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INTERSPECIFIC TRANSMISSION OF *BRUCELLA ABORTUS* FROM EXPERIMENTALLY INFECTED COYOTES (*CANIS LATRANS*) TO PARTURIENT CATTLE

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ABSTRACT: In four separate trials, 10 coyotes (*Canis latrans*) which had been individually fed mascerated bovine placental tissue experimentally inoculated with *Brucella abortus* strain 2308 were placed in a 1 ha isolation area with six parturient, non-*B. abortus* vaccinated, *Brucella* spp. seronegative Hereford heifers. During the second trial, three of the heifers became *Brucella* spp. seroreactive (as determined by the card, standard agglutination tube, rivanol, complement fixation, and enzyme labeled immunosorbent assay tests) 14 days after exposure to the *B. abortus* infected coyotes. Five of 10 coyotes in the second trial seroconverted to *Brucella* spp. positive by day 14 postexposure (PE) and by day 27 PE seven of the 10 coyotes were *Brucella* spp. reactive. The three *Brucella* spp. seroreactive heifers subsequently aborted on days 35, 64 and 66 PE and *B. abortus* strain 2308 was isolated from vaginal swabs, milk, and placental and fetal tissues. There was no serologic or bacteriologic evidence of transmission from *B. abortus* infected coyotes to the susceptible cattle recorded in the other three trials.

Key words: Coyote, *Canis latrans*, *Brucella abortus*, interspecific transmission, serology, domestic cattle, experimental study.

INTRODUCTION

Serologic evidence has indicated that timber wolves (*Canis lupus*) in Alaska can become naturally infected with rangiferine brucellosis (Neiland, 1970) and wolves have been experimentally infected by intraperitoneal inoculations of 1×10^8 colony forming units (CFU) of *Brucella suis* biovar 4 (Neiland and Miller, 1981). Rangiferine brucellosis also has been reported in arctic foxes (*Alopex lagopus*) and red foxes (*Vulpes vulpes*) in Russia (Pinigin and Zabrodin, 1970) and Alaska (Neiland, 1975). *Brucella suis* was found to occur naturally in red foxes in Bulgaria (Pavlov et al., 1960) and in Russia (Rementsova, 1964). In the United States, a serologic survey for *Brucella canis* conducted on seven species of wild carnivores from five states, detected positive reactions in a red fox from New York and two coyotes (*Canis latrans*) from Texas (Hoff et al., 1974). Serologic evidence of *Brucella abortus* infections has been reported in red foxes in Northern Ireland (McCaughey, 1968). Two species of wild foxes (*Dusicyon gymnocercus* and *D. griseus*) in Argentina were shown by serologic and bacteriologic methods to be naturally infected with *B. abortus* biovar 1 (Szyfres and Gonzales-Tome, 1966). Serologic evidence of *B. abortus* was reported in the black-backed jackal (*Canis mesomelas*), spotted hyena (*Crocuta crocuta*) and wild hunting dog (*Lycaon pictus*) in Tanzania (Sachs et al., 1968). Scanlan et al. (1984) successfully infected gray foxes (*Urocyon cinereoargenteus*) with 4.4×10^{10} CFU of *B. abortus* strain 2308.

Sera from coyotes collected in southern Texas were found to have a 6% (11 of 198) prevalence of *Brucella* spp. antibody as determined by the card test (Rahdhawa et al., 1977). Hoq (1978) in a serologic survey of *Brucella* spp. agglutinins in wildlife and sheep in California found 3% (five of 148) of the coyotes tested had a titer of >1:100 by the standard rapid plate agglutination test. In a serologic and bacteriologic survey for *B. abortus* in coyotes collected from eastern Texas, 18% (nine of 51) were serologically positive as determined by the card, rivanol, standard agglutination tube,

and cold complement fixation tests; *B. abortus* biovar 1 was isolated from various tissues from seven of 43 coyotes and congenital transmission was also documented (Davis et al., 1979). The present study was conducted to evaluate the efficacy of various serologic techniques to diagnose *B. abortus* infections in coyotes and to determine the transmission potential of *B. abortus* from experimentally infected coyotes to susceptible parturient domestic cattle under controlled conditions.

MATERIALS AND METHODS

Coyotes were trapped from 24 counties in eastern Texas (USA) by personnel of the Texas Animal Damage Control Service (USDA/APHIS–ADC, P.O. Box 604, Bryan, Texas 77806, USA) and transported to the Veterinary Research Park (College of Veterinary Medicine, Texas A&M University, College Station, Texas 77843, USA). The coyotes were tested for *Brucella* spp. antibodies by the card, rivanol precipitation (RIV), standard agglutination tube (SAT) (National Animal Disease Laboratory, Diagnostic Services, Ames, Iowa 50010, USA) cold complement fixation test (CCFT) (Jones et al., 1963), enzyme labeled immunosorbent assay (ELISA) (Heck et al., 1980, 1982; Nielsen et al., 1983), and hemolysis in gel (HIG) techniques (Nielsen et al., 1983). Only those coyotes shown to be serologically negative for *Brucella* spp. for 30 days postcapture were utilized in the study.

In each of the four repetitions of the experiment, 10 *Brucella* spp. serologically negative coyotes were individually fed for three consecutive days approximately 100 g daily of macerated bovine placental and fetal tissue which had been inoculated with 1×10^6 to 1×10^8 CFU *B. abortus* strain 2308 per g of tissue (Table 1). Prior to, and after, being fed the *B. abortus* inoculated bovine tissue, coyotes were maintained on an ad libitum diet of a commercial dog food ration (Purina Hi Pro, Ralston Purina Company, St. Louis, Missouri 63164, USA). After consumption of the *B. abortus* inoculated bovine tissue, the 10 coyotes were placed in a 1 ha pasture (which had been modified with high fences, buried aprons and electrified fences to provide suitable isolation and security) with six *B. abortus* susceptible parturient heifers. During each of four trials, the coyotes remained in the pasture with the susceptible cattle until the termination of all cattle pregnancies for that repetition of the study. The 24 parturient heifers used in the investigation were registered Herefords. They were *B. abortus* non-vaccinated, *Brucella* spp. seronegative and from a single *B. abortus*-free herd. At the time the heifers were exposed to the *B. abortus* infected coyotes the cattle were in the second trimester of gestation. Blood samples were collected from all of the animals (10 coyotes and six heifers) for each weekly repetition from 3 wk prior to the exposure of the cattle to the coyotes until the termination of pregnancy in all of the cattle. Coyotes were bled through the cephalic vein and the cattle were bled through jugular venipuncture. The blood samples were collected by sterile needle and syringes, transferred to sterile vacuum blood tubes, refrigerated, allowed to clot for 4 to 6 hr, and centrifuged at 600 *g* for 10 min. The resulting sera were decanted and stored at −20 C. The sera were evaluated for *Brucella* spp. antibodies by the card, RIV, SAT, CCFT, ELISA, and HIG tests. Heifers that became *Brucella* spp. seroreactive were separated immediately prior to parturition from the rest of the cattle so that cross transmission of *B. abortus* between cattle would not occur.

Medial retropharyngeal lymph nodes, palatine tonsils, spleen, superficial inguinal lymph nodes and the testes or uterus were collected aseptically from the coyotes at necropsy and stored at −20 C. Placental tissue, vaginal swabs and quarter milk samples from the four mammary glands were collected at the termination of pregnancy (either abortion or normal birth) from the cattle. Rectal swabs and blood samples were taken from all live-born calves. Aborted fetuses were necropsied and lung, abomasum, mediastinal lymph node and stomach swabs were collected. The tissue samples and swabs from the cattle were also stored at −20 C. For bacteriologic culture, the tissue or swab was thawed and streaked on agar media (Bacto Brucella Agar, Difco, Detroit, Michigan 48323, USA; BB1 Formula Agar, National Veterinary Services Laboratory, Ames, Iowa 50010, USA) and Farrell's Media (Farrell, 1974). Inoculated plates were incubated at 37 C in 10% CO_2, and after 3 days the cultures were examined. Bacteria from *Brucella* spp.-like colonies were characterized by a rapid slide agglutination test (National Animal Disease Laboratory). Those reacting to the slide agglutination test were inoculated onto agar slants and *B. abortus* isolates were identified by standard serologic and biochemical criteria (Alton et al., 1975). Subcultures were submitted to the National Veterinary Services Laboratory for confirmation.

RESULTS AND DISCUSSION

In all repetitions of the study, some of the coyotes became infected with *B. abor-*

TABLE 1. Summary of the results of four repetitions of oral exposure of coyotes to *Brucella abortus* strain 2308 and the subsequent serologic and bacteriologic, and interspecific transmission to susceptible cattle.

Replicate number	Number of CFU[a] *B. abortus*/300 g	Number of coyotes serologically positive PE[b]	Number of isolations of *B. abortus* from coyotes PE (number and tissue of isolation)	Number of cattle serologically positive PE	Number of isolations of *B. abortus* from cattle	Number of abortions (day PE)
1	3.0×10^{8}	7/10	no tissue isolations no fecal isolations	0/6	no isolations	0/6
2	1.2×10^{11}	10/10	7/10 coyotes (retropharyngeal LN[c]—6, inguinal LN—6, spleen—1, tonsil—4, and feces—2)	3/6	milk samples—3 vaginal swabs—3 placenta—3 fetal—2	3/6 (35, 64, 66)
3	1.5×10^{10}	9/10	4/10 coyotes (retropharyngeal LN—4, spleen—2, tonsil—1, and supramammary LN—1) no fecal isolations	0/6	no isolations	0/6
4	7×10^{7}	6/10	no tissue isolations no fecal isolations	0/6	no isolations	0/6

[a] CFU = colony forming units.
[b] PE = postexposure (days).
[c] LN = lymph node.

tus by ingesting the experimentally inoculated bovine placental and fetal tissues. The serologic responses observed in the coyotes corresponded to the number of viable *B. abortus* ingested (Table 1). During the first, third and fourth trials the number of organisms in the mascerated bovine tissue was $\leq 5 \times 10^7$ CFU *B. abortus* strain 2308/g. In the second trial (in which the coyote to cattle transmission was achieved) the number of *B. abortus* strain 2308 organisms/g of experimentally inoculated bovine tissue was $\geq 4 \times 10^8$ CFU. *Brucella abortus* biovar 1 was isolated from fecal samples collected from two of the 10 *B. abortus* exposed coyotes in second trial, 7 days postexposure (PE). *Brucella abortus* was not isolated from feces from any of the other 30 exposed coyotes during the three other trials. *Brucella abortus* strain 2308 was isolated from coyote tissues collected at necropsy in all four repetitions of the study (Table 1). Coyote tissues from which *B. abortus* was isolated included spleen, retropharyngeal lymph nodes, superficial inguinal lymph nodes and palatine tonsils.

Fourteen days after the *B. abortus* exposed coyotes were placed in the pen with the susceptible heifers in second trial, three of the heifers were positive to *B. abortus* by diagnostic criteria (Heck et al., 1982) as determined by at least two of the tests utilized. By day 28 PE, all three of the heifers were *B. abortus* serologically positive to the card, RIV, CCFT, SAT, ELISA, and HIG. An abortion occurred in a reactor heifer 35 days PE. *Brucella abortus* strain 2308 was isolated from milk, vaginal swabs, placental, and fetal tissues collected on the day of the abortion. Subsequent abortions occurred in the other two *B. abortus* seroreactive heifers on days 64 and 65 PE, and *B. abortus* strain 2308 was isolated from milk and tissue samples collected.

The various serologic tests utilized on the coyotes were evaluated by several criteria, such as the earliest to react after exposure to the *B. abortus* organisms, agreement with bacteriologic results, agreement with the other serologic tests and sensitivity of the test. No single serologic test was determined to be the most efficacious in diagnosing *B. abortus* infections in coyotes. The card test seems to be a reliable screening test, but it should be compared or performed in conjunction with the ELISA or the HIG test both of which seem to be able to detect lower antibody levels and for a longer period.

The epidemiologic significance of the transmission of *B. abortus* from coyotes to cattle should not be overstated. The animals in the investigation were in an artificially crowded situation by experimental design (six cattle and 10 coyotes in a 1 ha area) that would be unusual in nature. A successful coyote to cow transmission of *B. abortus* occurred only in three of 24 cattle and only once in four attempts. However, the present study did show that it is possible under certain conditions for coyotes to become infected orally and to maintain and excrete *B. abortus* in sufficient quantities for a period adequate to cause infections in other animals in close contact with, or proximity to, them. Earlier researchers have stated that "*Brucella* organisms are not readily transmitted from the preferential host to dissimilar hosts" (Meyer, 1964). The present study does support this conclusion but it also indicates that transmission from "dissimilar hosts" such as predators to the "preferential host" is possible under certain conditions.

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LITERATURE CITED

ALTON, G. G., L. M. JONES, AND D. E. PIETZ. 1975. Laboratory techniques in brucellosis, 2nd ed. World Health Organization, Geneva, Switzerland, pp. 121–124.

DAVIS, D. S., W. J. BOEER, J. P. MIMS, F. C. HECK, AND L. G. ADAMS. 1979. *Brucella abortus* in coyotes. I. A serologic and bacteriologic survey in eastern Texas. Journal of Wildlife Diseases 15: 367–372.

FARRELL, I. D. 1974. The development of a new selective medium for the isolation of *Brucella abortus* from contaminated sources. Research in Veterinary Science 16: 280–286.

HECK, F. C., B. L. DEYOE, AND J. D. WILLIAMS. 1982. Antibodies to *Brucella abortus* in sera from strain 19 vaccinated and non-vaccinated cows as determined by enzyme linked immunosorbent assay and conventional serologic methods. Veterinary Immunology and Immunopathology 3: 629–634.

———, J. D. WILLIAMS, AND J. PRUETT. 1980. Interpretation of spectrophotometric absorbance values to define results of enzyme-linked immunosorbent assays. Journal of Clinical Microbiology 111: 398–401.

HOFF, G. L., W. J. BIGLER, D. O. TRAINER, J. G. DEBBIE, G. M. BROWN, W. G. WINKLER, S. H. RICHARDS, AND M. REARDON. 1974. Survey of selected carnivore and opossum serums for agglutinins to *Brucella canis*. Journal of the American Veterinary Medical Association 165: 830–831.

HOQ, M. A. 1978. A serologic survey of *Brucella* agglutinins in wildlife and sheep. California Veterinarian 32: 15–17.

JONES, L. M., J. B. HENDRICKS, AND D. T. BERMAN. 1963. The standardization and use of the complement fixation test for the diagnosis of bovine brucellosis with a review of the literature. American Journal of Veterinary Research 24: 1143–1151.

MCCAUGHEY, W. J. 1968. Brucellosis in wildlife. Symposia of the Zoologic Society of London 24: 99–105.

MEYER, M. E. 1964. The epizootiology of brucellosis and its relationship to the identity of *Brucella* organisms. American Journal of Veterinary Research 25: 553–557.

NEILAND, K. A. 1970. Rangiferine brucellosis in Alaskan canids. Journal of Wildlife Diseases 6: 136–139.

———. 1975. Further observations on rangiferine brucellosis in Alaskan carnivores. Journal of Wildlife Diseases 11: 45–53.

———, AND L. G. MILLER. 1981. Experimental *Brucella suis* type 4 infections in domestic and wild Alaskan carnivores. Journal of Wildlife Diseases 17: 183–189.

NIELSEN, H. H., F. C. HECK, J. M. STILLER, AND B. ROSENBAUM. 1983. Interaction of specifically purified isotypes of bovine and antibody to *Brucella* antibody to *Brucella abortus* in the hemolysis in gel test and enzyme-linked immunosorbent assay. Research in Veterinary Science 35: 14–18.

PAVLOV, P., D. TCHILEV, M. MATEEN, M. MILANOV, B. TATROV, AND V. KRASTEV. 1960. Recherches sur des reservoirs de brucella chez le porc vivant en liberte. Bulletin de Office Internationale des Epizooties 53: 1511–1526.

PINIGIN, A. F., AND V. A. ZABRODIN. 1970. On the nidality of brucellosis. Vestnik Sel'skokhozyaistvennoi Nauki (Moscow) 7: 96–99.

RAHDHAWA, A. A., V. P. KELLY, AND E. F. BAKER. 1977. Agglutinins to *Coxiella burnetti* and *Brucella* spp., with particular reference to *Brucella canis* in wild animals of southern Texas. Journal of the American Veterinary Medical Association 171: 939–942.

REMENTSOVA, M. M. 1964. La brucellose des animoux sauvages. Bulletin de Office Internationale des Epizooties 61: 99–112.

SACHS, R., C. STAAK, AND C. M. GROOCOCK. 1968. Serological investigation of brucellosis in game animals in Tanzania. Bulletin of Epizootic Diseases in Africa 16: 91–100.

SCANLAN, C. M., G. L. PIDGEON, L. J. SWANGO, S. S. HANNON, AND P. A. GALIK. 1984. Experimental infection of gray foxes (*Urocyon cinereoargenteus*) with *Brucella abortus*. Journal of Wildlife Diseases 20: 27–30.

SZYFRES, B., AND J. GONZALES-TOME. 1966. Natural *Brucella* infection in Argentine wild foxes. Bulletin of the World Health Organization 34: 919–923.

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