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## Experimental *Brucella suis* Biovar 4 Infection in a Moose

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**ABSTRACT:** A moose (*Alces alces gigas*) was inoculated with *Brucella suis* biovar 4 to better understand the effects of brucellosis in this species. Serum antibody titers increased rapidly and peaked within 21 to 56 days. Fever, leukocytosis, recumbency, anorexia and depression were observed starting 42 days post inoculation. *Brucella suis* biovar 4 was isolated from blood, lymph nodes, liver and spleen.

**Key words:** Moose, *Alces alces*, brucellosis, *Brucella suis* biovar 4, experimental study.

Brucellosis has been confirmed rarely in free-ranging moose (*Alces alces*) (Fenstermacher and Olsen, 1942; Jellison et al., 1953; Corner and Connell, 1958). *Brucella abortus* was implicated in those cases confirmed by bacterial isolation. There has been little serologic evidence of exposure in moose from areas where *B. abortus* was enzootic (Hudson et al., 1980). Some investigators have postulated that the disease is so severe in moose that few survive acute infection to be detected by serologic surveys at a later date (Corner and Connell, 1958; Dieterich, 1981).

Brucellosis caused by *B. suis* biovar 4 is enzootic in Alaskan reindeer and caribou (*Rangifer tarandus*) herds and serologic prevalence of antibodies is  $\leq 30\%$  (Dieterich, 1985). In many areas of Alaska, moose, reindeer, and caribou share common ranges. It is unusual to find seropositive moose even in areas where prevalence of antibodies in reindeer and caribou is high (Zarnke, 1983). Four (0.4%) of 1,119 moose sera collected from 1969 through 1989 were positive for antibody to *Brucella* spp.

A 9-mo-old male moose (*A. alces gigas*) was housed in an isolation room (10.5 m<sup>2</sup>) with wood shavings as bedding material at the University of Alaska Agricultural and Forestry Experiment Station (Fairbanks, Alaska 99701, USA). Feed (Quality Tex-

ture, Fisher Mills Inc., Seattle, Washington 98124, USA) and water were provided ad libitum. The light-dark cycle in the room was adjusted to simulate natural conditions. Public Health Services/CDC-recommended biosafety level 3 techniques were used throughout the experiment.

The moose was inoculated by placing  $1.7 \times 10^7$  colony forming units (CFU) of *B. suis* biovar 4 (in 0.1 ml of physiologic saline solution) in a conjunctival sac. This strain was originally isolated from a reindeer on the Seward Peninsula of Alaska and was passed one time through a guinea pig before being used as the inoculum. The inoculum of  $1.7 \times 10^7$  CFU was chosen because that dose was the minimum number of CFU's found to routinely infect reindeer (Dieterich, 1981).

Blood was collected before inoculation and then weekly for hemoculture, serology, and complete blood counts (CBC). Jugular blood (5 ml) was inoculated into Trypticase Soy Agar (Baltimore Biological Laboratories, Cockeysville, Maryland 21030, USA) slant bottle overlaid with 20 ml of tryptose broth with 1% sodium citrate for hemoculture. The standard plate (SP), buffered *Brucella* sp. antigen (BBA), and rivanol (Riv) tests were conducted at the University of Alaska (Fairbanks, Alaska 99701, USA) while the standard tube (ST), mercaptoethanol (ME), and complement fixation (CF) tests were conducted at the National Animal Disease Laboratory (Ames, Iowa 50011, USA) (United States Department of Agriculture, a, b). The CF test was done according to Hill's method (Hill, 1963). Prior to experimental exposure, the moose had no serologic evidence of previous contact with *Brucella* spp. Blood was sterile on culture. Packed cell

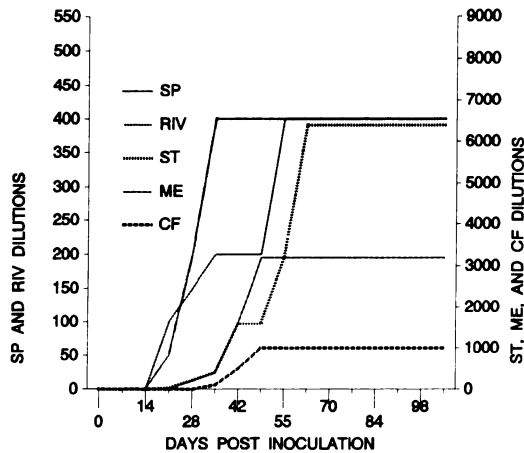


FIGURE 1. Serum antibody response of a 9-month-old male moose experimentally exposed to *Brucella suis* biovar 4. End point dilutions were: SP test (1:400), Riv test (1:400), ST test (1:6,400), ME test (1:3,200), and CF test (1:1,000).

volume (PCV), white blood cell count (WBC), and differential WBC count were within normal reference values (Dieterich, 1971). The moose was euthanized and necropsied 103 days post inoculation (PI). Tissues were collected for bacteriologic and histopathologic examination using standard procedures (Dieterich et al., 1981; United States Department of Agriculture, c).

The SP and Riv titers were first detected 14 days post infection (PI) and rapidly increased. The ST, ME and CF titers rose rapidly 21 days PI. All tests peaked by 56 days PI (Fig. 1). The BBA test became positive on day 35 PI and remained so until the moose was necropsied.

Complete blood counts were normal until day 55 PI when the WBC count increased from  $7-9 \times 10^3/\mu\text{l}$  to  $15-16 \times 10^3/\mu\text{l}$  and remained at that level until necropsy. The PCV decreased from 35 to 40% to 22 to 28% during the same period of time. There was a slight increase in the percentage of neutrophils and a slight decrease in lymphocytes. The percentages of other leukocytes remained stable.

On day 42 PI, the moose was observed lying down most of the time and would rise only when urged. He was anorexic for

3 days, then resumed eating approximately one-half his normal ration. Body temperature increased to 39.5 C from a normal of 38 C during the same time period. The moose remained very depressed for several more days. Normal appetite and activity resumed by day 58 PI.

At necropsy, the mediastinal, left pre-femoral, left and right popliteal, left pre-scapular, and right mandibular lymph nodes were enlarged and edematous. The epididymides and spleen were slightly enlarged. The spleen was friable with several 1 to 2 mm white foci on the surface and in the parenchyma. No other gross lesions were observed in other organs. Histologic examinations of tissues revealed focal areas of necrosis and inflammatory reaction in the spleen. Minor perivascular mononuclear infiltration was observed in the lung. Literature reports on *B. abortus* infection in moose describe primarily pericarditis, necrotic splenic foci, edematous lymph nodes, and peritonitis (Fenstermacher and Olsen, 1942; Jellison et al., 1953; Corner and Connell, 1958).

*Brucella suis* biovar 4 was isolated from spleen, liver, mandibular, retropharyngeal, mediastinal, superficial cervical, popliteal, superficial inguinal, and parotid lymph nodes. The organism was isolated from blood from day 28 PI through day 103. Other pathogens were not isolated on culture.

In our opinion, this moose would have died if it had been in the wild. The animal might have died of dehydration or been easy prey. This study lends support to the theory that brucellosis is a serious disease in moose. Occurrence of the disease in moose in brucellosis enzootic areas remains unknown.

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