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# EXPERIMENTAL STUDIES ON *BRUCELLA ABORTUS* IN MOOSE (*ALCES ALCES*)

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ABSTRACT: Four moose (*Alces alces*) were inoculated conjunctivally with *B. abortus* biovar 1 to determine their susceptibility to brucellosis, and to describe the serology, bacteriology, hematology, clinical chemistry, and pathology associated with infection. All moose became infected. Two moose were killed at day 70 post-exposure, one (83F) died acutely at day 85, and one was killed at day 166. None of the moose had clinical signs, except for 83F immediately before death. Infected moose were readily detected serologically by the buffered antigen plate test, Brewer card test, standard tube agglutination test, and complement fixation test as used for brucellosis in cattle. With the exception of samples from 83F taken 24 hours before death, clinical chemistry, and hematology results were stable for all moose, and similar to normal values seen in cattle. Lesions seen in all moose were indicative of endotoxemia, and moose 83F died of acute endotoxic shock. *Brucella abortus* biovar 1 was isolated from several tissues in all moose, most notably from lymph nodes where counts often exceeded $4 \times 10^4$ colony forming units per g of tissue. Thus infection with *B. abortus* will kill moose, and progression of the disease is likely rapid under field conditions. Moose appear to be a dead-end host for brucellosis.

*Key words:* Brucellosis, *Brucella abortus*, moose, *Alces alces*, experimental infection, pathogenesis, endotoxemia.

## INTRODUCTION

The role of moose (*Alces alces*) in the epizootiology of bovine brucellosis is unclear. Significant antibody titers to Brucellae among free-ranging moose populations in North America have not even been found in areas where moose could come in contact with infected cattle (Diesch et al., 1972; Hudson et al., 1980; Bourque and Higgins, 1984). *Brucella abortus* has been recovered from only four wild moose between 1937 and 1985 (Fenstermacher and Olsen, 1942; Jellison et al., 1953; Corner and Connell, 1958; Tessaro, 1988). In three of the four reported cases, moose were associated with infected primary hosts for *B. abortus*, either cattle (Fenstermacher and Olsen, 1942) or bison (*Bison bison*) (Corner and Connell, 1958; Tessaro, 1988). The four infected moose were described as thin, weak, and in some cases with abnormal behavior. The most frequently observed pathology included lymphadenitis, pleuritis, peritonitis, pericarditis, myocarditis, orchitis, hepatitis, and nephritis. Lesions reported on a singular basis included congested meninges, splenic congestion, pulmonary adhesions (Fenstermacher and Olsen, 1942), and pulmonary congestion (Corner and Connell, 1958). *Brucella abortus* was isolated from numerous tissues and body fluids including heart blood, liver, lung, kidney, spleen, and testicle (Fenstermacher and Olsen, 1942), various lymph nodes (Fenstermacher and Olsen, 1942; Tessaro, 1988), and pleural and pericardial fluid (Jellison et al., 1953). All three moose which were tested had strong seroconversion on tests used for brucellosis in cattle (Fenstermacher and Olsen, 1942; Jellison et al., 1953; Corner and Connell, 1958). Thus brucellosis may be a fatal disease in moose, but experimental information on the significance of brucellosis in moose is lacking. Interactions between the moose,



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their environment, and various other infectious agents could not be ruled out as factors contributing to the clinical signs and lesions observed. In the cases reported, debilitation may have been a prerequisite for infection. The recovery of *B. abortus* from a moose cohabiting range with infected bison in Wood Buffalo National Park (58°05′ to 60°40′N, 111°05′ to 115°30′W), Alberta and Northwest territories, Canada (Tessaro, 1988) has raised questions regarding the epizootiological significance of moose in programs to control the disease in the region.

Our objective was to confirm the susceptibility of moose to infection by *B. abortus*, to determine the clinical effects, and to describe the associated serology, bacteriology, hematology, clinical chemistry, and pathology of brucellosis in moose.

## MATERIALS AND METHODS

Five captive-reared adult moose obtained from the University of Alberta, Edmonton, Alberta, Canada were used in this study. The three year old animals included three females (74F, 83F, 84F) and two males (77M, 80M). They were housed in a biocontainment facility for the duration of the experiment. The moose were divided into three groups in separate rooms: moose 77M and 80M (infected); 74F and 83F (infected); and 84F (control). A pelleted deer ration (Welch et al., 1989), good quality alfalfa hay, and freshly cut willow (Salix sp.) browse were fed ad libitum. Rooms were cleaned daily. Observations were made without disturbing the animals by viewing them through windows from an elevated observation deck. Moose were observed a minimum of twice in the morning, twice in the afternoon, and twice in the evening on week days, and three times daily on weekends. Xylazine (Rompun®. Bayvet Division, Chemagro Limited, Etobicoke, Ontario, Canada.) administered intramuscularly at a dose of 0.3 mg/kg body weight, was used as necessary to provide restraint for inoculation, blood sampling, and euthanasia. Sodium pentobarbitol (Euthanyl Forte®. M.T.C. Pharmaceuticals, Canada Packers Inc., Cambridge, Ontario.) administered intravenously was used for euthanasia at a dose of 100 mg/kg body weight.

*Brucella abortus* biovar 1, isolated from a bison in Wood Buffalo National Park, was used to challenge moose 83F, 74F, 77M and 80M. Each moose received $2.68 \times 10^8$ organisms administered by placing 50 µl of *Brucella* sp. suspension in each conjunctival sac, and holding the eyes closed for 3 min. Control moose 84F received identical volumes of 0.85% physiological saline in each conjunctival sac.

Blood samples were taken from each moose before infection, and weekly thereafter until day 63 post-inoculation (PI), after which they were bled approximately every 2 wk and at postmortem. Fresh feces were collected daily. Moose 77M, and 80M were killed on day 70 PI; moose 74F, and 84F on day 166 PI. Moose 83F died on day 85 PI. Tissues with visible abnormalities, all major body lymph nodes, and samples of organs were collected at necropsy (Table 1). Additional specimens included urine (77M, 80M, 83F), seminal vescicles (77M, 80M), and synovial fluid from the left carpal (77M, 80M, 83F, 74F), coxofemoral (84F), and hock joints (77M, 80M). Specimens for bacterial culture were frozen at −20 C immediately after collection. A portion of each tissue was fixed in 10% neutral buffered formalin.

Tissues for bacterial culture were trimmed of fat and connective tissue, dipped in ethanol, flamed, incised several times with sterile scissors or a scalpel, and placed in a sterile Stomacher bag (A. J. Seward, Bury St Edmunds, Suffolk, England.) with an equal volume of 0.85% physiological saline. Samples were homogenized in a reciprocating paddle blender (Model 80 or 400 Stomacher® blender, A. J. Seward) for 2 to 5 min depending on sample type. A 250 µl aliquot of homogenate was inoculated onto each plate. The inoculum was spread evenly over the entire surface of the plate. Blood clots also were homogenized and spread onto plates in an identical fashion, but without added saline. Fluid samples, such as urine and joint fluid, were also spread onto plates in 250 µl aliquots. Swabs were applied directly to plates. The inoculum from each tissue was placed onto: two 5% sheep blood agar plates (Trypticase Soy Agar, BBL, Becton Dickenson and Company, Cockeysville, Maryland, USA) custom poured in our laboratory; two plates of the Kuzdas and Morse formulation of *Brucella* medium (Alton et al., 1975), one with ethyl violet (J. T. Baker Chemical Co., Phillipsburg, New Jersey, USA) (1:800,000) and one without; and two plates of the U.S. Department of Agriculture (USDA) formulation of *Brucella* medium (Alton et al., 1975), one with ethyl violet (1:800,000) and one without. Blood clots were cultured using two or three plates of USDA medium with ethyl violet and two or three plates without, depending upon the volume of homogenized clot available. Urine, uterine fluid, and cerebrospinal fluid were cul-

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TABLE 1. Location and number[a] of *B. abortus* organisms for experimentally infected moose.

| Source | Moose | | | |
|---|---|---|---|---|
| | 77M | 80M | 83F | 74F |
| Mandibular ln[b] | 8,000 | 72,000 | 12,700 | 47,300 |
| Parotid ln | 40,000 | 80,000 | 50,300 | 30,300 |
| Retropharyngeal ln | 8,000 | 56,000 | 18,800 | 48,000 |
| Suprascapular ln | 8,000 | 64,000 | 77,000 | 46,000 |
| Prefemoral ln | 8,000 | 96,000 | 14,600 | 46,600 |
| Supramammary ln | NA[c] | NA | 19,300 | 45,200 |
| Superficial inguinal ln | 8,000 | 40,000 | NA | NA |
| Popliteal ln | 8,000 | 56,000 | 78,300 | 58,900 |
| Mesenteric ln | 400 | 800 | 1,560 | 272 |
| Hepatic ln | 40,000 | 40,000 | 27,800 | 56,000 |
| Bronchial ln | 40,000 | 56,000 | 13,600 | 59,300 |
| Internal iliac ln | 8,000 | 40,000 | 42,900 | 50,100 |
| Lumber ln | 800 | 176 | 16,700 | —[d] |
| Renal ln | 800 | 800 | 3,616 | 56,000 |
| Axillary ln | 1,600 | — | — | — |
| Tracheal ln | — | — | 15,800 | — |
| Mediastinal ln | — | — | 3,656 | 74,800 |
| Reticular ln | — | — | 944 | — |
| Ileocecal ln | — | — | 1,064 | — |
| Palatine tonsil | — | — | 800 | — |
| Liver | 800 | 400 | 288 | — |
| Spleen | 8,000 | 8,000 | 58,392 | 15,600 |
| Lung | 16 | 40 | 24 | — |
| Kidney (pooled L and R) | 8 | 16 | 52 | 168 |
| Bladder | 0 | 0 | 0 | 24 |
| Testicle | 24 | 16 | NA | NA |
| Uterus | NA | NA | 16 | 32 |
| Ovary | NA | NA | 8 | — |
| Udder | NA | NA | 8 | 160 |
| Brain | 0 | 0 | 56 | — |
| Cerebrospinal fluid | — | — | <8 | — |
| Adrenal gland | — | — | 16 | — |
| Peritoneal fluid | — | — | 8 | 0 |
| Left carpal bursal fluid | — | — | — | 4,192 |
| Left carpal synovium | — | — | — | 3,088 |
| Right carpal joint fluid | 0 | 0 | 4 | 612 |
| Right carpal bursal fibrin | — | — | — | 400 |
| Right carpal synovium | — | — | 8 | — |
| Stifle joint fluid | 0 | 0 | 4 | — |

[a] Colony forming units per g of tissue or per ml of fluid.
[b] Ln = lymph node.
[c] NA = not applicable.
[d] Dash (—) indicates the specimen was not cultured.

tured on the same media as tissues; as was joint fluid, and joint swabs for 83F, 74F and 84F. Joint swabs from 77M and 80M were inoculated onto three 5% sheep blood agar plates, and three tryptose (Difco Laboratories, Detroit, Michigan, USA) agar plates containing 1% dextrose (Difco) and 5% bovine serum (Cansera, Rexdale, Ontario, Canada). Fecal samples were frozen at −20C, thawed, mixed with an equal volume of sterile 0.85% physiological saline, and then homogenized and inoculated onto plates as described for tissues. Six plates of Farrell's medium (Robertson et al., 1980) were used for culture. Formalin-fixed tissues were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for light microscopy. The buffered antigen plate test (BAPT), the Brewer card test (BCT), the standard tube agglutination test (STAT), and the complement fixation test (CFT) were per-

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formed on all sera as described by Stemshorn et al. (1985). Visible agglutination on the BAPT and BCT was interpreted as positive. Sera for the STAT and CFT were titrated to their endpoints.

The white blood cell count (WBC), red blood cell count (RBC), hemoglobin concentration (HGB), packed cell volume (PCV), mean red cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), coefficient of variation in red cell size (RDW), platelet count (PLT), packed platelet volume (PCT), mean platelet volume (MPV) and coefficient of variation in platelet size (PDW) were determined using a Model S Plus IV hematology system (Coulter Electronics Inc., Hialeah, Florida, USA). Red blood cell morphology, platelet morphology, white blood cell differential counts and fibrinogen were determined following procedures described by Jain (1986).

Serum sodium, potassium, chloride, carbon dioxide, calcium, phosphorus, magnesium, urea, creatinine, glucose, total bilirubin, alkaline phosphatase (ALP), creatinine phosphokinase (CK), aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), as well as total protein, and albumin were measured using the Discrete Analyzer with Continuous Optical Scanning (DACOS system) (Coulter Electronics Inc.) with anion gap. The albumin to globulin (A/G) ratio was calculated from these values.

## RESULTS

Moose 77M and 80M were killed on day 70 PI, moose 83F died on day 85 PI, and moose 74F and control moose 84F were killed on day 166 PI. At the time of death, moose 74F and 84F appeared thinner than the others, but at necropsy all moose had extensive subcutaneous and internal fat deposits. All moose except 83F were clinically normal for the entire experiment. The right conjunctiva of moose 83F became hyperemic 48 hr before death; 24 hr later, 83F was depressed, her mucous membranes were congested and brick red in color, and rectal temperature was 40.5 C. The animal was weak, but remained ambulatory, and drank large amounts of water; 12 hr before death, 83F was reluctant to rise, showed no resistance to handling, and appeared oblivious to her surroundings. Rectal temperature was 40.7 C, respiration 100, and heart rate 24. Blood samples taken at this time clotted quickly, but showed poor clot retraction. Two hours before death, 83F was moribund and in sternal recumbency. The animal died before euthanasia could be carried out.

The most severe pathological signs were observed in 83F (died acutely) and 74F (longest period of infection). Less advanced lesions were present in moose 77M and 80M. Control moose 84F had no significant lesions, but did have a low level lungworm infection.

The most consistently observed lesions were in lymph nodes. The moose killed at 70 days PI (77M, 80M) had grossly normal lymph nodes, but histologically there was generalized mild to moderate follicular hyperplasia with low numbers of giant cells in the sinusoids and subcapsular sinuses. Sinus histiocytosis was present in 77M. The moose killed at 166 days PI (74F) and the moose that died acutely (83F) had marked lymph node enlargement due to severe follicular hyperplasia and edema. In addition, the lymph nodes of 83F were congested, with fibrin deposits within the distended subcapsular sinus and medullary sinusoids, foci of hemorrhage and cortical necrosis, thrombi, and increased numbers of macrophages and giant cells.

The presence of fibrin and fibrous tags was related to duration and severity of infection. Moose 77M did not have this type of lesion. Moose 80M had a few fibrous tags on the pleural surface of lungs, diaphragm and liver. Moose 74F had fibrin on the the pulmonary pluera, liver, intestines and synovial membrane of the left carpal joint as well as fibrous tags on the colon, uterus and lung. Moose 83F had the most extensive involvement. There was fibrin and fibrous tags on the pulmonary pluera, omentum, liver and other abdominal viscera, as well as fibrin on the synovium of both carpal joints, with fibrosis beneath.

Liver lesions were also associated with duration and severity of infection. Moose

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77M did not have liver lesions. Grossly, moose 80M had a few fibrous tags on the liver surface, moose 74F had foci of necrosis and hemorrhage on the capsule, and moose 83F had an enlarged liver with foci of necrosis on the capsule. Histologically, moose 80M, 74F, and 83F had multiple small foci of necrosis in the hepatic parenchyma and cuffs of marcrophages and lymphocytes around the portal triads. These changes were most severe in moose 83F.

Two of the moose had joint lesions. Moose 83F had an organized, subcutaneous hematoma over the cranial aspect of the right carpal joint and a lesser degree of subcutaneous hemorrhage over the the cranial aspect of the left joint. The synovial fluid in the right joint was amber colored. The synovium of both joints had fibrin on the surface, foci of hemorrhage, and fibrosis beneath, but relatively few inflammatory cells. Moose 74F had a swelling over the cranial aspect of the right carpus which consisted of dense fibrous connective tissue. The left carpus had an increased amount of clear, yellow, viscid fluid in the joint, as well as the bursae and sheaths of the extensor tendons. Grossly, the synovium was hyperemic with marked villus hypertrophy and pannus formation. Microscopically, there was villus hypertrophy with some necrosis, infiltration with large numbers of plasma cells, and organized fibrin on the synovial surface. There was granulation tissue, hemorrhage, and numerous hemosiderin-laden macrophages in the sub-synovial connective tissue.

Moose 83F had additional lesions at necropsy that were not seen in the other infected moose. Mucous membranes were cyanotic. There was marked injection of the blood vessels in the serosa of the intestines and colon, mesentery, omenta, mucous membranes, and meninges. There were petechiae, a few small granulomas, and pervascular cuffs of mononuclear cells on the greater omentum. Grossly, the lungs were wet and rubbery with an irregular, lobular pattern of congestion. There was edema fluid in some alveoli and thrombi in some pulmonary vessels. The intestine and colon contained brown, watery fluid.

The clinical chemistry results for individual moose were remarkably stable (Table 2). Variability within each test range for each moose was small (coefficient of variation $\leq$20%) except for total bilirubin, ALP, CK, AST and GGT. The only consistent difference between the three infected moose and the control moose was a moderate elevation of urea and creatinine in the infected moose. Glucose values for all five moose usually were above the normal bovine range, while AST results were usually lower. Results were either within acceptable limits for cattle or very close to them.

Moose 83F had a sudden change in values 24 hr before death as compared to the stable status of the previous 11 tests. Except for an increase in GGT from $\leq$ 20 U/l to $\geq$ 41 U/l during the preceding two weeks, clinical pathology did not predict the onset of clinical signs or death. Total bilirubin, CK, AST, GGT and total protein showed abrupt increases 24 hr before death with values of 7 mmol/l, 242 U/l, 444 U/l, 79 U/l and 92 g/l respectively. At death, values had increased further to 21 mmol/l, 11930 U/l, 670 U/l, 229 U/l and 109 g/l, respectively. Twenty-four hours before death, urea (9.2 mmol/l) increased to the upper portion of the previously defined range, while creatinine (251 µmol/l) increased slightly above this range, and calcium (2.51 mmol/l), $CO_2$ (14 mmol/l) and ALP (5 U/l) decreased below it. At death, ALP (18 U/l) had increased to near the upper range limit, and calcium (3.33 mmol/l) to slightly above the limit. $CO_2$ (6 mmol/l), and glucose (1 mmol/l), at death, were well below the range. Phosphorus (5.2 mmol/l), magnesium (2.57 mmol/l), urea (16.9 mmol/l), creatinine (502 µmol/l), and potassium (29.1 mmol/l) had increased to above the range at the time of death.

The range of hematology results was similar for all moose (Table 3). During the

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TABLE 2. Clinical chemistry values for four moose infected with *B. abortus*, one noninfected control moose, and normal cattle.

| Test | Cattle[a] (normal) | Moose[b] 84F control ($n$ = 17) | 77M ($n$ = 11) | 80M ($n$ = 11) | 74F ($n$ = 18) | 83F[c] ($n$ = 11) |
|---|---|---|---|---|---|---|
| Sodium (mmol/l) | 135–151 | 136–144 | 134–142 | 134–142 | 135–147 | 134–141 |
| Potassium (mmol/l) | 3.90–5.90 | 2.9–6.4 | 2.97–7.41 | 3.02–7.14 | 3.30–5.90 | 2.39–7.81 |
| Chloride (mmol/l) | 96–110 | 95–105 | 92–103 | 89–101 | 95–105 | 93–101 |
| Carbon dioxide (mmol/l) | 20–32 | 24–35 | 24–36 | 26–42 | 25–34 | 25–35 |
| Anion gap (mmol/l) | 14–26 | 7–23 | 8–23 | 0–27 | 9–22 | 8–25 |
| Calcium (mmol/l) | 2.11–2.75 | 2.26–2.96 | 2.51–3.05 | 2.37–2.78 | 2.43–3.18 | 2.56–2.93 |
| Phosphorus (mmol/l) | 1.08–2.76 | 0.49–2.30 | 0.94–2.25 | 1.10–2.16 | 0.39–1.94 | 0.59–2.64 |
| Magnesium (mmol/l) | 0.80–1.32 | 0.46–1.25 | 0.73–1.21 | 0.67–1.33 | 0.47–1.38 | 0.67–1.31 |
| Urea (mmol/l) | <7.5 | 3.3–8.8 | 6.1–15.7 | 6.1–13.6 | 3.4–10.8 | 1.8–10.9 |
| Creatinine (μmol/l) | 67–175 | 112–152 | 245–321 | 177–262 | 154–325 | 165–247 |
| Glucose (mmol/l) | 1.80–3.80 | 2.40–7.69 | 2.83–8.86 | 2.12–7.68 | 5.35–8.07 | 3.75–8.19 |
| Total bilirubin (mmol/l) | 0–30 | 0–7.3 | 0–5.9 | 0–3.6 | 0–7.7 | 0–3.5 |
| Alkaline phosphatase (U/l) | <121 | 5.1–21.9 | 5.6–22.9 | 7.2–24.7 | 2.9–18.9 | 6.0–20.0 |
| Creatinine kinase (U/l) | <350 | 0–219 | 0–250 | 0–234 | 0–387 | 0–263 |
| Aspartate aminotransferase (U/l) | 46–118 | 19.4–56.8 | 19.0–53.3 | 11.9–59.3 | 29.4–62.2 | 0–133 |
| Gamma glutamyl transferase (U/l) | <31 | 0–25.0 | 5.0–18.9 | 5.7–12.3 | 4.4–16.9 | 0–46.2 |
| Total protein (g/l) | 66–78 | 62–81 | 65–79 | 62–72 | 69–92 | 73–84 |
| Albumen (g/l) | 23–43 | 20–33 | 29–35 | 26–29 | 24–44 | 27–35 |
| Albumen : globulin ratio | 0.66–1.30 | 0.46–0.73 | 0.68–0.94 | 0.61–0.78 | 0.33–1.26 | 0.53–0.79 |

[a] The values for normal cattle ($n$ > 100) were determined for each test protocol by the Department of Clinical Pathology, Western College of Veterinary Medicine, Saskatoon, Saskatchewan, Canada.

[b] Range limits were defined as the mean ± 2 SD which accounted for >94% of results. Outlyers are marginally outside this range.

[c] Results for 83F do not include samples taken 24 hours before death or at death.

course of the experiment, moose 83F had a slightly higher total WBC count than moose 84F. One week prior to the onset of clinical signs, moose 83F had a rise in WBC count from 6.2 to 11.5 × $10^9$ cells/l. This was still within the normal range for cattle, and the relative proportion of cells in the differential count remained unchanged. There were no other consistent differences separating the negative control (84F), or the moose that died acutely (83F), from the other three moose. Moose 83F, 24 hr before death, had a decrease in the total WBC count from 11.5 to 4.0 × $10^9$ cells/l. The actual decrease may have been greater; a rise in the total RBC count (8.7 × $10^{12}$/l), PCV (0.57 l/l) and total protein (98 g/l) was evidence that some degree of dehydration was present. Relative proportions of WBC's remained close to previous values. The percentage of segmented neutrophils rose from 43% to 47%, and lymphocytes decreased from 54% to 42%. The difference was primarily made up by an increase in band neutrophils from 0% to 8%.

*Brucella abortus* biovar 1 was first recovered from blood clots on day 29 PI in moose 83F and 74F, and on day 43 PI in moose 80M and 77M. Initial isolations consisted of less than one colony forming unit (CFU) per ml of clot material. Thereafter, a *Brucella* sp. bacteremia was present in all infected moose for the duration of the experiment. Counts ranged from 1 to 26 CFU/ml of clot material.

*Brucella abortus* biovar 1 was present in high numbers in lymphoid tissue and was isolated from all lymph nodes (LN) collected from each infected moose (Table 4). Moose 80M averaged over 46,000 CFU/g of tissue from 13 LN (range 176 to

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TABLE 3. Hematology values for four moose infected with *B. abortus*, one noninfected control moose, and normal cattle.

| Test | Cattle[a] (normal) | Moose[b] 84F control ($n$ = 14) | 77M ($n$ = 10) | 80M ($n$ = 10) | 74F ($n$ = 15) | 83F[c] ($n$ = 10) |
|---|---|---|---|---|---|---|
| White blood cells ($10^9$/l) | 4–12 | 4.2–9.5 | 3.3–8.5 | 4.1–5.6 | 3.9–8.3 | 4.8–11.6 |
| Red blood cells ($10^{12}$/l) | 5–10 | 4.1–5.2 | 3.3–5.5 | 3.8–4.6 | 3.6–5.0 | 3.9–5.5 |
| Packed cell volume (l/l) | 0.23–0.46 | 0.26–0.32 | 0.22–0.32 | 0.25–0.29 | 0.25–0.37 | 0.25–0.36 |
| Segmented neutrophils ($10^9$/l) | 0.6–4 | 2.1–6.0 | 0.6–5.2 | 1.5–2.9 | 1.4–5.1 | 1.7–6.1 |
| Segmented neutrophils (%) | 15–45 | 43–74 | 28–67 | 33–58 | 34–70 | 33–60 |
| Band neutrophils ($10^9$/l) | 0–0.12 | 0 | 0.04[d] | 0 | 0 | 0.18[d] |
| Band neutrophils (%) | 0–2 | 0 | 1[d] | 0 | 0 | 3[d] |
| Metamyelocytes ($10^9$/l) | 0 | 0 | 0 | 0 | 0 | 0 |
| Eosinophils ($10^9$/l) | 0–2.4 | 0–0.56 | 0.03–0.71 | 0–0.83 | 0.03–0.08[e] | 0–0.51 |
| Eosinophils (%) | 2–20 | 0–8 | 0–14 | 0–17 | 1[e] | 0–6 |
| Basophils[f] ($10^9$/l) | 0–0.2 | 0.05–0.09 | 0.04–0.08 | 0.04 | 0.16 | 0.04–0.09 |
| Basophils[f] (%) | 0–2 | 1 | 1 | 1 | 2 | 1 |
| Monocytes[g] ($10^9$/l) | 0.03–0.84 | 0.11–0.69 | 0–0.51 | 0–0.45 | 0.03–0.24 | 0–0.54 |
| Monocytes[g] (%) | 2–7 | 1.9–10.5 | 0–7.6 | 0.4–8.9 | 0.3–4.6 | 0–7.2 |
| Lymphocytes ($10^9$/l) | 2.5–7.5 | 1.0–3.3 | 1.6–3.3 | 1.5–2.7 | 1.6–3.9 | 1.9–5.7 |
| Lymphocytes (%) | 45–75 | 18–45 | 23–62 | 29–58 | 27–64 | 32–61 |
| Hemoglobin (g/l) | 80–150 | 88–113 | 76–109 | 84–102 | 89–130 | 89–125 |
| Mean corpuscular volume (fl) | 40–60 | 61–64 | 61–62 | 61–65 | 69–77 | 65–66 |
| Mean corpuscular hemoglobin (Pg) | 11–17 | 20–23 | 21–22 | 21–23 | 24–27 | 23 |
| Mean corpuscular hemoglobin concentration (g/l) | 300–600 | 324–368 | 341–355 | 337–360 | 324–380 | 340–358 |
| Coefficient of variation in red cell size (%) | 17–23 | 12–22 | 18–19 | 11–23 | 9–26 | 16–18 |
| Metarubricytes ($10^9$/l) | 0 | 0 | 0 | 0.04[h] | 0 | 0.06[h] |
| Metarubricytes (%) | 0 | 0 | 0 | 1[h] | 0 | 1[h] |
| Platelets ($10^9$/l) | 100–800 | 188–415 | 136–293 | 166–469 | 89–257 | 126–312 |
| Total protein (g/l) | 57–81 | 69–78 | 61–77 | 62–72 | 67–86 | 72–81 |
| Fibrinogen (g/l) | <6 | 2.2–8.3 | 0–5.8 | 2.0–6.9 | 0–6.4 | 1.0–6.4 |

[a] The values for normal cattle ($n$ > 100) were determined for each test protocol by the Department of Clinical Pathology, Western College of Veterinary Medicine, Saskatoon, Saskatchewan, Canada.

[b] Range limits were defined as the mean ± 2 SD, which accounted for >95% of results. Outlyers were marginally outside of this range.

[c] Results for 83F do not include samples taken 24 hours before death or at death.

[d] Band neutrophil results for 77M and 83F are each based on a single observation.

[e] Eosinophil results for 74F are based on five observations.

[f] Basophil results for 84F are based on two observations; 77M, three; 80M, two; 74F, one; 83F, four.

[g] Monocyte results for 84F are based on 12 observations; 77M, six; 80M, eight; 74F, nine; 83F, eight.

[h] Metarubricyte results for 80M and 83F are each based on a single observation.

>96,000; SD = 30,617), moose 77M over 12,000 CFU/g from 14 LN (range 400 to >40,000; SD = 15,058), moose 83F over 22,000 CFU/g from 18 LN (range 800 to >78,000; SD = 24,487), and moose 74F over 46,000 CFU/g from 13 LN (range 272 to >59,000; SD = 17,245).

Moose 80M and 77M had normal joints at necropsy; *B. abortus* was not isolated from carpal, stifle, or hock joint fluid. Moose 83F and 74F had carpal joint abnormalities at necropsy, although neither moose had been lame. *Brucella abortus* was present at less than 4 CFU/ml in carpal and stifle fluid of 83F. Synovial membrane from the right carpus of this moose had <8 CFU/g of tissue, while a hematoma from the same joint contained 12 CFU/ml about the same count observed in clotted vascular blood. Moose 74F lived 81 days longer than 83F, and had more pronounced carpal joint lesions at necropsy. A

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swab of synovial fluid from the left carpal joint was negative, but bursal fluid contained over 4,000 CFU/ml, and synovium over 3,000 CFU/g of tissue. Synovial fluid from the right carpal joint contained 612 CFU/ml, and fibrin from the carpal swelling contained 400 CFU/ml.

Culture of postmortem urine from 80M, 77M, and 83F was negative. There was no urine present in the bladder of 74F at the time of necropsy. Moose 74F, 84F, 80M, 77M, and 83F had 54, 54, 54, 52, and 50 fecal samples cultured, respectively; all were negative for *B. abortus*.

There was no histologic evidence of viral infection in any of the moose. Routine virus isolation procedures were done on lung, liver, and kidney tissue from moose 83F, and were negative.

The BAPT detected antibodies to *B. abortus* earlier than the BCT. It was positive at 15 and 21 days PI in moose 74F and 83F respectively, and at 29 days PI in 80M and 77M. The BCT was positive at 21 days PI in 74F, 29 days PI in 83F and 80M, and 35 days PI in 77M. Once seroconversion occurred, all four infected moose remained positive on both tests for the duration of the experiment.

Anti-*Brucella* antibody was detected 1 to 3 wk earlier with the STAT than the CFT. The STAT and CFT titers rose rapidly and, without exception, were maintained at very high levels for the rest of the experiment. The earliest STAT reactions occurred at day 15 PI in moose 77M, 80M and 74F. They were ≤31 IU; this is well below the positive level of ≥125 IU set for cattle. By day 35 PI all moose except 80M were positive, and by day 43, 80M was positive as well. At death, 77M, 80M, 83F and 74F had 1,000, 1,000, 2,000 and 8,000 IU of anti-*Brucella* antibody in their sera, respectively. The earliest CFT titer was 1/10 observed in 74F on day 21 PI. A reaction of 1/10 is significant using criteria for cattle. By day 35 PI, 77M, 83F and 74F had titers of 1/5, 1/100, 1/100, respectively. Moose 80M did not have a CFT titer until day 43, at which time it was 1/10. At death, 77M, 80M, 83F and 74F had titers of 1/200, 1/200, 1/800 and 1/1,600, respectively.

## DISCUSSION

*Brucella abortus* biovar 1 was distributed widely in the tissues of infected moose. In the absence of other possible causes, an endotoxemia associated with the massive systemic load of *B. abortus* accounted for the clinical signs in 83F and lesions observed in all four moose. The sudden death and lesions in 83F were consistent with acute endotoxic shock (Cheville, 1988). It was remarkable that the lesions in 77M, 80M and 74F were not more severe considering they had tissue loads of *B. abortus* similar to that of 83F. This was probably due to differences in individual susceptibility. The bacterial load, progressive lesions, and absence of a protective response were evidence that these moose would have developed more severe lesions if the experiment had been extended.

Lymphoid tissue contained very high numbers of *B. abortus*; thus concentration and multiplication of the bacteria were occurring faster than killing and clearance by the host. Lesions were present in the lymph nodes of all four infected moose, and progressed over time. Lymph node lesions were the most frequent abnormalities reported in previous field cases (Fenstermacher and Olsen, 1942; Jellison et al., 1953; Corner and Connell, 1958; Tessaro, 1988).

The pleural lesions were compatible with a Gram-negative septicemia resulting in endotoxin-mediated damage to endothelial cells and subsequent fibrin deposition. Similarly, liver lesions were characteristic of those seen in a number of infectious processes including bacterial septicemia (Cheville, 1988; Kelly, 1985) The pleuritis and hepatitis were attributed to brucellosis because of the high numbers of *B. abortus* present, and the absence of other pathogenic microorganisms. Pleuritis and peritonitis, varying in degree, were previously described in three cases of *B.*

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*abortus* infection in wild moose (Fenstermacher and Olsen, 1942; Corner and Connell, 1958; Tessaro, 1988).

*Brucella abortus* was recovered from the carpal joints of moose 83F, and 74F. Joint lesions have been associated with *B. abortus* in cattle and bison, and with *B. suis* biovar 4 in reindeer and caribou (Blood and Radostits, 1989; Tessaro et al., 1990; Forbes, 1991). The pathogenesis of these lesions remains speculative; joint trauma may be a predisposing factor (Doyle, 1935; Bracewell and Corbel, 1980), and live organisms in the joint may not be a prerequisite for lesions. In humans, the presence of anti-nuclear antibodies, rheumatoid factor, and immune complexes in synovial fluid, and the fact that live organisms are seldom recovered, have lead to the suggestion that it is an immune-mediated mechanism that causes the damage, as opposed to a true infectious mechanism (Alarcon et al., 1987). Similar observations were made in cattle (Bracewell and Corbel, 1980; Wyn-Jones et al., 1980; Corbel et al., 1989a, b). In the case of moose 83F, the lesions and the few *Brucella* sp. isolated supported an etiology of simple trauma with hemorrhage into the joints, probably caused by kneeling on a hard surface. Moose 74F, however, had a pronounced plasmacytic synovitis together with large numbers of *Brucella* sp. in the joints; this was evidence for a more active specific inflammatory response with a strong immune-mediated component.

Testicular lesions associated with *Brucella* sp. have been reported in a number of animals (Enright, 1990) including moose (Fenstermacher and Olsen, 1942; Corner and Cornell, 1958). In this experiment *B. abortus* was recovered from the testicles of both male moose, but in very low numbers relative to other tissues, and with no associated lesions. These findings probably reflected the passive presence the organism in blood, but may also have been related to the short duration of the experiment, the lack of a co-factor such as stress, or a combination the above. If these moose had been maintained for a longer period, testicular lesions might have developed.

Pericarditis, myocarditis and nephritis have been described in field cases of brucellosis in moose. (Fenstermacher and Olsen, 1942; Jellison et al., 1953; Corner and Connell, 1958). These conditions were not observed in this experiment. Low numbers of brucellae were present in the kidneys of 77M, 80M, 83F and 74F, and in the empty bladder of 74F. They were attributed to bacteria in residual blood as there were no signs or lesions indicative of infection in the kidneys or the bladder. The extensive systemic infection present in the experimental moose without associated renal, pericardial or myocardial involvement is evidence that the lesions described in previous field cases may have been due to causes other than brucellosis.

Clinical chemistry and hematological values for moose were compared to those of normal cattle because the laboratory equipment was standardized against a livestock database. These values are comparable to those previously reported in moose (LeResche et al., 1974; Franzmann and LeResche, 1978; Kitchen, 1986), and were notable because they remained stable despite the presence of infection and they were similar in magnitude to bovine normal values. The only exception was moose 83F which died acutely. This moose had values similar to the others until 1 wk before death, at which time the leukocyte count nearly doubled. This did not appear significant at the time because there were no clinical signs, WBC counts remained within normal limits for cattle, chemistry and other hematology values remained stable, and a similar range of values had been observed in the control moose. At the onset of clinical signs, 24 hr before death, neither the signs nor the hemogram and chemistry results reflected the severity of disease. However, the rapid progression of signs that followed, and the change in hematology and chemistry values, were com-

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patible with acute systemic collapse leading to death.

The inability of the moose to control and eliminate *B. abortus* exposed them to high levels of lipopolysaccharide (LPS) which caused the lesions observed. From our data, moose tolerate very high systemic levels of (LPS) before exhibiting effects. Moose may have adaptive physiological mechanisms that are eventually exhausted by continued exposure, or they may simply have a high threshold tolerance. The widespread and progressive lesions, persistent bacteremia, and failure to show signs of recovery are evidence that all infected moose would eventually have died. The disease would likely have progressed much faster in moose subjected to a variety of stressors under field conditions.

The high antibody titers were evidence that a humoral immune response occurred, but neither humoral nor cellular immunity was protective. In contrast, cattle have a strong humoral immune response but cell mediated immunity is responsible for protection, and the disease is rarely fatal.

The serological tests used to detect *B. abortus* infection in cattle were adequate for moose. The initial serological response of moose to infection with *B. abortus* was typical of that seen in cattle; the BAPT, BCT, and STAT, as a group, reacted earlier (15 to 29 days) than the CFT (21 to 43 days). Titers increased rapidly, and did not decline during the experiment.

Moose from areas where brucellosis occurs in other species have seldom had reactions on serological tests for brucellosis (Diesch et al., 1972; Hudson et al., 1980; Zarnke, 1983; Bourque and Higgins, 1984; Kocan et al., 1986; Dieterich et al., 1991; Jellison et al., 1953). Only four free ranging moose have been observed with high titers, and all four had signs of severe disease (Fenstermacher and Olsen, 1942; Jellison et al., 1953; Corner and Connell, 1958). The rarity of antibody titers and clinical cases in free-ranging moose is evidence that either moose are rarely exposed to *B. abortus*, that diagnostic methods lack sensitivity, or that infected moose die so quickly that they are not detected and do not transmit the disease within the population. The work of Fenstermacher and Olsen (1942), Jellison et al. (1953), Corner and Connell (1958) as well as our data support the hypothesis that moose die of brucellosis before detection and with no intraspecific transmission. Dissemination of *Brucella* spp. into the environment by infected moose does not appear to be a significant risk factor given the rapid course of the disease, the solitary nature of moose, the lack of draining lesions, and the lack of fecal and urinary output of the organism. Dissemination of the organism at parturition is unlikely as females are most likely to become infected in the spring, and either die, or be too debilitated to breed during the fall rut. A more precise risk assessment would require research to determine the minimum infective dose, survival time of infected moose in the wild, and effects on reproduction.

Moose appear to be dead-end hosts for *B. abortus*, but are highly susceptible to the disease. The effect of brucellosis on the population dynamics of moose cohabiting range with infected reservoir species such as bison or elk has not been studied. It should be noted that, under such conditions, infected moose could present a substantial zoonotic risk to hunters.

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