7 colony forming units of *B. suis* biovar 4 in the conjunctiva and serologically evaluated over 6 wk by the card test (CARD), rivanol test (RIV), serum agglutination test (SAT), complement fixation test (CFT), particle concentration fluorescence immunoassay (PCFIA), and competitive enzyme linked immunosorbent assay (c-ELISA), as routinely used for bovine samples. Six weeks postinoculation the animals were euthanized and cultured for *B. suis* biovar 4. One deer became serologically positive 4 wk postinoculation on CFT, CARD, PCFIA, and c-ELISA. At 6 wk postinoculation, CFT was positive in four infected deer, CARD was positive in three deer; RIV, SAT, and PCFIA was positive in two deer; and cELISA was positive in one deer. Only the CFT was 100% sensitive. At necropsy *B. suis* biovar 4 was isolated from four of five deer, and representative colonies were biologically similar to the challenge organism.

Key words: *Brucella suis* biovar 4, brucellosis, diagnosis, *Odocoileus virginianus* serology, white-tailed deer.

Brucellosis caused by *Brucella suis* biovar 4 is enzootic in reindeer (*Rangifer tarandus*) populations throughout the arctic regions of the world (Meyer, 1966). Reindeer and caribou (*Rangifer tarandus*) are considered the primary hosts for *B. suis* biovar 4 (Broughton et al., 1970). Clinical signs associated with the disease in reindeer are abortions, birth of weak calves, orchitis, arthritis, and bursitis (Davidov, 1961; Neiland et al., 1968). The disease is transmitted by ingestion of the organism in aborted fetal membranes, or uterine discharges (Davidov, 1961). Diagnosis is based on clinical signs and serology and is confirmed by isolation of the bacterium from tissues collected at necropsy or slaughter.

In Alaska (USA), a large number of free-ranging native reindeer herds are infected with *B. suis* biovar 4. Several serological surveys for brucellosis have been conducted with the highest prevalence of 30% in caribou herds in the arctic (Dieterich, 1981). Therefore, there may be a potential problem if infected animals are exported to *Brucella*-free areas. Large numbers of reindeer are being moved from Alaska and Canada to parts of the USA, because there is an increasing market for game farming of cervids. In 1994, 1,053 reindeer were imported from Alaska of which a large number went to Texas (T. Scheib, pers. comm.). If native cervids, the most common species being white tailed deer (*Odocoileus virginianus*) in Texas, come in contact with reproductive products from infected reindeer disease transmission might occur. White-tailed deer are known to be susceptible to *Brucella abortus* infections, and it was shown that their antibody response was similar to that of cattle (Youatt and Fay, 1959). Nothing is known about susceptibility of white-tailed deer to *B. suis* biovar 4 infections if standard serological techniques are effective.

The rationale for this study was to (1) observe experimental *B. suis* biovar 4 infections in white-tailed deer, (2) to evaluate conventional serological tests for brucellosis, and (3) to re-isolate and biotype the organism from infected deer.

Five 2-yr-old male, hand-raised white-tailed deer, which were serologically negative on the *Brucella* spp. antigen card test (CARD) were held in a bio-safety facility at Texas A&M University (College Station, Texas, USA). They were maintained with a daily diet of pelleted feed (TAMU Custom Ration, Producers Cooperative, Bry-

an, Texas) and water *ad libidum.* The light-dark cycle in the room was adjusted to simulate natural conditions during the summer, when male deer are sexually inactive. The deer were challenged with an inoculation of 5×10^6 colony forming units (CFU) of *B. suis* biovar 4 in 0.05 ml given bilaterally in each conjunctival sac (total dose of 1×10^7 CFU). This route and dose of inoculation is the standard technique used in other species. The *B. suis* was originally isolated from the carpus of a reindeer shot near Nome (Alaska, USA) and had been tested for virulence by single passage through lemmings (*Lemmus sibiricus*) (Bevins et al., 1996). Deer were observed for 6 wks. Blood samples were analyzed serologically at Texas A&M University using CARD, rivanol precipitation test (RIV), serum agglutination test (SAT) (National Animal Disease Laboratory, Diagnostic Reagents Manual 65E and F, Ames, Iowa, USA), and the competitive enzyme linked immunosorbent assay (c-ELISA) (Rylatt et al., 1985). The RIV and the SAT were considered positive at a titer $\geq$ 1:50, while the cELISA was considered positive at $\geq$70% and suspect at 40 to 70%. In addition the complement fixation test (CFT) (Jones et al., 1963) and the particle concentration fluorescence immunoassay test (PCFIA) (Snyder et al., 1990) were done at the Texas Animal Health Commission Laboratory (Austin, Texas, USA). These tests were considered positive at a titer of $\geq$1:10 for CFT and a channel count reading of $<$0.6 for PCFIA. The animals were examined biweekly for clinical signs of disease. At 6 wks postinoculation all animals were euthanised with an overdose of pentobarbitone (D. Buthanasia Schering Plough Animal Health, USA; dose 1ml/5kg body weight given IV) and necropsied. Twenty one different lymph nodes and eleven organ samples were collected in sterile plastic bags from each animal, and stored at -70 C for subsequent bacteriological examination. These tissues included right and left atlantal, axillary, internal iliac, mandibular, parotid, popliteal, prefemoral, prescapular, and suprapharangeal lymph nodes; samples of the hepatic, mediastinal and mesenteric lymph nodes; and tissue samples of both epididymis, kidney, liver, lung, spleen, both tonsils, and both the testis. Thawed tissues for bacteriology were flamed and cut to expose an inner surface of the tissue. This tissue was then streaked over plates containing *Brucella* spp. selective media (SSR083A, Oxoid Selective Supplements, Unipath Ltd., Basingstoke, Hamsphire, England). All colonies were Gram stained and examined microscopically. Representative colonies, suspected of being *B. suis,* were confirmed as biovar 4 biotyping analysis (Alton et al., 1988).

Clinical signs of infections were not seen in any animal. One deer sustained an injury (broken leg) and was euthanised during the fifth week of the experiment and necropsy was performed as per the protocol. Gross lesions were not seen in any deer. *Brucella suis* was isolated from four of five deer. Bacteria were isolated mostly from lymph nodes, liver or spleen. Tonsils, lungs, testis and epididymis from all deer were negative. The biotyping analysis of these organisms were similar to the challenge organism, and were similar to the classification of *B. suis* biovar 4. The CARD, CFT, FIA and cELISA tests were positive for one deer 4 weeks postinoculation. At 6 wks postinfection, CFT was positive in four deer; the CARD was positive in three deer; RIV, SAT, and FIA were positive in two deer; and cELISA was positive in one deer (Table 1). Therefore, *B. suis* biovar 4 established infections in white-tailed deer, and conventional serological tests for brucellosis can be used to diagnose infections after 4 wks postinfection. Only the CFT correlated 100% with bacterial isolation.

This study was unfortunately limited to 6 wks. However, this study demonstrates that *B. suis* biovar 4 can infect white-tailed deer under experimental conditions and conventional tests for bovine brucellosis can successfully diagnose infections. Tests

TABLE 1. Diagnosis of *Brucella suis* biovar 4 antibodies in serum of experimentally infected white-tailed deer (*Odocoileus virginanus*) using conventional tests routinely used for bovine samples.

Week	ID No.	CARD[a]	RIV[b]	SAT[c]	CF[d]	PCIFA[e]	cELISA[f]
0	NT[g]	N[h]	N	N	N	N	N
	15	N	N	N	N	N	N
	16	N	N	N	N	N	N
	17	N	N	N	N	N	N
	18	N	N	N	N	N	N
2	NT	N	N	N	N	N	N
	15	N	N	N	N	N	N
	16	N	N	N	N	N	N
	17	N	N	N	N	N	N
	18	N	N	N	N	N	N
4	NT	N	N	N	N	N	N
	15	N	N	N	N	N	N
	16	P	N	N	P	P	P
	17	N	N	N	P	N	N
	18	N	N	N	P	N	N
6	—	—	—	—	—	—	—
	15	P[i]	N	P	P	N	N
	16	P	N	N	P	P	P
	17	N	P	N	P	N	N
	18	P	P	P	P	P	N

[a] Card test.
[b] Rivanol test.
[c] Serum agglutination test.
[d] Complement fixation test.
[e] Particle concentration fluorescence immunoassay.
[f] Competitive enzyme linked immunosorbent assay.
[g] Sample for deer number NT was not available at week 6.
[h] Negative.
[i] Positive.

that use whole cell *B. abortus* antigen were better in diagnosing *B. suis* biovar 4 infections in white-tailed deer than c-ELISA that used processed antigens. The cELISA was not optimized for white-tailed deer or *B. suis* biovar 4. In this study the tests used were based on antigens of whole *B. abortus* except cELISA which uses purified lipopolysaccharide antigens. As reviewed by Wright and Nielsen (1990), CARD determines the IgG 1 reaction using a buffered antigen, RIV precipitates IgM and detects the IgG 1 and 2 reactions, while the other tests, SAT, CFT, PCFIA, detected a mixed immunoglobulin response. Of these tests, only the CFT has the highest sensitivity and specificity for the diagnosis of bovine brucellosis and we observed that the CFT correlated best with bacteriological results. A sensitive and specific test for diagnosis of *B. suis* biovar 4 infections is needed not only for reindeer, but other potential hosts. Other studies have shown that current tests lack sensitivity in diagnosis of *B. suis* biovar 4 in cattle (Forbes and Tessaro, 1993). In an attempt to improve the diagnostic tests, Bevins (1993) developed an indirect ELISA which was more sensitive than the standard agglutination tests currently used to diagnose *B. suis* biovar 4. This test was designed to discriminate between infected and vaccinated animals based on the differential response to the A and M polysaccharide antigens. These are surface antigens found on smooth strains of *Brucella* spp. routinely used in tests to differentiate species based on dominance of either A, M, or both antigens (Alton et al., 1988). In the present study, we used commercially available tests that were developed for the diagnosis of *B. abortus* in cattle. We recommend that a battery of several serological tests be used for diagnosis of *B. suis* infections in white-tailed deer.

Behavioral and management differences between reindeer and white-tailed deer make it unlikely that white-tailed deer will be exposed to infected reindeer, but the possibility exists. *Brucella suis* biovar 4 is endemic in most Alaskan reindeer herds and without effective testing and quarantine measures for screening these reindeer when they move, there is a possibility that infected reindeer could be imported into white-tailed deer range. Imported reindeer are usually kept in enclosures, but some may escape. Reindeer differ from white-tailed deer in their feeding habits, migratory behavior and gregariousness. Yet, white-tailed deer could be exposed to uterine discharges or placenta of *B. suis* biovar 4 infected live or aborted reindeer calves. We know that *B. suis* biovar 4 infections can be established in white-tailed deer, but do not know if these infections are maintained in these animals and if

these infections cause reproductive disease. We do not know if transmission of *B. suis* biovar 4 between white-tailed deer is possible or likely. Such transmission would be necessary for the organism to become established in a free-ranging population. Should infections be established in the free-ranging populations of white-tailed deer, control and eradication of the disease would be difficult if not impossible.

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