ARTICLE

A survey of brucellosis and tuberculosis in bison in and around Wood Buffalo National Park, Canada

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Abstract

Examinations of complete or partial remains of 72 bison found dead in and around Wood Buffalo National Park, Canada, revealed evidence of brucellosis in 18 (25%) and tuberculosis in 15 (21%), with a combined prevalence of 42%. Urease-positive and urease-negative strains of *Brucella abortus* biovar 1, and strains of biovar 2, were isolated from tissues of bison, including synovium and exudate from severe arthritic lesions. *Mycobacterium bovis* was isolated from a range of granulomatous lesions that were similar to those reported in tuberculous cattle. Diseased bison had a broad geographical distribution, and were found outside the park on at least three natural corridors. The diseases have a deleterious effect on this population of bison, and pose a health risk to other bison herds, livestock, and native hunters in the region.

Résumé

Une enquête sur la présence de brucellose et de tuberculose chez le bison dans la région du parc national Wood Buffalo, Canada

Des examens de cadavres complets ou partiels de 72 bisons trouvés morts dans les environs du parc national de Wood Buffalo, Canada, ont démontré la présence de brucellose chez 18 (25 %) bisons et de tuberculose chez 15 (21 %) bisons. La prévalence combinée des deux maladies est donc de 42 %. Des souches de *Brucella abortus* biovar 1 uréase négatives et uréase positives et biovar 2, furent isolées des tissus de bisons, incluant la synovie et l'exsudat de lésions articulaires sévères.

***Mycobacterium bovis* fut isolé d'une variété de lésions granulomateuses similaires à celles rencontrées chez des bovins atteints de tuberculose. Les bisons malades se retrouvaient dans une distribution géographique étendue de même que sur au moins trois corridors naturels à l'extérieur du parc. Ces maladies ont un effet important sur la santé et la population des bisons et posent un risque de contamination à la fois pour les bisons, les troupeaux de bovins et les chasseurs de la région.**

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Introduction

In 1985, Canada's national cattle population was declared free of bovine brucellosis, and bovine tuberculosis is expected to be eliminated by the end of 1989. Consequently, extraneous sources of these diseases have become increasingly important because of the risk of their reintroduction to cattle. The free-ranging bison (*Bison bison*) population in and around Wood Buffalo National Park (WBNP) (Figure 1) is the only known reservoir of bovine brucellosis and tuberculosis in Canada. The diseases and the hybrid status of the bison population are legacies of a misinformed decision to transport 6,673 infected plains bison (*B. bison bison*) to WBNP during the 1920's. These bison interbred with the estimated 1,500 wood bison (*B. bison athabascae*) that were native to the park region. Concern about the bison has increased as livestock and game ranching enterprises move closer to the park and as three recently introduced, healthy populations of wood bison develop in the region.

Past information on brucellosis and tuberculosis in bison in WBNP was based on gross lesions, serological tests, and intradermal (caudal fold) tuberculin test results in bison that were driven into corrals and slaughtered at two sites in the park prior to 1967 (1-3). Bacteriological and histological evaluations were not reported, and biotyping methods for *Brucella* were not available. The purpose of the present study was to correlate gross, histological, and bacteriological findings on brucellosis and tuberculosis in bison, and to determine if *Brucella* biotyping would provide useful epidemiological markers for tracking brucellosis in the region.

Materials and methods

The study was strictly limited to opportunistic sampling in accordance with research permits issued by the

Health of Animals Laboratory, 116 Veterinary Road, Saskatoon, Saskatchewan S7N 2R3 (Tessaro, Forbes); Animal Diseases Research Institute, P.O. Box 11300, Station H, Nepean, Ontario K2H 8P9 (Turcotte).

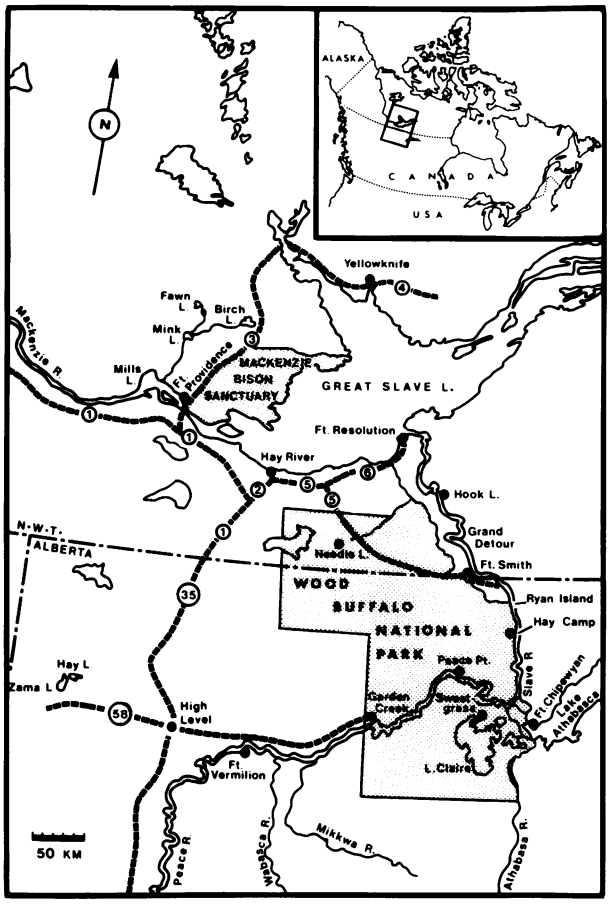

Figure 1. Map showing the location of Wood Buffalo National Park. Broken lines and circled numbers represent highways and highway numbers.

Canadian Parks Service and the Northwest Territories Department of Renewable Resources. Necropsies were to be done on complete or partial remains of bison that died as a result of hunting, poaching, predation, diseases, or natural accidents. Dead bison were located by ground and aerial surveys, and from information provided by local hunters, trappers, park wardens, and wildlife officers.

All major lymph nodes and portions of major viscera were collected whenever possible, based on condition and completeness of the carcasses. A small portion of each tissue was fixed in 10% neutral buffered formalin for histological evaluation, and the remainder was frozen at −20°C for subsequent bacteriological assessment. When possible, blood samples were collected from the jugular vein, carotid artery, or ventricles of the heart; blood was centrifuged, and the serum was collected by pipette and stored at −20°C for later serological testing.

Formalin-fixed tissues were processed to paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin for light microscopic examination. Duplicate sections were stained by the Ziehl-Neelsen method whenever any gross or microscopic lesions were seen. Sections of *Mycobacterium avium*-infected tissues were used as controls for the Ziehl-Neelsen stain. A minimum of 100 fields were examined at 1000 × magnification for acid-fast bacilli in granulomatous lesions.

Sera were tested for *Brucella* antibodies using the standard tube agglutination test (STAT), the buffered plate antigen test (BPAT), and the direct complement fixation test (CFT) as described by Forbes (4). For bacteriological studies of *Brucella*, each tissue was trimmed, flamed with absolute ethanol, and incised with sterile scissors before being double-bagged with an equal volume of 0.85% saline. The samples were then homogenized in a Colworth Stomacher Blender (A.J. Seward, Medical Division of UAC International Limited, 3 Cavendish Road, Bury St. Edmunds, Suffolk, England) and swabbed over the entire surface of two blood agar plates and four *Brucella* agar (5) plates with the following additives:

a) one plate of *Brucella* agar containing 25,000 IU of bacitracin, 6,000 IU of polymyxin B sulfate, and 100 mg of cyclohexamide per liter of serum tryptose agar basal medium.

b) one plate of *Brucella* agar containing the same antibiotics as (a) plus ethyl violet at a final concentration of 1:800,000.

c) one plate of *Brucella* agar containing 7,500 IU of bacitracin, 1,800 IU of polymixin B sulfate, and 30 mg of cyclohexamide per liter of serum tryptose agar basal medium.

d) one plate of *Brucella* agar containing the same ingredients as (c) plus ethyl violet at a final concentration of 1:800,000.

The plates were incubated in 10% CO_2 at 37.5°C and examined daily for up to 10 days. Suspect colonies were identified to species and biovar using standard procedures (5). The identity of *Brucella* isolates was confirmed by the Animal Diseases Research Institute in Nepean, Ontario, and by the world reference center at the Central Veterinary Laboratory in Weybridge, England.

Tissues with gross or microscopic lesions suggestive of mycobacteriosis were submitted for mycobacteriological evaluation. The tissues were trimmed free of fat and connective tissue. One gram of tissue was mixed with 5.0 mL of a 15 M aqueous phosphate buffer (pH 7.0). The sample was then homogenized in a Ten Broek grinder (Fisher Scientific, 112 Colonnade Road, Nepean, Ontario) and 5.0 mL of the resulting suspension were mixed with 10.0 mL of 2% NaOH containing phenol red indicator (0.1 g phenol red per 500 mL of 2% NaOH). The suspension was allowed to stand for a maximum of 12 min and was then neutralized with 2N HCl. Sterile distilled water was added to give a final volume of 35.0 mL, the sample was centrifuged at 1780 *g* for 30 min, and the supernatant discarded. The sediment was used to prepare a smear and to inoculate culture media. As a control, a duplicate sample of each tissue was treated the same way as the first sample but omitting the NaOH decontamination step.

Sediment smears were heat-fixed and stained by the Ziehl-Neelsen method, and 100 oil-immersion fields were examined for acid-fast bacilli. The decontaminated and nondecontaminated sediments were inoculated into one tube each of Middlebrook 7H-9 medium, Herrold egg yolk medium with 2.0 mg/L mycobactin, Lowenstein-Jensen medium, and Stonebrink medium. These were incubated at 37°C. Two additional tubes

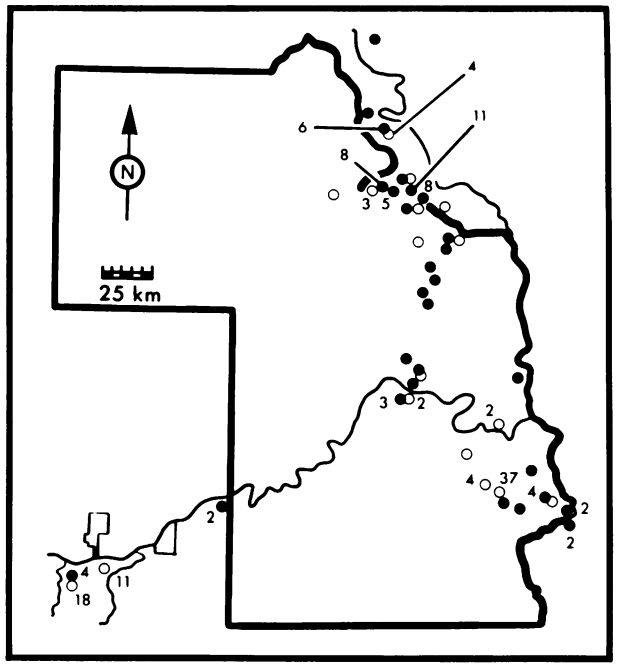

Figure 2. Map of Wood Buffalo National Park showing the location of bison carcasses. Solid circles represent locations where carcasses suitable for sampling were found; open circles indicate sites where carcasses were found but were too decomposed or too scavenged for diagnosis of disease. Numbers are provided where more than one dead bison was found at a given location.

of Lowenstein-Jensen medium were inoculated: one was incubated at 30°C and the other at 42°C. The media were examined for up to 90 days. Suspect colonies were identified by morphological characteristics and standard biochemical tests (6).

Results

The complete or partial remains of 164 bison were found in and around WBNP between June 1983, and October 1985. Of these, 72 were suitable for disease analyses and 92 were unsuitable because they were too decomposed and/or too heavily scavenged. The sample had a broad geographical distribution, and included animals that had died outside the park boundary on the northeast, southwest, and southeast (Figure 2). The majority of the bison were necropsied during winter months when ground transportation was easiest (snowmobile), hunting and poaching activity were highest, and dead bison were most easily found because of lack of leaf cover and the ease of tracking hunters and poachers on snow.

The 72 animals included 23 bulls, 27 cows, 16 calves, two yearlings, and four adults of indeterminate sex. Nine of the 27 mature cows were pregnant in late fall and in winter. The sample was comprised of 44 bison killed by hunters, 12 bison killed by poachers, five bison killed by motor vehicles, four bison seen being killed by wolves, three debilitated bison shot by park wardens, two bison that died of diseases, and two bison that died of undetermined causes. The number of tissues collected from each bison varied between one and 14, with an average of 7.4.

Brucella abortus was isolated from the tissues of nine adult and two yearling bison. Positive BPAT results and high antibody titers on STAT (1:50 to 1:400, median 1:200) and CFT (1:5 to ≥1:50, median ≥1:50) were found in an additional seven adult bison from which the bacterium was not isolated. Sera obtained from five of the 11 culture-positive bison had high titers on STAT (1:400 to 1:3200, median 1:1600) and on CFT (≥1:50). None of the 16 calves was culture-positive, and sera obtained from four of these did not react on serological tests for brucellosis.

The isolates of *B. abortus* from the 11 bison included eight urease-positive strains of biovar 1, two urease-negative strains of biovar 1, and one strain of biovar 2. Isolates were obtained from one or more of the following tissues: retropharyngeal, submandibular, bronchial, hepatic, internal iliac, superficial inguinal, suprascapular, and precrural lymph nodes; liver, kidney, spleen, synovium, synovial fluid, and exudate from a subcutaneous abscess. The relative frequency of isolation of *B. abortus* from each tissue, and the sensitivity and specificity of serological tests, could not be evaluated because of the small number of animals and because a complete collection of serum and tissues from each bison was not possible.

Lesions associated with *B. abortus*-infection (Figure 3) were seen in four bulls. One bull had a 5 cm diameter subcutaneous abscess on the caudal aspect of the left stifle joint; *B. abortus* biovar 1 was isolated from the suppurative exudate. Each of the three other bulls had one severely swollen stifle joint, and one of these also had a 29 cm long, 10 cm diameter hygroma on the cranial aspect of the left carpal and metacarpal region. *Brucella abortus* biovar 1 was isolated from all of these lesions. One of these bulls had died of starvation secondary to severe arthritis.

Arthritis of the stifle joints was characterized grossly by loss of articular cartilage with eburnation or lysis of bone, synovial villus hypertrophy and pannus formation, thickening and distention of the joint capsule, and a large volume (200–1000 mL) of exudate. In two cases, the exudate was a translucent, viscous, yellow fluid containing small flecks of debris; it was found in the joint space and in distended tendon sheaths distal to the joint. In the third case, there was a thick, white, suppurative exudate within the joint space.

Two emaciated bison cows had identical lesions of serous arthritis involving stifle joints, but brucellae were not isolated from joint exudate. One of these cows had very high *Brucella* antibody titers on STAT (1:400) and CFT (1:25); serum was not available from the other cow, and the hunter had removed part of the joint capsule while butchering the animal, leaving little exudate or tissue for bacteriological examination. One cow was killed by a park warden because it was severely crippled and starving.

Histologically, animals with serous arthritis had large numbers of plasma cells and lymphocytes, and multifocal aggregates of hemosiderin-laden macrophages, within the synovium. There was marked proliferation of synovial villi and many of these were acellular and sclerotic. There was necrotic debris, but few inflammatory cells, on the synovial surface. There were foci of necrosis and mineralization within the synovium and joint capsule, and some of the deeper foci were large and surrounded by epithelioid cells,

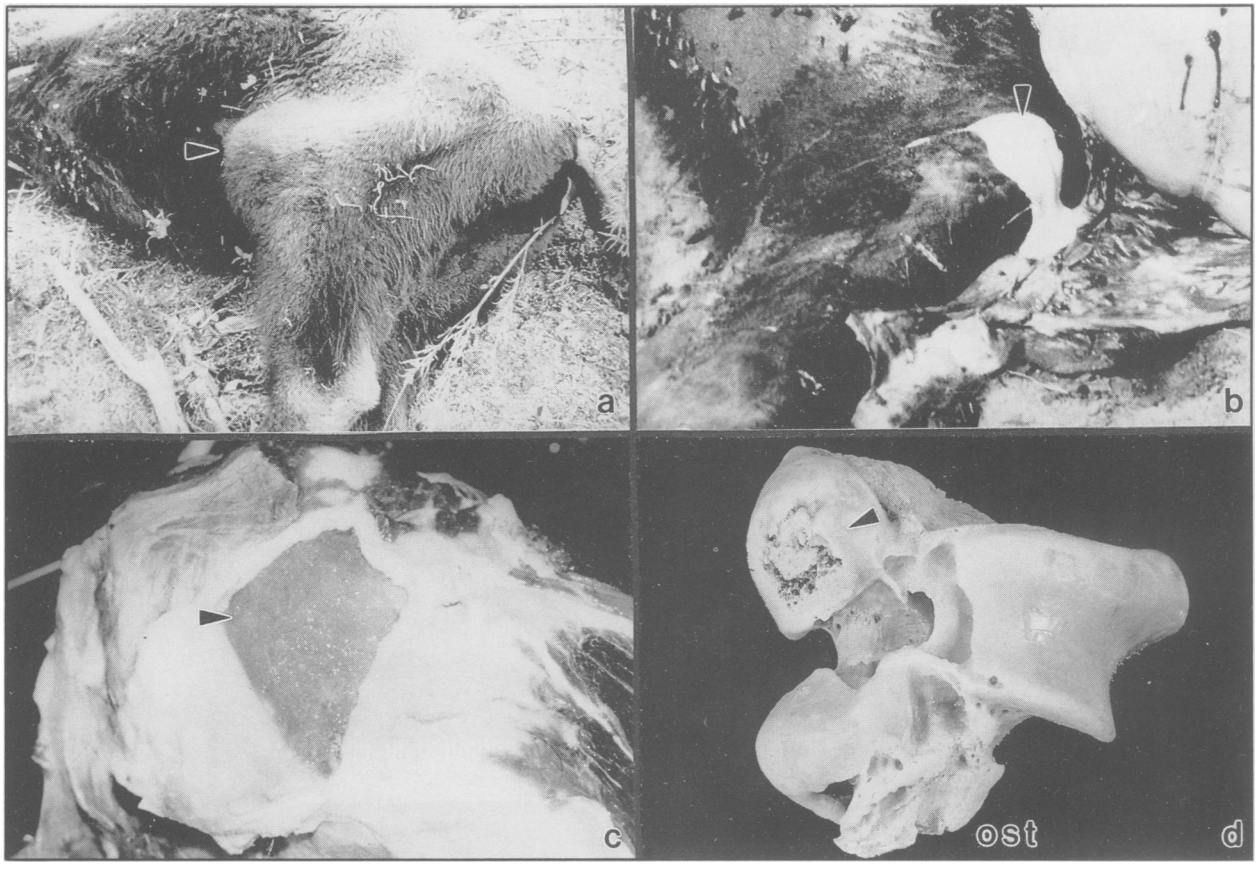

Figure 3. Articular lesions of bison from which *Brucella abortus* was isolated. a. Swollen left stifle joint (arrows) of a bison with serous arthritis. b. Suppurative arthritis of the left stifle joint (arrow). c. Serous exudate (frozen *in situ*) within the distended capsule of a stifle joint (arrow). d. Eburnation, bone lysis (arrow) and osteophytes (ost) on the distal articular portion of the left femur of a bison with brucellar arthritis.

macrophages, lymphocytes, and fibroblasts. Giant cells were occasionally present in these granulomas. No consistent, attributable macroscopic or microscopic lesion was found in lymph nodes that were culture-positive for *B. abortus*.

Mycobacterium bovis was isolated from lesions in seven adult bison, and another seven adults and one calf had gross and microscopic lesions indicative of tuberculosis but were culture-negative. Other species of mycobacteria were not found. Both *B. abortus* and *M. bovis* were isolated from the tissues of two adult bison; a third adult bison that reacted on STAT (1:50) and CFT (1:5) for brucellosis had advanced lesions typical of pulmonary tuberculosis but was culture-negative.

Tuberculous lesions (Figure 4) were confined to the lymph nodes in 10 of the 15 affected bison: five had lesions restricted to the lymph nodes of the head; two had lesions only in bronchial lymph nodes; one had lesions in bronchial and suprascapular lymph nodes; one had lesions in retropharyngeal, bronchial and mesenteric lymph nodes; one had lesions in one precrural lymph node. The other five bison had lymphoid and visceral lesions: two had pulmonary tuberculosis with involvement of bronchial and retropharyngeal lymph nodes; two had generalized tuberculosis involving thoracic and abdominal viscera (lungs, pleura, pericardium, ribs, liver, kidneys, testes, and epididymi), and multiple lymph nodes; one calf had granulomatous encephalitis (tuberculoma). In 14 of the 15 tuberculous bison, retropharyngeal and/or bronchial lymph nodes were consistently affected.

Isolates of *M. bovis* were obtained from lesions in retropharyngeal, bronchial, parotid, mandibular, mediastinal, mesenteric, renal, internal iliac, and superficial inguinal lymph nodes, and from lung and liver.

Histologically, tuberculosis in the lymph nodes and organs of bison was characterized by typical granulomas composed of macrophages, epithelioid cells, lymphocytes, and Langhans' giant cells, with variable amounts of caseation, mineralization, and fibrosis. Aggregates of neutrophils were occasionally present in these lesions but only rarely in large numbers. The granulomas could be seen individually or in confluent clusters. They ranged from microscopic, poorly delineated foci with little or no caseation and no fibrosis, to large caseous foci, and to highly mineralized fibrotic lesions. This range of lesions could be found within individual animals as well as among different animals. Complete regression and scarring of these lesions were not seen in any of the bison; those with highly mineralized, fibrotic lesions also had numerous foci of more acute granulomatous lesions.

Acid-fast bacilli were rarely seen in granulomatous lesions, even when the lesions were culture-positive. When found, the bacilli were in macrophages or giant cells surrounding caseous foci where there was little or no mineralization.

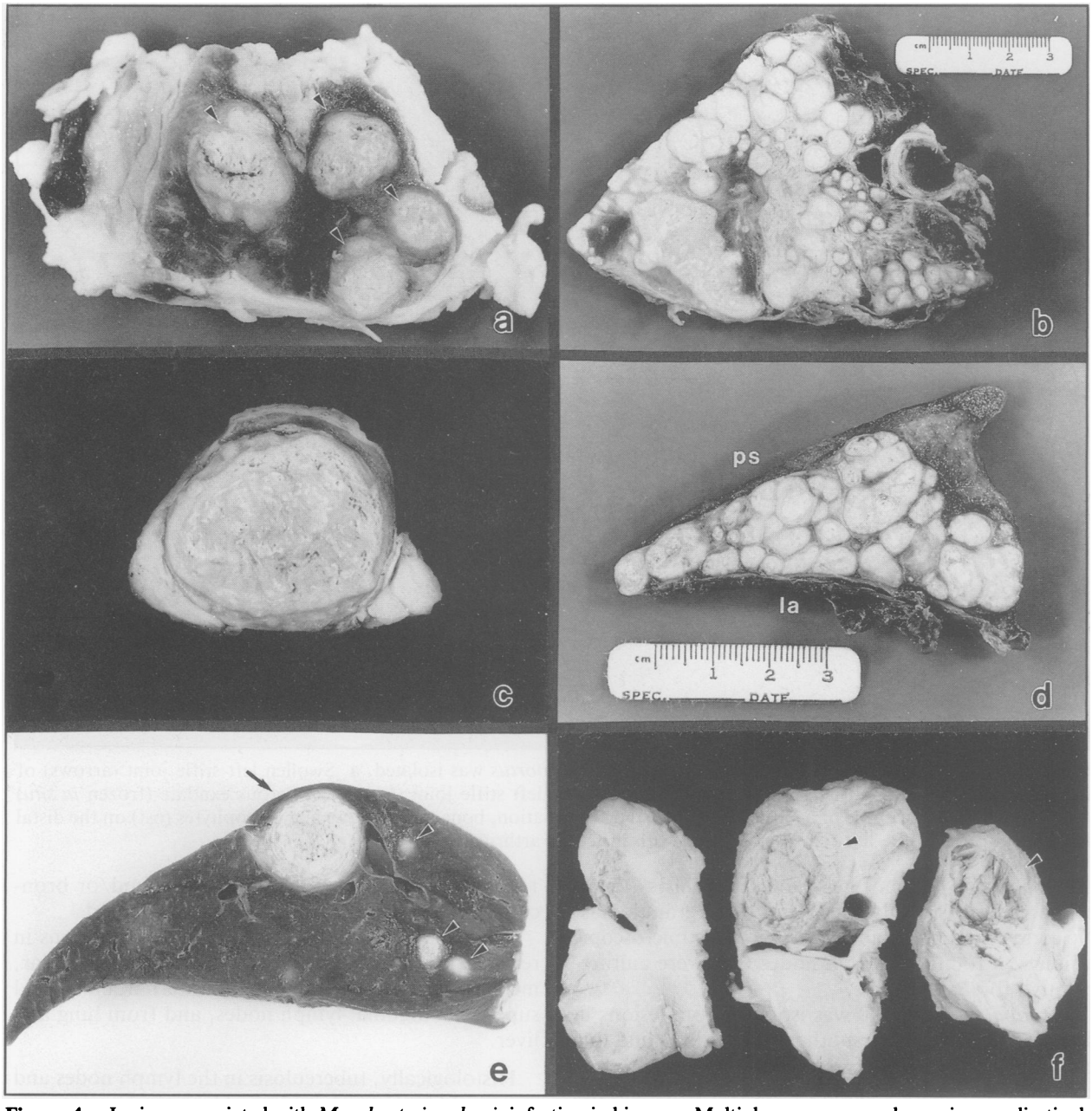

Figure 4. Lesions associated with *Mycobacterium bovis* infection in bison. a. Multiple caseous granulomas in a mediastinal lymph node. b. Cross-section of left caudal lobe of the lung of a bison that died of advanced pulmonary tuberculosis. c. Granulomatous lymphadenitis in a retropharyngeal lymph node; only a thin rim of lymphoid tissue remains around the central area of partially mineralized, caseous necrosis. d. Granulomatous pericarditis with adhesions between the pericardial sac (ps) and the epicardial surface of the left auricle (la). e. Multiple caseating granulomas (arrows) in a cross-section of liver. f. Focal granulomatous encephalitis (arrows) in the left cerebral hemisphere of a bison calf.

Bison with evidence of one or both diseases were widely distributed in the park and were also found outside the park (Figure 5). Of the 56 bison killed by native people, 15 (27%) had evidence of brucellosis and nine (16%) had lesions of tuberculosis. Three of the four bison seen being killed by wolves were severely debilitated by advanced lesions of tuberculosis; the fourth was an apparently healthy young calf. One bison cow had died of generalized tuberculosis and one bison bull died as a result of emaciation secondary to severe brucellar arthritis. Of the three disabled bison killed by park wardens, two had broken legs (trauma) and the other was incapacitated and starving because of severe arthritis; there was no evidence of brucellosis or tuberculosis in the first two bison, but the third animal, with serous arthritis of the left stifle, had very high antibody titers to *Brucella* on STAT (1:400) and CFT (1:25). Three of the five bison killed by motor vehicles were healthy and the other two had small tuberculous lesions in retropharyngeal and bronchial lymph nodes. The three bison with advance tuberculosis and two bison with severe debilitating brucellar arthritis were emaciated, but the other 67 bison in the sample were in good body condition, based on subcutaneous and internal fat deposits, regardless of the season in which they were killed.

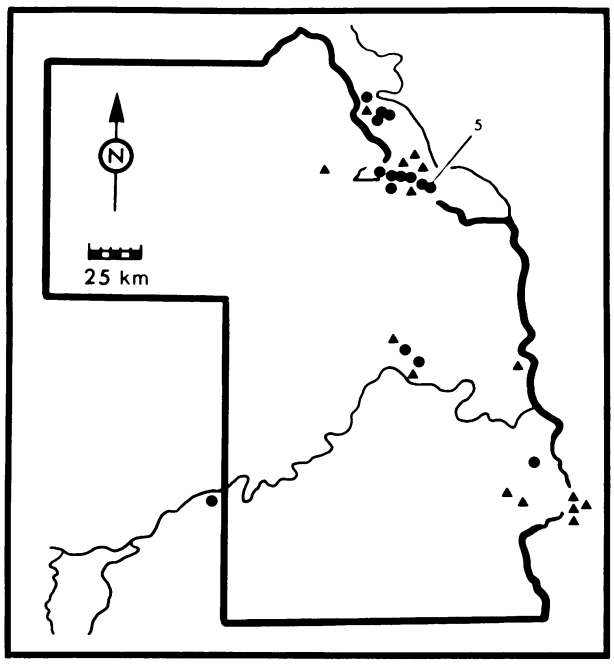

Figure 5. Map of Wood Buffalo National Park showing the locations of bison with bovine brucellosis (circles) and tuberculosis (triangles). Numbers are provided where more than one infected animal was found at the same site.

Discussion

Acquisition and quality of samples were good considering the inherent limitations of an opportunistic collection, the great size of the study area, and the logistical difficulties of retrieving samples. The 164 bison found dead in the study did not reflect total mortality in the population during that period: as many as 372 dead bison were reported in conversations with hunters, trappers and wildlife personnel, but many of these reports could not be verified.

Of the 72 bison necropsied, 18 (25%) had evidence of brucellosis and 15 (21%) had evidence of tuberculosis, with a combined prevalence of 42%. This is likely a conservative estimate of the prevalence of the diseases in the sample because some carcasses yielded few tissues for analyses.

The sample was essentially a hunting survey, since 78% of the bison were collected by hunters and poachers. Hunters preferred younger bison that were in good body condition, a process of selection that biased against finding diseased animals. Because of many years of poor production and recruitment of calves (7), the age distribution of the population of bison in the park region is likely skewed towards an older mean age, whereas the harvest by hunters was biased in favor of younger animals. Consequently, the hunter-killed portion of the sample likely underestimated the prevalence of the diseases in the general population.

The small portion of the bison in the sample that were killed by vehicles or by park wardens, or which died of undetermined causes was not obviously biased in relation to disease. Despite the small sample size, the results suggested a correlation between predation by wolves and advanced tuberculosis. The diseases predispose bison to predation, and this should be taken into account when considering proportional mortality due to wolves. The availability of large numbers of debilitated, diseased bison may artificially maintain a large population of wolves which, in turn, likely exacerbates the problem of poor recruitment of calves into the bison population.

Based on this survey, the 95% confidence intervals taken from binomial distribution tables (8) indicated that the prevalence of brucellosis in the bison population was 16–37% and that the prevalence of tuberculosis was 12–32%, with a combined prevalence of 31–55%. These results compared well with prevalence values obtained from bison that were driven into corrals and slaughtered in the park between 1959 and 1967; 27.4% of 1,789 bison were positive on brucellosis agglutination tests and 42.4% of 1,090 bison had lesions of tuberculosis (3).

One bison died of tuberculosis and three others would have died of the disease had wolves not intervened; one bison died of starvation, and another was euthanized because of starvation, both secondary to severe brucellar arthritis. Thus the deaths of six (8%) bison in the sample could be attributed to the two diseases. This observation concurs with a previous estimate of 4–6% mortality based on finding generalized tuberculosis, and 2% prevalence of arthritis, in adult bison commercially slaughtered in the park during the 1950's (9).

Brucella abortus was isolated from a wide range of lymphoid tissues from bison, similar to the distribution of the pathogen in infected cattle (10–14). Because of this wide range of potentially infected tissues, a broad collection of samples should be done in order to find the bacterium in bison. The arthritis and hygromas associated with brucellosis in bison were similar to those reported in cattle (10,15–18). These lesions caused marked lameness and contributed to the emaciated condition of affected bison. A quantitative assessment of the contribution of brucellosis to the poor calf production in the park bison (7,9) was not possible from an opportunistic study of this kind.

The biovars of *B. abortus* isolated from bison were similar to field strains reported in cattle, and no strain that could be considered unique to bison was found. Urease-positive strains of biovar 1 were the most common isolates when brucellosis occurred in cattle in Canada (Forbes, unpublished). Biovar 2 has been detected only once in cattle in Canada (19) and has been found in a small percentage of infected cattle in the USA (20). Urease-negative strains of biovar 1 have never been reported in cattle in Canada, but have been found occasionally in cattle from various other countries (21). The inability to split urea is a stable characteristic of strain 544, the type strain of *B. abortus* (22). The rarity of biovar 2 and urease-negative strains of biovar 1 in cattle in Canada and the USA makes them widely useful epidemiological markers for tracking brucellosis; identification of urease-positive strains of biovar 1 is also useful, but on a more localized basis.

All strains of *B. abortus* isolated from bison and elk in the USA have been biovar 1 (23,24). The presence of additional strains of *B. abortus* in bison in the

WBNP region suggests that the bison were historically exposed to more than one source of infection.

The distribution and characteristics of lesions of bovine tuberculosis in bison were the same as those described in cattle (25–30). As in cattle (29), alimentary and aerogenous infection both would produce lesions in the lymph nodes of the head of bison. The greater number of bison that had tuberculous lesions in bronchial lymph nodes and lungs, compared to the few bison with lesions in abdominal lymph nodes and viscera, may suggest that the respiratory tract is the more important route of infection in adult bison.

Of the 56 bison that were killed and butchered for human consumption by hunters and poachers in the park region, 15 had brucellosis and nine had tuberculosis. Hunters did not wear gloves or take any other special precautions when processing dead bison. They were seen puncturing hygromas and arthritic joints, opening subcutaneous abscesses, incising caseous retropharyngeal lymph nodes, and contaminating their hands and knives, and the meat. *Brucella abortus* and *M. bovis* were subsequently isolated from these lesions. There is obvious potential for human infection.

The broad geographical distribution of bison with brucellosis and/or tuberculosis indicated that the diseases are enzootic in the population. This was not unexpected given the wide-ranging movements and gregarious behavior of bison, and the chronic, highly infectious nature of these diseases. During this survey, bison were seen up to 75 km outside the southwest corner of WBNP. Gainer (31) has documented sporadic sightings of bison in and near the agricultural zone of Fort Vermilion, Alberta. The high prevalence of brucellosis and tuberculosis in these bison, the distances over which they roam, and the increasing proximity of livestock and other bison populations present a growing risk of the spread of the diseases to uninfected herds of bison and cattle.

Acknowledgments

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