EVIDENCE OF EXPOSURE TO *BRUCELLA SUIS* BIOVAR 4 IN NORTHERN ALASKA MOOSE

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ABSTRACT: Historically, some moose (*Alces alces*) of northern Alaska have been tested for serologic evidence of exposure to viral and bacterial diseases. Recently, in response to poor recruitment and declining numbers of moose along the Colville River and associated drainages on the North Slope of Alaska (near Umiat), 42 cows and 5 bulls were serologically tested for *Brucella* spp. Surprisingly, 8 (19%) cows had antibody titers $\geq$ 400 to *Brucella* spp. No bulls tested positive. This antibody prevalence is higher than published reports. Western blot analysis indicates antibody is specific to *Brucella suis* biovar 4. Three of 94 bulls (3%) and 1 of 69 cows (2%) sampled during 1992-97 in the Noatak River drainage had titers $\geq$ 100 (4 of 163 or 2%). The 12 positive moose detected since 1992 had relatively high titers. No significant antibody titers to *Brucella* spp. were detected in moose from the Seward Peninsula (34 cows), Selawik River (46 cows, 46 bulls) or the Arctic National Wildlife Refuge (38 cows, 14 bulls). Blood samples taken in 1996 from the Colville River moose were not optimal for recovery of viable bacteria and culture for *Brucella* organisms was unsuccessful.

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Moose (*Alces alces*) became established in Alaska north of the Brooks Range, Kotzebue Basin, and Seward Peninsula in the 1940s. Moose populations in these areas generally increased from that time until approximately 1990-95. During 1990-95, moose populations in the Colville River and associated drainages, Noatak drainage, and Seward Peninsula suffered sharp declines. Subsequent studies demonstrated that adult mortality and poor recruitment contributed to this population decline. Over 30 carcasses were located in the summer of 1995 near the Colville River. Causes of poor recruitment were unclear, but adverse weather, increasing predation, and deteriorating range were possibilities. The acute population decline and suspected poor calf or yearling recruitment (survival or production) prompted investigation of bacterial and viral abortifacients. Blood samples were collected from adult moose during capture operations to evaluate serum antibody prevalence of selected pathogens. We summarize results of serologic tests for *Brucella* spp. in moose from 5 populations: (1) Colville River and associated drainages (North Slope); (2) middle and lower Noatak River drainage (Kotzebue Basin); (3) Selawik River drainage (Kotzebue Basin); (4) Fish River drainage (south-central Seward Peninsula); and (5) Sheenjek/Chandler/Old Crow Flats (eastern Brooks Range) in the Arctic National Wildlife Refuge (ANWR).

Some moose in northern Alaska disperse during summer and occupy tundra habitats throughout the region, some as far north as the arctic coast. In the winter most moose congregate in narrow bands of riparian shrubs along streams draining the north slopes of the Brooks Range and other areas. Because most moose congregate along

the rivers, censuses are conducted each spring, which allows assessment of population and calving trends. In this paper we will focus on the serum antibody prevalence of *Brucella* spp. in moose from these areas.

Brucellosis is a disease caused by bacteria from the genus *Brucella* which cause "contagious abortion" or "Bang's Disease" in cattle, "fistula of the withers" or "poll evil" in horses, and "undulant fever" in humans (zoonotic). *Brucella* spp. are pathogenic in a variety of mammals (Fraser 1991) and have been recently described in marine mammals (Foster *et al.* 1996, Nielsen *et al.* 1996). Many ungulates which transmit, harbor, and develop infections can show dramatic clinical signs, but, there is limited information on moose brucellosis.

A common finding with brucellosis is an aborted fetus. The contaminated tissues are a common source of exposure. *Brucella* organisms remain viable in contaminated forage and water up to 2 months when conditions are dark and cool (Fraser 1991). Milk from infected females may contain *Brucella* organisms. Another major pathologic effect is grossly enlarged joints. Other effects include retained placenta, stillborn calves, reduced milk production, infected and affected male sex organs (i.e. orchitis), lameness, and occasionally death or extreme debilitation. Transmission of *Brucella* spp., as known for cattle and suspected for reindeer, is mostly oral (mucosal) and rarely, if at all, venereal (Rausch and Huntley 1978, Fraser 1991). Diagnosis is typically based on serology and culture. However, more specialized tools such as western blot analyses and polymerase chain reaction (PCR) amplification can identify the species and biovar of *Brucella* spp. by detecting specific antibodies or characteristic sequences of DNA, respectively.

Previous reports of natural *Brucella* spp. infection in moose are from isolated cases. Past diagnoses of brucellosis were in a young bull moose (Fenstermacher and Olsen 1942), and in a young female moose from Montana (Jellison *et al.* 1953). Serology tests revealed evidence of exposure in 9 of 44 moose killed by hunters in Montana during 1951 (Jellison *et al.* 1953). Studies at Elk Island National Park (Edmonton, Alberta), showed serologic evidence of exposure in 2 moose and *B. abortus* was cultured (Corner and Connell 1958). These previous studies documented that *B. abortus* could cause severe disease in free-ranging moose, but no indication of relative occurrence or predisposing factors could be made.

Experimental inoculation of *B. suis* biovar 4 in a moose (Dieterich *et al.* 1991) indicated that serum antibody peaked day 21 to 56. Clinical signs appeared on day 42. *Brucella suis* biovar 4 infection was documented in a debilitated free-ranging adult female moose from the Mackenzie River, Northwest Territories, Canada which had large fluctuant carpal masses (bilateral bursitis and osteomyelitis) (Honour and Hickling 1993). *Brucella suis* biovar 4 was shown to cause severe disease in a free-ranging moose, but, no conclusions were developed relating *B. suis* biovar 4 exposure and potential adverse health effects to populations of moose.

Researchers have speculated that moose are very susceptible to brucellosis and that individuals would die before transmitting the bacteria to other moose hosts, thus resulting in a very low antibody prevalence in a population (Hudson *et al.* 1980, Zarnke 1983, Dieterich *et al.* 1991). We present serologic, pregnancy, calving, and mortality data for a population of moose on the Colville River with a higher than expected rate of *Brucella suis* biovar 4 seropositive animals when compared to serologic data from other populations of moose across northern Alaska. We also discuss public health concerns for consumers that

should be considered as well (Chan *et al.* 1989).

METHODS

Capture and Sampling

In April 1996, moose from the Colville River area were captured, examined, collared with VHF transmitters and evaluated for pregnancy, serologic evidence of disease, minerals status (serum and hair), fecal indicators of parasites, standard blood indices, and others. Moose from other regions had serum collected. All moose from each of the areas reported here were captured using standard helicopter and chemical immobilization techniques. In general, moose were immobilized using 3.6-4.2 mg carfentanil citrate (Wildnil@ 3mg/ml, Wildlife Pharmaceuticals, Inc., Fort Collins, CO) and 160 to 170 mg of xylazine (Sedazine@ 100mg/ml, Wildlife Pharmaceuticals, Inc., Fort Collins, CO). These drugs were injected intramuscularly using a 3 ml dart with a 2.5 cm needle fired from a Captur rifle using brown wad external charges (Palmer Chemical & Equipment Co., Douglasville, GA). If immobilization was not achieved within 15 minutes, the use of a second injection was considered and was filled with a "half dose" (1.5 mg carfentanil and 80 mg xylazine). Naltrexone (50 mg/ml, Wildlife Pharmaceuticals, Inc., Fort Collins, CO) was given (400 mg for each ml of carfentanil) intramuscularly to reverse the effects of the carfentanil once procedures were completed or if the moose showed signs of distress. In 1992, 600 mg of naloxone for each ml of carfentanil was used on the Noatak moose. Blood samples were taken by syringe from the jugular vein and placed into tubes for serum collection. Tubes were prevented from freezing.

Brucellosis Testing

Serum was separated from blood and frozen. Most serologic testing was conducted at the Institute of Arctic Biology, University of Alaska Fairbanks (IAB-UAF). Two standard serologic methods were used: (1) the buffered *Brucella* antigen card test (BBA or card test), and (2) the standard plate test (SPT). The BBA results were recorded as simply either positive or negative for the presence of antibody to *Brucella* spp. For the SPT, titers $\geq$ 50 were considered indicative of previous exposure (Alton *et al.* 1975). Supportive tests for the Colville River moose population included cold complement fixation (CF) (Alton *et al.* 1975) and particle concentration fluorescence immunoassay (PCFIA) (Reynolds 1987). The term anti-complementarity indicates that the serum has an inherent, nonspecific ability to affect the CF assay and specific antibody for *Brucella* spp. are not able to be tested. Minimum criteria for diagnostically positive reactions for these tests are listed in APHIS (1992). The CF assay was conducted at the State Federal Laboratory, 4501 Springdale Rd., Suite B, Austin, TX 78723-9983, and the PCFIA was done by the Division of Animal Health, Dept. of Agriculture, PO Box 630, Jefferson City, MO 65102. Western blot analysis involves immunoprecipitation of specific antigens (epitopes A and M of the O side chain) by the test serum antibody and was performed at Louisiana State University. If the test serum antibody only reacts with the A epitope, then *B. suis* is not suspected. If both A and M epitopes (proportionally more M antigen) react to antibody then *B. suis* biovar 4 antibody is present. Clinical signs and lesions were evaluated as best as possible.

Pregnancy Testing

Pregnancy-specific protein B (PSPB) testing was performed by Biotracking Inc. (Moscow, ID 83843) on serum samples of moose from the Colville River area. The test measures the amount of PSPB in serum

which is determined by the competitive radioimmunoassay and the amount of ^{125}I-PSPB bound. If less than 93% of the ^{125}I-PSPB binds, then more of the unlabeled PSPB is present and consequently the diagnosis of pregnancy (Sasser *et al.* 1986). Serum estradiol (E2) and progesterone (P4) were measured by the Animal Reproduction and Biotechnology Laboratory at Colorado State University, Fort Collins, CO (80523-1683) (Niswender 1973, Thompson *et al.* 1978). The sex steroid analyses confirmed the PSPB diagnostic test.

RESULTS

Five populations of moose from northern Alaska were serologically tested for the presence of antibody to *Brucella* spp. using the BBA and SPT assays (Table 1 and Figure 1). Three of 94 bulls (3%) and 1 of 69 cows (2%) sampled during 1992-97 in the Noatak River drainage were positive by the BBA and SPT tests (4 of 163 or 2%, Table 2). Eight Colville River cow moose (42 cows and 5 bulls tested) were positive by the BBA test had relatively high titers (titers >400) using SPT (Table 1). No bulls tested positive. Therefore, for the Colville River specifically, 17% of the moose tested were positive and 19% of the female moose tested were positive (Table 1 and 2). No significant antibody titers to *Brucella* spp. were detected in moose from the Seward Peninsula (34 cows), Selawik River (46 bulls, 46 cows) or the ANWR (38 cows, 14 bulls) (Table 1). Four of the 6 positive collared cow moose from the Colville River in 1996 were pregnant based on PSPB test results. All 4 pregnant moose produced calves, 2 of which survived at least through April 1997. Ten calves from 30 collared cows survived through April, 1997; of these, 3 calves were from 3 untested cows, 5 calves were from 21 *Brucella* spp. negative cows, and 2 calves were from the 6 positive cows. During 1997, 2 out of 15 cows from the Colville River area were positive for *Brucella* spp. and pregnant. Of the *Brucella* spp. positive moose from the Noatak River 2 bull moose were killed by hunters, one bull mortality

Table 1. *Brucella* spp. serologic test results for moose from northern Alaska, 1992-1997, for each region by year.

Location/year[1]	#samples	BBA+[2]	SPT+[3]
Noatak River			
1992	57	1	1(400)
1993	17	1	1(400)
1994	16	0	0
1995	52	2	2(400, 100)
1997	21	0	0
TOTAL	163	4 (2%)	4 (2%)
Selawik River			
1994	60	0	0
1996	14	0	0
1997	18	0	0
TOTAL	92	0	0
Seward Peninsula			
1995	24	0	0
1996	10	0	0
TOTAL	34	0	0
ANWR			
1995	52	0	0
Colville River			
1996	32	6 (19%)	6(19%)(>400)
1997	15	2	2 (>400)
TOTAL	47	8 (17%)	8 (17%)

[1] For each year the number (%) of moose positive for antibody to *Brucella* spp. by region, and summarized for all years as TOTAL for each region.

[2] BBA+, number of serum samples positive by buffered *Brucella* antigen card test.

[3] SPT+, number of serum samples positive by standard plate test (titer).

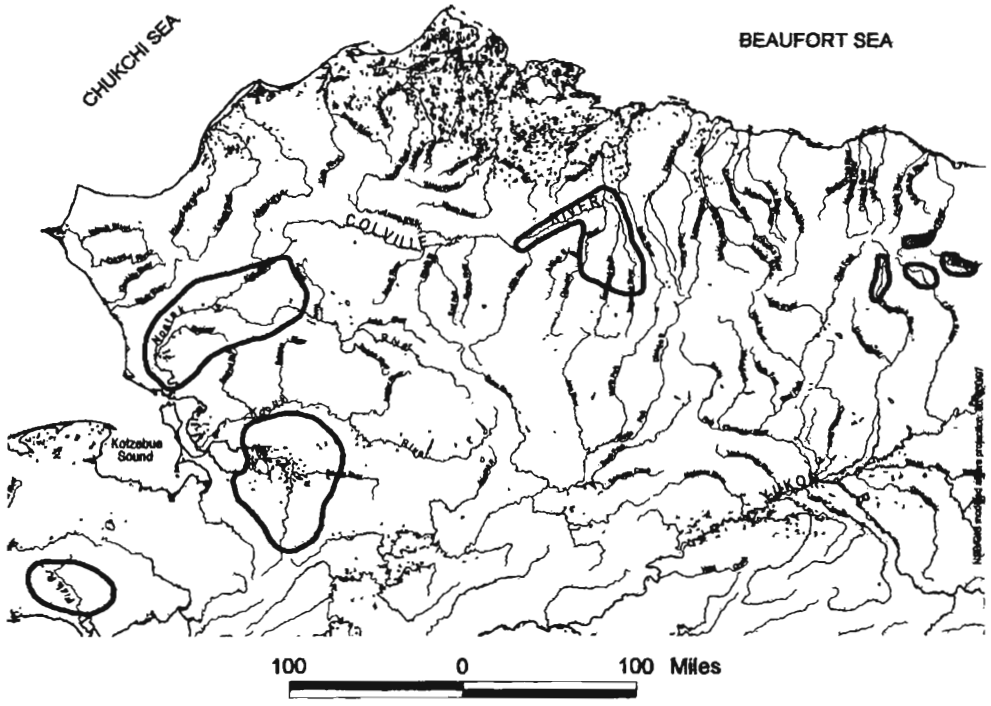

Fig. 1. Areas in Northern Alaska where moose were captured and tested for exposure to *Brucella* spp.

Table 2. Percentage, number (in parentheses), and binomial confidence intervals for prevalence of *Brucella* spp. positive animals by gender for each region based on BBA and SPT tests.

Location/sex	# sampled	% positive[1]	80th	90th	95th
Noatak River/male	94	3.2 (3)	1.2-7.0	0.9-8.0	0.7-9.3
Noatak River/female	69	1.5 (1)	0.2-5.5	0.1-6.7	0.0-7.8
Selawik River/male	46	0			
Selawik River/female	46	0			
Seward P./female	34	0			
ANWR/female	38	0			
ANWR/male	14	0			
Colville R./female	42	19.1 (8)	11.4-29.1	9.8-31.8	8.6-34.1
Colville R./male	5	0			

[1] Based on BBA (positive by buffered *Brucella* antigen card test) and SPT (positive by standard plate test) results as presented in Table 1.

was a suspected wolf predation, and the one cow was alive and showed no evidence of producing a calf. However, calf recruitment has been low in this region for 4-5 years. The 12 positive moose (Colville and Noatak Rivers) detected since 1992 had relatively high titers, which may indicate an ongoing or recent infection as opposed to a historic exposure. Subsequent testing using PCFIA indicated the same 6 Colville River (1996 tested only) moose were positive, and CF indicated that two were anti-complimentary (unable to be tested), 2 were positive, and 2 had insufficient sample to be tested. Western blot analyses of antibodies indicated *B. suis* biovar 4 was the primary agent resulting in the high *Brucella* spp. titer for the Colville River moose. Bacterial culture of *Brucella* spp. from these samples proved unsuccessful, perhaps because a potentially bactericidal anticoagulant (EDTA) had been used for blood collection (citrate or heparin is the preferred anticoagulant for culture of *Brucella* spp). However, the samples were suitable for polymerase chain reaction (PCR) amplification of any *Brucella* spp. genetic material for species identification and have been submitted for testing. No indication of enlarged joints, recent abortions, or other unusual pathology were noted at the time each moose was handled.

DISCUSSION

Antibodies to *Brucella* spp. may be more prevalent in northern Alaska moose populations than previously thought. Previous rates indicated a prevalence rate of 3% (1 out of 39) (Zarnke 1983) and 0.4% (4 out of 1131) (R.L. Zarnke, *unpubl.*) for antibody to *Brucella* spp. in moose statewide. Our results show prevalence rates of 3% and 17% for the moose sampled in the Noatak River and Colville River regions, respectively. Moose from the Noatak and Colville Rivers were not represented in the earlier study by Zarnke (1983). Western blot analysis indicates that *B. suis* biovar 4 was responsible for eliciting these immune responses. This conclusion was based on an evaluation of antibody to both A and M epitopes of the O side chain of the *Brucella* spp.

The role of moose in *Brucella* spp. epizootiology is not well understood. Brucellosis is a zoonotic disease (Chan *et al.* 1989) with public health concerns and proper notification of the public is essential and was accomplished jointly by local wildlife managers and public health officials when the serologic test results were returned. *Brucella abortus* has been found in documented cases of only 5 wild moose between 1937 to the present. Previous reports on *Brucella* spp. in moose are from isolated cases or small sample sizes. *Brucella abortus* was recovered from a moose cohabiting with infected bison (*Bison bison*) in Wood Buffalo National Park, Alberta and Northwest Territories, Canada (Forbes *et al.* 1996). Prior diagnoses of brucellosis were in a young bull moose (Fenstermacher and Olsen 1942) and in a young (3 y) cow moose from Montana (Jellison *et al.* 1953) which showed marked pericarditis and lymphadenitis, and died outright (cultured *Brucella abortus*, agglutination test at 1:20,000). Studies from Elk Island National Park (Edmonton, Alberta), showed 124 seronegative moose (agglutination test), and 2 "sick" moose were positive. The 1.5 y old male was emaciated and had an active, suppurative pneumonitis, pericarditis, peritonitis, and lymphadenitis and the agglutination test was >1:12,800 (strong reactor). A 4.0 y old bull had non-suppurative pneumonitis, pericarditis, and lymphadenitis and was a strong sero-reactor. *Brucella abortus* was cultured from this animal (Corner and Connell 1958). The previous studies documented that *B. abortus* could cause severe disease in free-ranging moose. However,

they provided no indication of prevalence or predisposing factors.

Four moose in a laboratory study were inoculated with *B. abortus* (Forbes *et al.* 1996). All 4 became infected. One developed clinical signs and died. The other 3 were killed for sampling. Serologic test results confirmed exposure. *Brucella abortus* was isolated from lymph node lesions, carpal joints, and lesions associated with pleuritis, peritonitis, and hepatitis. The authors concluded that *B. abortus* in moose will result in a rapid progression of disease culminating in death (Forbes *et al.* 1996). Joint involvement has been speculated to be mostly immune-mediated or requiring the presence of *B. abortus*, however, this is not clear. One moose intentionally inoculated with *B. abortus* developed clinical signs and one week before death the leukocyte count doubled. No other changes were detected until 24 h before death when acute systemic collapse was evident (Forbes *et al.* 1996). Four free-ranging moose have been observed with high titers, and all had severe signs of disease. Therefore these authors (Forbes *et al.* 1996: 103) concluded "the rarity of antibody titers and clinical cases in free-ranging moose is evidence that either moose were rarely exposed to *B. abortus*, methods lack sensitivity for detection, or infected moose die quickly and did not transmit the disease". This was apparently not the case with Noatak and Colville River moose exposed to *B. suis* biovar 4. However, past impacts cannot be assessed and limited monitoring will attempt to describe any pathology or population impacts associated with brucellosis in the future.

A moose experimentally infected with *B. suis* biovar 4 developed a severe, fatal septicemia (Dieterich *et al.* 1991). This experiment indicated that serum antibody peaked day 21 to 56, with clinical signs appearing on day 42. There were edematous lymph nodes, an enlarged and friable spleen, and following euthanasia *B. suis* was isolated from many tissues. This case and those reported by Honour and Hickling (1993) suggest that *B. suis* biovar 4 can cause chronic and severe manifestations of disease in free-ranging moose. However, no epizootiologic conclusions or theories could be developed from isolated cases relating *Brucella* spp. exposure and potential adverse health effects to populations of moose.

Many have speculated that moose are very susceptible to brucellosis (severe disease) and that individuals die before transmitting the bacteria to other moose, thus resulting in a very low antibody prevalence in a population (Corner and Connell 1958, Hudson *et al.* 1980, Dieterich 1981, Zarnke 1983, Dieterich *et al.* 1991). Based upon our findings this may not be true for *B. suis* biovar 4 in 6 cow moose we have monitored since April, 1996. All 6 cows have survived for one year, 4 of them produced viable calves and 2 of these calves have survived the winter (April, 1997). However, in combination with another stressor (malnutrition, genetic predisposition, large parasite burden, etc.) *Brucella* spp. may cause pathology as described above.

Reindeer or caribou may be the ultimate source of exposure for these *Brucella* spp. antibody positive moose. Transmission by contact from reindeer to cattle and other species has been documented to occur for *B. suis* biovar 4 (Rausch and Huntley 1978, Morton 1986, Forbes and Tessaro 1993); however, the authors caution that under natural conditions there may be a higher degree of specificity for *Rangifer* (Rausch and Huntley 1978). Serologic reactions to *B. suis* biovar 4 were detected in red foxes (*Vulpes vulpes*), arctic foxes (*Alopex lagopus*), grizzly bears (*Ursus arctos*), and arctic ground squirrels (*Spermophilus parryi*) in areas of high prevalence for reindeer. Reindeer confined with infected

foxes became infected (Morton 1986). *Brucella suis* biovar 4 has been isolated from a wild muskox (*Ovibos moschatus*) with bursitis (Gates *et al.* 1984) from the District of Keewatin, Northwest Territories, and from red and arctic foxes of Alaska (Morton 1986). Direct or indirect (i.e. predators) transmission should be considered as a potential path from *Rangifer* to moose for *B. suis* biovar 4. However, considering the high prevalence of brucellosis in reindeer on the Seward Peninsula it would be expected that moose from this region would have evidence (0 of 34 in our study) of *B. suis* biovar 4 exposure if this *Rangifer* source was the key. If caribou were the key, then we should not see a difference in prevalence rates for the Colville, Noatak and Selawik Rivers as these regions are all within the range of the Western Arctic and Teshekpuk Lake (some years) caribou herds.

Many authors have discussed the origin of *B. suis* biovar 4 in reindeer and two theories have been postulated (Rausch and Huntley 1978) regarding its establishment in North America. The first is that the organism was introduced by reindeer shipped from Siberia at the end of the last century. The second considers *B. suis* biovar 4 to be holarctic dating from faunal exchanges across Beringea during late Pleistocene time. This holarctic distribution is consistent with known arctic parasite-host assemblages that are identical on both continents and would have occurred as a intercontinental dispersal of host-specific parasites (including microbes) (Rausch 1972, Rausch and Huntley 1978). Comparisons of *Brucella* spp. isolates from reindeer in North America and Eurasia have shown a single biotype (biovar) is involved. Using standardized methods, the isolates were designated as *Brucella suis* type (biovar) 4 (Meyer 1966). Questions remain as to whether this organism has been recently introduced to moose of northern regions (or moose hosts introduced to an enzootic area), enzootic to them, or an anomaly of our sampling. Work will continue to better characterize prevalence rates, pathologic changes and potential population impacts in moose of northern Alaska.

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