Brucellosis in Free-ranging Bison (Bison bison) in Yellowstone, Grand Teton, and Wood Buffalo National Parks: A Review

Authors: Meyer, Margaret E., and Meagher, Mary

Source: Journal of Wildlife Diseases, 31(4) : 579-598

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-31.4.579

Journal of Wildlife Diseases, 31(4), 1995, pp. 579–598

LETTER TO THE EDITOR . . .

Brucellosis in Free-ranging Bison (*Bison bison*) in Yellowstone, Grand Teton, and Wood Buffalo National Parks: A Review

Brucellosi , caused by *Brucella abortus*, is enzootic among the free-ranging bison (*Bison bison*) herds in Yellowstone and Grand Teton National Parks (Wyoming, USA), and Wood Buffalo National Park (Alberta, Canada). In contrast to previous assumptions, we present evidence that the response of bison to infection with *B. abortus* differs significantly from that of cattle. One marked difference is in their response to immunization with strain 19. In contrast to the 65 to 80% efficacy of strain 19 in the immunization of bovine calves, it stimulated no immunity in bison calves. Additionally, when used for immunizing adult cattle, it caused less than 2.5% to abort, while in adult bison it caused 70% to abort. Additional differences between cattle and bison occurred in the low frequency of abortions in natural infections among bison and also in the comparatively few recoveries of *B. abortus* from tissues of seroagglutination positive bison. Among seroagglutination-positive cattle, *B. abortus* can be recovered from 86 to 95% of tissue samples. Among 218 Yellowstone National Park bison shot during the winter of 1992, *B. abortus* was recovered from 26 of 109 seroagglutination positive animals and 22 (85%) of the isolates were from subadults. Based on available data, we believe the risk of transmission of brucellosis from free-ranging bison to cattle herds is very low.

INTRODUCTION

Among the four major free-ranging bison (*Bison bison*) herds in North America, those in Yellowstone National Park (YNP) (Mohler, 1917), Grand Teton National Park (GTNP) (Williams et al., 1993), both in Wyoming (USA), and in Wood Buffalo National Park (WBNP) (Tessaro, 1987) in Alberta and the Northwest Territories (Canada), are enzootically infected with *Brucella abortus*. The herd in the Mackenzie Bison Sanctuary in the Northwest Territories is brucellosis free (Tessaro et al., 1993).

A significant event in the history of brucellosis in free-ranging bison of these herds occurred when Mohler (1917) reported positive seroagglutination tests for two bison cows that had aborted and a negative test on a third cow that had not aborted. This established that bison are susceptible to brucellosis, that some of the YNP herd were infected, and that the seroagglutination test used for detecting *Brucella* antibodies in cattle serum also could be used to detect them in bison serum. Since this initial report, workers have interpreted bison serum antibody titers as recommended for infected cattle (Anonymous, 1932). In recent reports, brucellosis in bison was referred to as bovine brucellosis of wildlife (Tessaro, 1986) and later and more specifically, as bovine brucellosis of bison (Tessaro, 1992). This implies that investigators assumed the host response of bison to infection with *B. abortus* was similar to that of cattle. However, no data have been published to support this concept. Our purpose in this review is to evaluate available data and ascertain if *B. abortus* infection in bison is, in fact, mimetic of that in cattle.

BRIEF HISTORY OF FREE-RANGING BISON HERDS

Yellowstone National Park herd. The long history of bison in and around YNP has been documented extensively (Skinner and Alcorn, 1942; Alcorn, 1947). The breeding stock of the herd consisted of 18

adult females from the Pablo-Allard herd in Montana (USA) and three adult males from the Goodnight herd in Texas (USA) that were imported in 1902, to eventually interbreed with the remnant native herd of approximately 25 animals. Since 1902 no other bison have been introduced from the outside and this remains a closed herd. Topographic and environmental barriers effectively preclude a GTNP bison from entering YNP. In a recent legal case (Parker Land and Cattle Co. Inc. v. United States) this possibility was denied (Keiter and Froelicher, 1993).

While there has been no addition of bison to YNP, there have been substantial removals from the population. Until 1967, management policy was to maintain the herd at an estimated carrying capacity for park range land. The first removal of a significant number of bison (n = 109) occurred in 1925 (Skinner and Alcorn, 1942). By 1967, when the policy became one of minimal human interference, 9,016 bison had been removed, mostly by slaughter (Meagher, 1973a). Thereafter, there were records through 1992 of an additional 1,055 being shot after straying out of the park (M. Meagher, unpubl.). Thus, since 1925, 10,071 animals have been removed from the herd. At the termination of population control in 1966, the population was 397 bison (Meagher, 1973a). On 21 January 1994, 3,551 bison were counted by aerial survey (M. Meagher, unpubl.). Although the bison have been enzootically infected with *B. abortus* since at least 1917, population reduction operations within the park were not for brucellosis control.

Wood Buffalo National Park herd. These bison also are descendants from the interbreeding of native and introduced bison. The introduced bison originated from the Pablo-Allard herd of Montana, over 700 of which were purchased by the Canadian government in 1906, and from 1907 to 1912, more than half were moved to the newly-created Buffalo National Park at Wainwright, Alberta (Corner and Connell, 1958). Descendants of the Pablo-Allard bison are believed to have acquired brucellosis at Wainwright (Tessaro et al., 1990; Carbyn et al., 1993). Later, between 1925 and 1928, 6,673 bison were transported from Wainwright to WBNP (Carbyn et al., 1993). A major consequence of this bison transfer was the probable introduction of brucellosis into the WBNP herd. However, it was not diagnosed until the late 1950's (Corner and Connell, 1958; Choquette et al., 1961).

Grand Teton National Park (Jackson) herd. Historic details of bison in the Grand Tetons and Jackson Hole area were reported in an environmental assessment document (Grand Teton National Park, 1994). However, only the recent events in this herd are pertinent to its infection with *B. abortus*. In 1968 the small herd of 15 or 16 bison escaped from a fenced enclosure in GTNP and became free-ranging. During the winter of 1976 most of the herd began to winter on National Elk Refuge lands just south of GTNP, where elk (*Cervus elaphus*) were supplementally fed in winter. In 1980 the bison began to consume feed intended for the elk. Since then, in winter the bison have been supplementally fed along with 7,000 to 9,000 elk (Grand Teton National Park, 1994). It has been known since about 1930 that these elk are infected with *B. abortus* (Thorne et al., 1978) and transmission of *B. abortus* among elk aborting while on the feedgrounds results in the maintenance of a high annual prevalence of brucellosis (Thorne et al., 1979). Brucellosis was found in the GTNP bison herd in 1989, and while the source of infection has not been determined definitively, it was presumed to have been infected feedground elk (Williams et al., 1993).

SOURCES OF DATA

Data on the early investigations of brucellosis in the YNP herd are in the scientific journals. However, most of the data were collected between 1925 and 1967 during herd reductions. These data were accumulated as routine, in-house reports on the

current status of the herd. With the end of herd size controls data were obtained opportunistically. These data are preserved in the YNP archives, which is an official branch of the National Archives in Washington, D.C. A copy of any unpublished report cited in this paper may be obtained from the Archivist, National Park Service, P.O. Box 168, Yellowstone National Park, Wyoming 82190 (USA).

Data pertaining to bison in the Canadian national parks also have been stored in government files. Tessaro (1987) cited 111 unpublished reports compiled largely by personnel in Parks Canada and the Canadian Wildlife Service.

INVESTIGATIVE METHODS (1917 to 1993)

Mohler (1917) did not provide details on how he made the antigen for the seroagglutination test. However, before any more test results were reported on bison sera, recommendations to standardize the seroagglutination tube test on cattle sera (Anonymous, 1931) were adopted by the United States Livestock Sanitary Association. The following year, the first dilution of each serum dilution series was lowered to 1:25. Dilution titers of 1:25 and incomplete reactions at 1:50 were to be interpreted as suspicious; complete reactions at 1:50 and above were considered positive (Anonymous, 1932). These recommendations became part of the 1947 Uniform Methods and Rules for Eradication of Bovine Brucellosis established by the U.S. Department of Agriculture (Becton, 1977).

Prior to standardization of interpreting seroagglutination titers and production of a standardized antigen in 1939 (Becton, 1977) there undoubtedly were variations in test results. However, results reported by LaNoue (1932), Rush (1932a, b), Tunnicliff and Marsh (1935) and Skinner (1941) were performed under the supervision of, or by, Dr. Hadleigh Marsh in the Veterinary Research Laboratory (now the Montana Livestock Diagnostic Laboratory) at Bozeman, Montana; thus, there should have been uniformity in the methods used during those years. The possibility that Dr. Marsh used a rapid whole blood field agglutination slide test in the YNP herd was suggested by Quortrup (1944). Such an agglutination test was developed for field use (Welch and Marsh, 1935) but was used in the YNP herd only to screen live animals for shipment.

Although Tunnicliff and Marsh (1935) did not report the methods used for identification of the *B. abortus* isolate from the testicle of an adult male bison, the only method available at that time was that used by Creech (1930) for identifying *B. abortus* that he had isolated from an infected adult male bison on the National Bison Range, Moiese, Montana. After 1978, methods for isolating *B. abortus* from tissue and identifying the species and biovar of the organism were those of Meyer (1978, 1984). Some investigators made minor modifications in standardized methods in choice of antibiotics for inclusion in the culture media and in choice of method or apparatus for preparing tissue for culture (Ewalt, 1989). No additional isolates of *B. abortus* were reported from infected bison until 1985.

In GTNP and WBNP all tissue and blood samples were obtained from opportunistically available carcasses. Bison of the GTNP herd were hunter-shot for population reduction on National Elk Refuge lands (Williams et al., 1993). In WBNP, bison deaths were due to a variety of causes (Tessaro, 1987). In YNP, from 1925 through 1966 blood samples were obtained during herd reductions. Thereafter, samples were obtained from bison shot after straying into Montana.

Tissue samples obtained during the exodus of YNP bison into Montana in the winter of 1991 and 1992 were collected according to a protocol established by the Montana Department of Livestock (Aune and Schladweiler, 1993). Samples of blood, a variety of lymph nodes, and either seminal vesicles or the uterus were obtained on 218 freshly killed bison. The Montana Livestock Diagnostic Laboratory, Boze-

man, Montana, performed the serologic tests. Bacteriologic culturing of the tissues for recovery of *B. abortus* was done by the U.S. Department of Agriculture (USDA), National Veterinary Services Laboratory, Ames, Iowa (USA).

Age categories used herein are those presented in the original data source. Prior to development of field aging techniques for bison (Fuller, 1959) ages presented in reports and publications probably were comparable to those used for cattle, generalized as calves (up to 1 yr old), yearlings (1 to 2 yr old), subadults or young animals, and adults. Tooth eruption and tooth wear patterns (Fuller, 1959; Frison and Reher, 1970), and the development of cementum aging techniques (Matson's Laboratory, P.O. Box 308, Milltown, Montana) allowed more refinement. The anatomically immature subadult category includes some individuals that mature sexually prior to adult age status. However, in field situations, males categorized as subadults were usually 2 to 5 yr old, females were 2 to 3 yr old. A generalized adult category for animals older than calves was used in some source material.

The bison census in the GTNP (Jackson) bison herd was opportunistic (Grand Teton National Park, 1994). Counts for WBNP and YNP were by aerial surveys as described by Carbyn et al. (1993) and by Meagher (1973a, 1989, 1993), respectively.

CAUSAL ORGANISM OF BRUCELLOSIS IN CATTLE AND BISON

While *B. abortus* long has been known to be the usual causative organism of brucellosis in cattle (Bang, 1897), it was not identified in bison until Creech (1930) isolated it from the testicle of an infected adult male at the National Bison Range. *Brucella abortus* was recovered from the genitals of an infected adult male from the YNP herd in 1933 (Tunnicliff and Marsh, 1935).

To ensure that organisms in the genus *Brucella* are correctly identified, members of the International Committee on Nomenclature of Bacteria, Subcommittee on the Taxonomy of the Genus *Brucella* (Stableforth and Jones, 1963) and of the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Brucellosis (1964) recommended that oxidative metabolic patterns be used to establish species identity and conventional determinative methods be used to establish biotype (biovar) identity. That these recommendations be followed was (and still is) particularly essential for definitive identification of brucellae isolated from previously unreported hosts or geographic areas.

In accordance with these recommendations, additional isolates recovered from bison from 1933 to 1935 were examined by the conventional determinative methods and for their oxidative metabolic patterns (Meyer and Cameron, 1961) and found to be typical for *B. abortus* biovar 1 (Meyer, 1965). All of the *B. abortus* isolates from bison (Williams et al., 1993) and elk (Thorne et al., 1978) have been identified as biovar 1. While no full studies have been published, isolates of *B. abortus* biovar 1 recovered from YNP bison are infective and pathogenic for cattle (Davis, 1990). Thus, the strain or strains of *B. abortus* biovar 1, enzootic in bison in YNP and GTNP, are identical to *B. abortus* biovar 1 isolated from cattle by all available methods for identifying and characterizing strains of *B. abortus*. Except for one isolate of *B. abortus* biovar 2 the same is true of *B. abortus* isolated in WBNP (Tessaro, 1987).

SEROLOGIC RESPONSE OF BISON AND CATTLE EXPOSED TO *B. ABORTUS*

Prior to introduction of the Uniform Methods and Rules for the Eradication of Bovine Brucellosis in 1947 (U.S. Department of Agriculture, 1992), the classification of seroagglutination titers into suspect or positive (seroreactor) categories was subjective. Thus, the number of animals investigators included in the suspect and

positive categories did not always represent animals with similar seroagglutination titers. Since the significance of serum agglutination antibody titers has not been evaluated for bison, and to present a worst case scenario for disease assessment as suggested by McCorquodale and DiGiacomo (1985) for wild ungulates, by Morton et al. (1981) specifically for elk, and by the USDA (General Accounting Office, 1992), we also combined the suspect and positive categories on all the serosurveys in each of the three national parks. When all titers of 1:25 and over were considered positive, the proportion of bison with positive seroagglutination titers was 795 (37%) of 2089 bison for WBNP (Corner and Connell, 1958; Choquette et al., 1961, 1978; Tessaro, 1987); eight (50%) of 16 bison in GTNP (Williams et al., 1993); and 1348 (40%) of 3364 bison in YNP (Clark and Kopec, 1985; Pac and Frey, 1991; James, 1992).

RELATIONSHIP BETWEEN SEROAGGLUTINATION TITERS AND RECOVERY OF *B. ABORTUS* FROM TISSUES

One essential component of the bovine brucellosis eradication program is serologic testing to identify infected cows and cows suspected of being infected. Among many reasons, but primarily because antibody level as measured by seroagglutination and presence of infection as determined by recovery of *B. abortus* from tissue are in close agreement (Manthei and Deyoe, 1970), the tube seroagglutination test is one of the most reliable and widely used of the serologic tests to identify infection in cattle. Lambert et al. (1960) reported *B. abortus* isolations from 90% of seroreactor cows, Herr et al. (1982) found an 86% agreement, and Manthei and Deyoe (1970) documented a 95% agreement, between culture and seropositivity. The sites from which recoveries were made most often were supramammary lymph nodes, udder (milk), and the iliac and retropharyngeal lymph nodes (Manthei and Deyoe, 1970).

Clark and Kopec (1985) reported that between 1917 and 1985, seroagglutination tests were conducted on 2,211 YNP bison, of which 998 (45%) had antibody titers of 1:25 or above. The tissues from 85 of these tested bison were cultured for *B. abortus* (James, 1985). Even though only seven (8%) were bacteriologically positive, Clark and Kopec (1985) concluded from the serological evidence that the YNP bison herd had been highly infected since 1917.

Since that time 301 bison, 218 from YNP, 67 from WBNP, and 16 from GTNP, have been tested simultaneously by seroagglutination and bacteriologic culture of their tissues. All specimens were obtained opportunistically. On most bison the age, sex, and pregnancy status were known.

The specimens from the YNP herd were obtained during the winter of 1991 to 1992 when the bison were shot after exiting the park. It is among these bison that the USDA (General Accounting Office, 1992) said that, based on serologic tests, the infection prevalence was about 50%. While 106 (49%) did have seroagglutination titers of at least 1:25, an analysis of the age, sex, and pregnancy status of these serotitered bison in relation to culture positivity of their tissues revealed an entirely different picture of the *B. abortus* infection in the YNP bison.

Among these 218 bison, 113 were males. Of these, 61 (54%) males had seroagglutination titers, and 17 (28%) of the 61 were culture positive. Among the males, 36 were adults over 5 yr old; 18 (50%) of these had seroagglutination titers, and two (11%) of the 18 were culture positive. Among the 77 subadult males, 43 (56%) had seroagglutination titers; 13 (30%) of these 43 bison were culture positive, and two that were seronegative were culture positive. Thus, among 77 subadult males, 15 (19%) were culture positive and seven (47%) of these 15 bison were calves and yearlings (James, 1992; National Veterinary Services Laboratory, 1992).

Among the 105 females evaluated, 45 (43%) had seroagglutination titers; nine

(20%) of these 45 bison were culture positive. Among the females, 68 were adults over 3 yr old except for one pregnant 2.5-yr-old animal. Among these 68 adults, 54 (79%) were pregnant; 26 (48%) of these 54 animals had seroagglutination titers, two of which were culture positive. From both of these pregnant bison, *B. abortus* was isolated only from lymph nodes. Among 14 adult, non-pregnant females, four had seroagglutination titers but none were culture positive. Thus, among 68 adult females, 30 (44%) had seroagglutination titers and two (7%) of those 30 bison were culture positive (James, 1992; National Veterinary Services Laboratory, 1992).

Included among the females were 37 subadults, 15 (40%) of which had seroagglutination titers. Seven (47%) of those 15 animals were culture positive.

Among the 218 bison there were 114 subadults, of which 58 (51%) had serotiters; 20 (34%) of the 58 bison were culture positive, and two male subadults were culture positive but seronegative. Thus, 22 (85%) of the 26 animals from which *B. abortus* was isolated were subadults.

Eighteen (24%) of the 74 bison tested by Tessaro (1987), were seroagglutination positive, culture positive, or both. Among the 12 bison on which both tests were performed, each had an elevated seroagglutination titer and five (42%) were culture positive. Of these five, four were males, yearlings, or both. Among the six bison that were culture positive but were lacking serologic results, three were males and one was a female yearling. Thus, among 11 culture positive bison, eight (72%) were either males, yearlings, or both.

Eight of 16 bison were seroagglutination positive in the GTNP herd (Williams et al., 1993). Of these, five were males, two of which were subadults and three were young (5-yr-old) adults. Among the eight with seroagglutination titers, three were culture positive. Two of the *B. abortus* isolates were from females, one of which had recently aborted, and one isolate was from a subadult male. In addition, one seronegative female calf was culture positive. Thus, two of the four culture positive bison were not adult.

Among the 126 bison from the three national parks that had seroagglutination titers, 34 (26%) were culture positive, and three culture positive bison were seronegative. Of the 34 culture positive bison, 30 (88%) either were males or subadults; most were calves and yearlings.

Validity of culture data has been refuted by members of the USDA (General Accounting Office, 1992) from YNP bison by arguing that failure to isolate the organism did not mean freedom from disease, only that there were no viable organisms in the particular tissues cultured. In view of the fact that USDA staff members long have believed that the YNP herd is not responding to brucellosis any differently than cattle herds with chronic brucellosis (Meagher, 1973b), it would seem that data would be offered to substantiate its position on lack of culture recovery. For example, if the organisms were present and simply not being recovered by direct culture, inoculation of tissue specimens into guinea pigs (*Cavia cobaya*) would increase the probability of recovering *B. abortus* (Meyer, 1981). If *B. abortus* organisms were present but not viable, various histologic staining techniques are available to identify *Brucella* spp. in tissues, such as immunofluorescence (Moulton and Meyer, 1957), or immunoenzymatic staining and other tissue staining techniques (Mayfield et al., 1990). Immunofluorescence staining of tissue impression smears (Meyer, 1966b) also can be used as an additional method to establish the presence of *Brucella* spp. in tissues.

There is a marked difference in percent of *B. abortus* isolated from seropositive cattle compared to bison, and this may reflect a difference in the circulating antibody response between these two species. Bison, however, are not the only Bovidae wherein the seroagglutination test is not correlated with infection. Renoux (1957) reported that 24% of bacteriologic-positive

sheep and goats were seronegative on both plate and tube agglutination tests.

SEROAGGLUTINATION IN RELATIONSHIP TO OTHER EVIDENCE OF *B. ABORTUS* INFECTION IN FREE-RANGING BISON

If the gap between the number of seroagglutination positive and culture positive animals is an artifact due to failure to recover *B. abortus* from infected bison, then there should be disease manifestations that not only identify the presence of brucellosis but also have a sufficient impact on the herds to substantiate these high percentages of serotiters. In individuals, this could include findings of pathologic lesions consequent to infection in both males and females. On a herd basis, this could include transmission of brucellosis to other herds of susceptible animals and to humans. Equally important is the long-term effect of brucellosis on herd population dynamics.

The pathologic consequences of brucellosis in individual animals include abortion, retained placenta, thickened and leathery placenta, necrotic cotyledons, vaginal discharge, metritis, and possible sterility (Jubb et al., 1985). In males, the consequences include epididymitis, orchitis, seminal vesiculitis, and possible sterility (Manthei and Deyoe, 1970).

During the era of population control in YNP, most bison were slaughtered, dressed out in the park, and hides and meat salvaged for allotment to Indian agencies (Skinner, 1941; Meagher 1973a). From 1935 through 1950, veterinarians present at slaughter operations performed at least 400 necropsies. In some population reductions, as many as 90% of the females were pregnant (Coburn, 1948; Rogers, 1950), many seropositive females were pregnant, and the percent of females pregnant was equal among seroagglutination reactors and nonreactors (Skinner, 1941). Rush (1932b) reported 74% of pregnant females were seropositive and 72% of seroagglutination-negative females were pregnant. In the 1992 winter exodus, 45% of the pregnant females had seroagglutination titers (Frey, 1992).

During the course of these necropsies, approximately 150 fetuses were examined. No evidence of disease was found by Quortrup (1944), who also commented that he had never observed necrotic cotyledons.

Brucella abortus was isolated from an enlarged epididymis from a YNP adult male cultured at Montana Veterinary Research Laboratory (Tunnicliff and Marsh, 1935). The other nine adult males in the group were culture negative. Quortrup (1944) commented that he found very few gross lesions in adult males and those were limited to a slight enlargement of the epididymis and mild orchitis. Among 94 adult males necropsied by Coburn (1948) seven adults had mild to moderate epididymitis or orchitis.

Few bison were reported to have had pathologic manifestations of *B. abortus*. The persistent high prevalence of *Brucella* antibody titers, as measured by seroagglutination, is not qualitatively related to the presence or severity of pathologic lesions, or apparently to abortions.

Documentation of abortion in the YNP herd is problematic. The park encompasses almost 900,000 ha and bison often inhabit remote locales, making abortions difficult to detect. In addition, we believe most fetuses would be devoured almost immediately by scavengers. Even so, in the last few years, the exodus of hundreds of bison across the park boundary into Montana has greatly increased the opportunity to observe abortions, to find fetuses, or those animals with protruding retained placentas or vaginal discharges. The winter exodus of YNP bison coincides with their mid-gestation period, from mid-December to late January (Meagher, 1973a). Abortions could be occurring any time from mid-December to termination of gestation, which is approximately mid-April to mid-June for most YNP bison. From 12 December 1988 to 20 March 1989 approximately 900 bison emigrated into Montana (Ferlicka, 1989). Of these, 569

were shot. Blood samples were obtained from 484 animals of which 279 were males, 202 were females, and three were of unknown sex (Pac and Frey, 1991). Among the 202 females, 89 (44%) were seropositive and were considered to be infected. While in Montana, they commingled with 810 head of cattle in 18 herds. All of the Montana cattle later tested seronegative on two consecutive tests (Ferlicka, 1989).

The 16 GTNP bison shot on the National Elk Refuge during a herd reduction were examined for both gross and microscopic lesions (Williams et al., 1993). Although 16 is a small sample, the pattern of response of these bison to exposure to *B. abortus* was similar to that found in the YNP bison; there was little relationship between elevated seroagglutination titers and presence of infection as measured by recovery of *B. abortus*. Three of eight seroagglutination reactors were culture positive. Among eight seronegative bison, one was culture positive.

In WBNP between 1950 and 1974, approximately 6,000 bison were slaughtered for use as meat. Selection of animals for slaughter was based, in part, on evidence for tuberculosis and brucellosis (Tessaro, 1987), but no brucellosis lesions were noted.

Among 72 carcasses obtained opportunistically by Tessaro (1987) and necropsied between June 1983 and August 1985, the only lesions found were an enlarged stifle joint in each of three adult males, one of which also had a carpal hygroma. Tessaro (1987) believed that the sample, which consisted of 23 adult males, 27 adult females, 18 calves, and six adults of unknown sex was sufficiently broad to represent the WBNP bison population; although 72 carcasses were reported, the total appears to be 74 animals. From data on these bison, he estimated the prevalence of brucellosis as 15 to 36%. Thus, as in the YNP and GTNP bison, the percent with seroagglutination titers was considered high, but few had pathologic lesions.

While presence of lesions and occurrence of abortions can provide a measure for the impact of brucellosis on a herd, transmission of the disease to nearby herds and to humans also is a useful gauge to herd infection status (Ray, 1977). There was no transmission from bison to cattle in the extensive contact between these two species during the winter of 1989 in Montana and there is no documentation that transmission from free-ranging bison to cattle or to humans has ever occurred. Between the time bison population reductions first started in the mid-1920's until the early 1950's, butchering of bison removed for human consumption was done in the park slaughterhouse by YNP employees (Meagher, 1973a). Thereafter the bison were either field-dressed or were taken to a commercial slaughterhouse in Livingston, Montana. Thousands of bison carcasses have been skinned, eviscerated, and handled by YNP employees and commercial butchers but no known cases of brucellosis have been reported from these slaughter operations. Winship (1989) alluded to cases of brucellosis occurring among a few veterinarians during the 1970's, supposedly arising from contact with YNP bison. However these were not reported to the Communicable Disease Center of Atlanta, Georgia (USA) so they cannot be confirmed. In contrast, one of the most common sources for *B. abortus* infection in humans is from handling and butchering bovine carcasses in slaughterhouses and meat packing plants (Steele, 1965). In view of the ample contacts between YNP bison and cattle, and between carcasses of YNP bison and humans, we conclude that these bison have not served as a reservoir of infection.

Another gauge to help ascertain if the prevalence of seroagglutination titers is approximately equivalent to the prevalence of infection is the effect of brucellosis on the population. In cattle herds infected with brucellosis, the impact of the disease on pregnancy, calf crop, and calf survival for the first several post-natal months can be observed and accurately measured. It

usually is difficult to attribute population declines in free-ranging bison herds to a specific cause unless the decline is precipitous or the cause is dramatic.

Among the three free-ranging infected bison herds, the WBNP herd for many years has had a declining population while both the YNP and GTNP herds have had increasing populations. The WBNP herd has had a marked and continuous decline since 1971 (Carbyn et al., 1993). In 1971 there were approximately 10,000 bison in WBNP, while in 1991 the number was estimated to be 3,000. This herd has been beset by a variety of problems, including massive drownings, wolf predation, possible loss of nutritive value of sections of grazing land due to placement of a dam external to the park, intermittent outbreaks of anthrax, and a concurrent enzootic problem with bovine tuberculosis (Tessaro et al., 1990; Carbyn, 1992). Some investigators believe that tuberculosis, and particularly brucellosis, is affecting the population dynamics by reducing fecundity and by increasing vulnerability of individuals to predation from lameness (Gates et al., 1992; Wobeser, 1992). In contrast, Carbyn (1992) believed that there is such an interplay of complex factors adversely affecting the WBNP bison population that it is not possible to sort out just a single factor.

Since 1980, the GTNP herd has been fed for up to 3 mo each winter on the National Elk Refuge, in association with brucellosis-infected and aborting elk (Grand Teton National Park, 1994). Even with probable repeated exposure to brucellosis, the herd has increased from 40 to 212 bison and there has been but one reported abortion (Williams et al., 1993).

There has been a continuous increase in the YNP bison population, from 397 in 1968 to 3,551 in January 1994 (Meagher, 1973a; M. Meagher, unpubl.). Despite 1,052 bison having been shot from 1968 through 1992 there has been a net increase of 4,250 animals exclusive of natural mortality and road kills (M. Meagher, unpubl.). This reproductive capability is being maintained in a herd made up of approximately 50% males, in environmental conditions that preclude every mature female from calving every year, and that adversely affects calf survival. The YNP population increased annually by an average of nearly 200 animals between 1974 and 1990 (M. Meagher, unpubl.). Calf mortality, which is largely due to winter mortality, reaches approximately 50% by the end of the second post-natal winter (Meagher, 1973a). Winter mortality, especially in severe winters, could even be higher. To recruit 200 animals into the YNP herd would require a estimated calf crop of about 400. If brucellosis had a measurable toll through reduced fertility or abortion as additive to winter mortality, it is doubtful that this rate of herd growth could be maintained. Interestingly, early investigators (Rush, 1932a; LaNoue, 1932; Tunnicliff and Marsh, 1935) commented that brucellosis did not seem to adversely affect herd growth.

IMMUNIZATION OF PREGNANT BISON COWS WITH *B. ABORTUS*, STRAIN 19

In a trial to assess the efficacy of strain 19 for immunizing adult bison, Davis et al. (1991) injected 92 pregnant adult female bison with the dose of strain 19 used for adult cattle. Following immunization, the inoculated bison were returned to the range to intermingle with 837 bred unvaccinated adult female bison. Abortions began in the immunized group 60 days post-immunization and continued for 3 mo, by which time 58 (63%) of the 92 bison had aborted. There were possibly five additional unobserved abortions. The calf crop among the immunized bison was 24%, and 93% among the remaining 837 females. Of 13 fetuses salvaged from the trial group, 12 were culture positive for strain 19.

The response of pregnant bison to administration of strain 19 differed markedly from that of pregnant cattle. In cattle abortion occurs only in 1.0 to 2.5% of cows that

are immunized during pregnancy (McDiarmid, 1951; Nicoletti, 1977). The 69% abortion among the 92 immunized bison was higher than the 50% induced by virulent *B. abortus*, strain 2308 used earlier in a bison susceptibility trial (Davis et al., 1990). Additional evidence for the pathogenicity of strain 19 for bison occurred in two of the immunized cows that aborted, wherein infection with strain 19 became chronic, and abortions occurred during subsequent pregnancies (Davis, 1993).

In cattle, the immunization of adult females, whether pregnant or not, commonly is used as a control measure in chronically infected herds. Among 14,800 cows in Florida (Nicoletti, 1981a) and approximately 8,500 cows in California (Breitmeyer et al., 1992) vaccinated as adults, less than 2% shed strain 19 in their milk; also, there were no abortions attributable to strain 19, and no chronic uterine infections. There is no documentation of chronic strain 19 uterine infection in cattle. Additionally, chronic uterine infections in cattle following abortions caused by virulent field strains of *B. abortus* occur only in approximately 5% of infected cows; these may abort a second time (Manthei and Carter, 1950; Stableforth, 1959).

Among the 837 bison that commingled with the 92 immunized and aborting bison cows, 14 seroconverted. Even though strain 19 was as pathogenic for pregnant bison as was virulent *B. abortus* strain 2308, no strain 19 organisms were recovered from any of these 14 animals, and Davis et al. (1991) concluded that lack of recovery of strain 19 was evidence that secondary exposure probably did not result in chronic strain 19 infections. In this situation the results of culture prevailed over serologic test results in coming to the conclusion that these 14 seropositive animals probably were not infected. In defining an animal as test positive, Davis et al. (1991) indicated that these 14 were seropositive on at least one of several serological tests including the card, rivanol, cold complement fixation, hemolysis in gel (HIG), and bison conjugated enzyme linked immunoassay (Bis-ELISA) tests. Significantly, Davis et al. (1990) concluded that, in combination, most of these tests reliably detected bison infected with *B. abortus* 10 wk post-inoculation, and that the card test, when combined with Bis-ELISA or HIG, would reliably diagnose brucellosis in bison at 8 wk post-inoculation. Thus, it would appear that the same results are simultaneously interpreted as both reliable and unreliable indicators of infection.

In free-ranging bison in the national parks, results of the serologic tests prevailed over bacteriologic results in determining the status of infection in individual bison and also of the percentage of infected animals within each herd. However, the pattern of response among free-ranging bison and the 14 bison cows that commingled with their aborting cohorts was similar. In both groups serologic results were considered diagnostic of infection, but there was little or no abortion and few cultures of *B. abortus* were recovered from their tissues.

In contrast, in both the adult immunization study (Davis et al., 1991) and the susceptibility study (Davis et al., 1990), 50% or more bison aborted and *B. abortus* was recovered from tissues of both dam and fetuses. Among the 12 animals in the susceptibility trial, there was 95% agreement between the bacteriologic and seroagglutination test results (Davis et al., 1990). Among the 92 strain 19 vaccinates, individual serologic test results were not given, but at 10 mo post-vaccination, 73% were still seroreactors (Davis et al., 1990). By the definitions of positivity as footnoted to table 1 of Davis et al. (1991), these should be called seroreactors. In any event, 69 to 74% had aborted post-immunization. Thus, percent abortion and percent seropositive animals were equivalent with strain 19.

The test results on bison differ according to route of exposure. Among those exposed by inoculation, as in the susceptibility study (Davis et al., 1990) and the immunization study (Davis et al., 1991), *B. abortus* was

recovered from 95% of the seropositive animals. However, among bison exposed orally, *B. abortus* was not recovered from any of the 14 bison that seroconverted following grazing with aborting herd members (Davis et al., 1991); among the three free-ranging bison herds it was recovered from only 34 (27%) of 126 seropositive animals. Route of exposure has a determinative effect on host response (Xin, 1986). This difference in response may be subtle. For example, among adult cattle administered strain 19, 1.4% vaccinated conjunctivally later shed strain 19 in their milk, 0.8% vaccinated subcutaneously became shedders, and among those vaccinated intradermally, none became shedders (Nicoletti, 1981b). However the difference in host response to route of exposure may be marked. For example, *B. suis* biovar 1, strain 2, is used in China as an oral immunizing agent in swine, cattle, sheep and goats (Xin, 1986), and is viewed as a safe and efficacious immunizing agent. While non-pathogenic when administered orally, subcutaneous injection into pregnant sheep and goats causes them to abort (Xin, 1986).

The evidence is circumstantial that the route of exposure to *B. abortus* may be one of the factors responsible for the differences observed between free-ranging and experimentally infected bison. However, it is the only discrete biological event that differs between these two bison groups. Well-designed and executed research projects are needed to answer this question and many others concerning brucellosis in bison.

DIFFERENCES BETWEEN BISON AND CATTLE IN RESULTS OF CALFHOOD IMMUNIZATION WITH STRAIN 19

Efficacy of immunizing agents for preventing or minimizing the effects of exposure to virulent strains of *B. abortus* is measured by the percentage protection against both abortion and infection (Manthei, 1959). Among bovine heifers immunized as calves with strain 19 and then challenged with virulent *B. abortus* in the second trimester of pregnancy, approximately 65 to 75% are protected against abortion (Manthei, 1959; Nicoletti, 1980) and of the 25 to 35% that become infected, many do not abort (Manthei, 1959).

Bison calves responded to strain 19 differently than bovine calves. Among 60 bison cows immunized with strain 19 when 8 mo of age and challenged with virulent *B. abortus* strain 2308 during their second trimester of pregnancy, 75% aborted and 91% became infected. This was similar to the non-immunized control group wherein 80% aborted and 87% became infected (Davis, 1993).

RESPONSE OF CALVES TO INJECTION AND INGESTION OF *B. ABORTUS*

Bovine heifer calves injected subcutaneously with strain 19 at 3 mo (King and Frank, 1961), 4 mo (Gilman and Wagner, 1959), and 8 mo of age (Lambert et al., 1961), developed seroagglutination titers within 21 days and reached a peak titer of 1:3200 before starting to recede. In 8-mo-old bovine calves, seroagglutination titers remained elevated in the 1:800 to 1:1,600 range for several months and in some instances did not recede below 1:100 to 1:200. When strain 19 is administered to bovine calves older than 9 mo old, persistent titers may interfere with the diagnosis of infection (Ray, 1979). Bison calves, however, respond quite differently. For example, in a group of 43 YNP yearling bison that had been immunized a year previously as calves, 18 were seroagglutination negative, three had titers of 1:100, and the remaining had titers of 1:25 or 1:50 (Coburn, 1948).

Exposure by ingestion produced opposite results. Based on field observations, Carpenter (1924) proposed that bovine heifer calves that nursed on infected dams rarely, if ever, became infected. This observation later was supported experimentally by feeding calves of various ages with known dosages of *B. abortus* organisms added to milk (Carpenter, 1924; Nagy and

Hignett, 1967). Infections did not become established in these calves, nor did they develop seroagglutination titers (Ray, 1977). These bovine calves did not respond to oral exposure by production of serum antibodies, or did so minimally and transiently. Yet, based on the data on relation of seroagglutination titer to bacterial culture we report herein, we believe that young bison have a humoral response to oral exposure. Because of differences between bison and cattle in the time required to reach sexual maturity, it is not possible to compare the two species simply by time elapsed from birth. While most bovine females become sexually mature as yearlings between 1 and 2 yr old, most female bison in YNP become sexually mature at 3 to 3.5 yr, and have their first calf during their fourth year (Meagher, 1973a). However, there may be variations at each end of this age spectrum; Williams et al. (1993) observed an abortion in a 2-yr-old GTNP female bison. Bison bulls mature sexually at ages comparable to females, but do not become sexually active until about 6 yr (Meagher, 1986). Among the YNP herd, calves, yearlings, and subadult animals periodically have been tested for antibodies. Tunnicliff and Marsh (1935) found that 43% of male and 25% of female subadult bison were seropositive. During the 1952 population reduction, 19 yearling males and seven yearling females were seropositive, as were 46 subadult males and six subadult females (Phillip, 1952). In the bison kill the winter of 1988 to 1989, 95 (59%) of 160 males 6 yr and under and 23 (34%) of 67 females 4 yr of age and under were seropositive (Pac and Frey, 1991).

INTRA-HERD EPIZOOTIOLOGY OF BRUCELLOSIS IN BISON

From the data available for the YNP herd, and from the GTNP herd, which has been observed during the midgestation-abortion period for each of the past 10 yr, abortion appears to be an infrequent occurrence. Only three abortions have been documented in the YNP herd, the two originally reported by Mohler (1917) and one that occurred in 1992 wherein *B. abortus* biovar 1 was recovered from the fetus (Rhyan et al., 1994). In GTNP, one abortion has been documented (Williams et al., 1993).

Because the antibody prevalences among subadult bison are remarkably high (56% in males, 44% in females), and because abortions in the YNP herd apparently occur infrequently, young bison must be exposed to the organism from sources other than abortion products. One possible route of exposure is through ingestion of infected milk. Infected bison cows excrete *Brucella abortus* in their milk within 3 wk of infection (Davis et al., 1990). Most infected females among domesticated livestock species excrete the organism for months or years following infection. For example, in goats, *B. melitensis* has been isolated from the udder 12 mo post-infection (Mediterranean Fever Commission, 1907). In cattle it was recovered throughout the first lactation period, and not uncommonly into the second (Brinley-Morgan and McDiarmid, 1960). We believe the most reasonable explanation for isolation of *B. abortus* from tissues of bison calves and yearlings was the excretion of the organism throughout most or all of the lactation period. Bison calves in the YNP and GTNP herds, and presumably in all free-ranging bison, nurse for 8 to 10 mo, with a few doing so as yearlings (Meagher, 1973a). When the dam is infected, we speculate that the calves receive an essentially ongoing, oral, low dose of exposure to *B. abortus* for the full length of their nursing period. Following this nursing period, most female bison calves have two more years before they reach sexual maturity.

As an explanation for the apparent infrequent occurrence of abortions in these bison herds, we hypothesize that in the interim between cessation of nursing and sexual maturity, many bison cows develop resistance to abortion. Natural transmission of the organism from dam to female calf may stimulate development of im-

munity to brucellosis infection as does the administration of *B. abortus* strain 19 for bovine heifer calves. Bison appear to develop increased protection against genital infection and, to a lesser degree, protection against extragenital infection. In fact, oral immunization with attenuated *B. suis* is widely and successfully used in China (Xin, 1986) for the protection of cattle, sheep, and goats against brucellosis. The oral route also has been successful for experimental immunization of cattle with strain 19 (Nicoletti and Milward, 1983).

Male bison do not become fully mature until they are about 6 yr old (Meagher, 1986). Subadult bison males consistently have a higher antibody prevalence than subadult bison females. However, the number of adult males observed with discernable lesions are few in number; thus, it seems that between nursing and maturity, males also may develop immunity following 8 to 10 mo of oral exposure.

STATUS OF THE HERDS

Those who support the concept that brucellosis of bison is indistinguishable from that of cattle maintain that the disease as manifested in free-ranging bison herds is no different than that seen in chronically infected cattle herds (General Accounting Office, 1992; Gates, 1993). Each of the three free-ranging herds has had brucellosis present for a long time, but there is little evidence they have the complex of manifestations typical for chronic brucellosis in cattle.

In chronic brucellosis, abortions occur cyclically in most chronically infected cattle herds (Stableforth, 1959), depending upon how rapidly the population is replenished. As new-born females mature and the number of susceptible animals increase, abortions will occur and then clinical manifestations of the disease will become unobtrusive until the cycle recurs. Cyclic occurrence of abortions has not occurred in the observed GTNP herd. It has grown by 212 new members since 1980. Even with deliberate reduction and natural mortality the herd census was 173 animals in January 1993 (Grand Teton National Park, 1994). Assuming a sex ratio of 50:50 among new born calves as in the adjacent YNP herd (Meagher, 1973a), we predict there now would be about 50 to 60 sexually mature susceptible females in the GTNP herd. However, no abortions or evidence thereof have been reported among live animals on the feed ground. The YNP herd has increased by approximately 200 to 300 animals each year for the last several years (Meagher, 1993). Thus there also is a sizeable pool of sexually mature susceptible females in this herd. Of the 102 adult females shot during the winter of 1988 to 1989 exodus, 74 (75%) were pregnant (Pac and Frey, 1991). In the winter of 1991 to 1992 exodus, 54 (79%) of 68 adult females were pregnant and 26 (48%) of these 54 bison were seropositive. Four of the pregnant cows were approximately 3 yr old and thus, each was probably in her first pregnancy. All were culture negative in the genital tract and only one was culture positive in the lymph nodes (James, 1992). The only isolation from a female genital tract was from a yearling approximately 18 mo old (James, 1992; National Veterinary Services Laboratory, 1992).

Among the 72 bison carcasses cultured by Tessaro (1987), 27 were mature females, nine of which were pregnant. He commented that none of the females had aborted, or had metritis or retained placentas. However, *B. abortus* was recovered from lymph nodes of three of these females.

As reported by the General Accounting Office (1992), workers from the Animal and Plant Health Inspection Service (APHIS) stated that the prevalence of brucellosis infection among YNP bison removed from the herd in the winter of 1991 to 1992 was 50%. Actually, of the 240 bison tested, 85 (35%) were seropositive and 24 (10%) were suspect positive animals, for a combined antibody prevalence of 45%. This percentage differed considerably from that reported for chronically infected cat-

tle herds. In Louisiana (USA), five chronically infected beef herds tested after having been free-ranging for 22 yr had an antibody prevalence between 3.1 and 16% (Enright, 1990).

In California (USA), antibody prevalence taken prior to the initiation of vaccination control programs were 16 to 17% for dairy cattle and 9% for beef cattle (Stuart et al., 1959; Wixom and Vanderwagon, 1965). In surveys prior to the initiation of control programs in each of ten west African and five east African countries, antibody prevalence of 10 to 16% occurred consistently in chronic brucellosis-infected cattle (Thimm and Nauwerk, 1974).

Gates (1993) also is a proponent of the concept that brucellosis in free-ranging bison herds, particularly in the WBNP herd, is identical to chronic brucellosis in cattle. In support of this position he stated that "lesions associated with brucellosis in bison in northern Canada are similar to those observed in cattle by Tessaro, 1988 [sic]". However, Tessaro (1987) reported that none of 27 bison bulls in his sample had epididymitis or orchitis and none of the bison cows had any lesions in the reproductive organs. He described four adult male bison with limb lesions, including a subcutaneous abscess and three adult males with arthritis in one stifle joint. One of these males also had a carpal hygroma.

Gates (1993) further supported his position by citing McDermott et al. (1987) on the association between abortion and hygromas in infected cattle in southern Sudan. In other areas of Africa, hygromas also were associated with numerous abortions (Domenech et al., 1980) and were breed-associated with Longhorn Sanga cattle in East Africa (Mortelmans and Kageruka, 1970). Hygromas in multiple sites, involving the neck, hips, forelegs, and hind legs were problems associated with brucellosis in the indigenous breeds of cattle in Rwanda-Burundi (Thienpont et al., 1961). Bovine brucellosis in the tropical regions of Africa differs both clinically and epizootiologically from that observed in the temperate zones (Ferney and Chantel, 1976); these differences have been associated with temperature, humidity, and solar radiation, and also to breeding conditions among the animals of nomadic people. When climatic factors and breeding conditions resembled those of the temperate zones, the clinical and epizootiologic aspects returned to the usual pattern of bovine brucellosis (Ferney and Chantel, 1976). Comparing one hygroma and three arthritic stifle joints in bison bulls in the WBNP herd to the chronic and serious abortion and hygroma problems in cows in Africa is highly questionable.

The third line of evidence Gates (1993) used to substantiate his position that brucellosis in free-ranging bison can be equated with chronic brucellosis in cattle was based on his belief that results of the study on the susceptibility of 12 captive bison to *B. abortus* (Davis et al., 1990) and of the study on immunization of pregnant bison cows with strain 19 (Davis et al., 1991) provided limited empirical information on herd population dynamics and that these dynamics paralleled those of cattle herds chronically infected with brucellosis.

CONCLUSIONS

There are marked differences between bison and cattle in their responses to infection with *B. abortus*. Seroagglutination titers of bison are neither reflective nor diagnostic of the status of infection of bison. Among 75% of seropositive bison the relationship between serotiter and infection could not be confirmed by recovery of *B. abortus* from appropriate organs. Other evidence to indicate the magnitude of infection in these herds, such as lesions of the reproductive tract, abortions, or occurrence of transmission, all of which would help validate the magnitude of infection rates, were rare. Thus, we propose that bacteriologic results provide a closer approximation of the amount of infection in these bison herds than do seroagglutination titers. Reliance on serologic results in the absence of supportive evidence is

considered a misuse of serology (Worthington, 1982).

Another difference between cattle and bison is source of recovery of *B. abortus*. Among bison, isolates were primarily from calves, yearlings, and subadults. In comparison, few isolations were made from mature animals, but these data provide strong evidence that infected milk of dams is a source of the organism and ultimately of what appears to be immunity to abortion. It also helps to explain the lack of human cases that normally arise in slaughterhouses. Young animals usually are not the ones slaughtered, and few older animals seem to harbor the organisms. This also helps explain the lack of transmission. Sexually immature animals are not the age group that spreads the infection.

A further difference between bison and cattle is the number of seropositive bison calves. While many of these animals probably develop titers from ingestion of infected milk, we also suspect an exposure source from non-fetal environmental contamination. *Brucella abortus* may pass through the ruminant digestive tract as it has been recovered from feces of calves that either nursed infected dams' milk or were fed artificially infected milk (Ranney, 1934). None of these investigators attributed further herd infections to contaminated feces, but Stableforth (1959) considered that such calves could be a source of infection.

In the YNP herd, most calves are born during a 6-wk period from 15 April to 31 May (Meagher, 1973a). The weather is cool, sometimes freezing, and the ground is damp, which is ideal for the environmental survival of *B. abortus* (King, 1957). Bison females with calves tend to socialize with each other rather than with cows without calves (Meagher, 1986). For the last several years, approximately 400 to 600 calves have been born in YNP yearly, as documented during aerial surveys (M. Meagher, unpubl.). It certainly seems that calves nursing infected dams and intermingling with the other calves may provide a source of exposure. This would be an excellent topic for experimental investigation.

The data available have provided insight into the nature of brucellosis in free-ranging bison, but additional data are needed. Tissues and fluids for testing now can be obtained selectively; the population segment that could furnish the reliable data are the females from 2.5 to 4 yr old.

Brucellosis of bison is not mimetic of bovine brucellosis. It appears that most, if not all, of the differences between their responses to infection with *B. abortus* are expressions of differences in immune responses. This also has been found in other host-parasite relationships involving *B. abortus* (Meyer, 1966a).

The immunological response of bison apparently differs significantly from cattle. The bacteriologic results from seropositive bison is evidence for a wide exposure to the antigen and a low level of actual infection. If a test and slaughter program, as used in infected cattle herds, were to be used in either the YNP or GTNP herds, it certainly would result in the eradication of animals with antibodies and probably would lead to eradication of the herds.

ACKNOWLEDGMENTS

We thank S. Broadbent and L. Mack for assistance with manuscript drafts. This publication was supported financially by the National Park Service and the National Biological Service.

LITERATURE CITED

ALCORN, W. B. 1947. Supplement, 1942–1947. Incl. history of the bison in Yellowstone Park. Archives, Yellowstone National Park, Wyoming, 7 pp.

ANONYMOUS. 1931. Report of committee on Bang's disease. Part I. Recommendations for a uniform technique for conducting the tube agglutination test for Bang's disease. Proceedings of the United States Livestock Sanitary Association 35: 323–325.

———. 1932. Report of committee on Bang's disease. Proceedings of the United States Livestock Sanitary Association 36: 335–336.

AUNE, K. E., AND P. SCHLADWEILER. 1993. Wildlife

laboratory annual report. December 1992. Montana Department of Fish, Wildlife, and Parks, Bozeman, Montana, 49 pp.

BANG, B. 1897. The etiology of epizootic abortion. Journal of Comparative Pathology and Therapeutics 10: 125–149.

BECTON, P. 1977. The national brucellosis program in the United States. *In* Bovine brucellosis, R. C. Crawford and R. J. Hildago (eds.). Texas A and M University Press, College Station, Texas, pp. 403–411.

BREITMEYER, R. D., D. W. HIRD, AND T. E. CARPENTER. 1992. Serologic and bacteriologic test results after adult vaccination with Strain 19 in three dairy herds infected with brucellosis. Journal of the American Veterinary Medical Association 200: 806–811.

BRINLEY-MORGAN, W. J., AND A. MCDIARMID. 1960. The excretion of *Brucella abortus* in the milk of experimentally infected cattle. Research in Veterinary Science 1: 53–56.

CARBYN, L. N. 1992. Wolves and bison. Wood Buffalo National Park—Past, present, and future. *In* Buffalo, J. E. Foster, D. Harrison, and I. S. MacLaren (eds.). Alberta Nature and Culture Series, University of Alberta Press, Edmonton, Alberta, Canada, pp. 167–178.

———, S. M. OOSENBRUG, AND D. W. ANIONS. 1993. Wolves, bison and the dynamics related to the Peace-Athabasca Delta in Canada's Wood Buffalo National Park. Circumpolar Research Series Number 4, Canadian Circumpolar Institute, University of Alberta, Edmonton, Alberta, 270 pp.

CARPENTER, C. M. 1924. *Bacterium abortum* invasion of the tissues of calves from the ingestion of infected milk. Cornell Veterinarian 14: 16–31.

CHOQUETTE, L. P. E., J. F. GALLIVAN, J. L. BYMES, AND J. PILAPAVICIUS. 1961. Parasites and diseases of bison in Canada I. Tuberculosis and some other pathological conditions in bison at Wood Buffalo and Elk Island national parks in the fall and winter of 1959–60. Canadian Veterinary Journal 2: 168–174.

———, BROUGHTON, J. G. COUSINEAU, AND N. S. NOVAKOWSKI. 1978. Parasites and diseases of bison in Canada IV. Serologic survey for brucellosis in bison in northern Canada. Journal of Wildlife Diseases 14: 329–332.

CLARK, W. W., AND J. D. KOPEC. 1985. Movement of Yellowstone Park brucellosis infected and exposed bison. Report of 15 October 1985, submitted to Brucellosis Committee, United States Animal Health Association. Archives, Yellowstone National Park, Wyoming, 7 pp.

COBURN, D. R. 1948. Special report: Report of field assignment at Yellowstone National Park, January 10–January 29, 1948. Archives, Yellowstone National Park, Wyoming, 30 pp.

CORNER, A. H., AND R. CONNELL. 1958. Brucellosis in bison, elk, and moose in Elk Island National Park, Alberta, Canada. Canadian Journal of Comparative Medicine 22: 9–21.

CREECH, B. T. 1930. *Brucella abortus* infection in a male bison. North American Veterinarian 11: 35–36.

DAVIS, D. S. 1990. Brucellosis in wildlife. *In* Animal brucellosis, K. Nielsen and J. R. Duncan (eds.). CRC Press Inc., Boca Raton, Florida, pp. 322–334.

———. 1993. Summary of bison/brucellosis research conducted at Texas A and M University 1985–1993. *In* Proceedings, North American Public Bison Herds Symposium. Custer State Park, Custer, South Dakota, Archives, Yellowstone National Park, pp. 347–359.

———, J. W. TEMPLETON, T. A. FICHT, J. D. WILLIAMS, J. D. KOPEC, AND L. G. ADAMS. 1990. *Brucella abortus* in captive bison. I. Serology, bacteriology, pathogenesis, and transmission to cattle. Journal of Wildlife Diseases 26: 360–371.

———, ———, ———, J. D. HUBER, R. D. ANGUS, AND L. G. ADAMS. 1991. *Brucella abortus* in bison. II. Evaluation of Strain 19 vaccination of pregnant cows. Journal of Wildlife Diseases 27: 258–264.

DOMENECH, J., P. LUCET, B. VALLET, C. STEWART, J. B. BONNETT, AND L. BERTAUDIERE. 1980. La brucellose en Afrique Centrale: II. Etude clinique et epidemiologique: Particularities regionale et problems de l'elevage semi-intensif. Review D'Elevage et De Medicine Veterinaire Des Pays Tropicaux 33: 277–284.

ENRIGHT, F. M. 1990. Mechanisms of self cure in *Brucella abortus* infected cattle. *In* Advances in brucellosis research, L. G. Adams (ed.). Texas A and M University Press, College Station, Texas, pp. 191–196.

EWALT, D. R. 1989. Comparison of three culture techniques for isolation of *Brucella abortus* from bovine supramammary lymph nodes. Journal of Veterinary Diagnostic Investigation 1: 227–230.

FERLICKA, D. P. 1989. Brucellosis and bison in Yellowstone National Park and Montana. Proceedings of the United States Animal Health Association 93: 674–675.

FERNEY, J., AND J. CHANTEL. 1976. Aspects cliniques et epidemiologiques de la brucellose bovine en Afrique tropicale. *In* International symposium on brucellosis. Developments in biological standardization, International Biological Standardization Council (eds.). S. Karger, New York, New York, pp. 274–278.

FREY, K. 1992. Summary of data collected from bison killed from November 14 to May 6 during winter season of 1991–1992. Wildlife Services Report to Montana Fish, Wildlife, and Parks. Archives, Yellowstone National Park, Wyoming, 4 pp.

FRISON, G. C., AND C. A. REHER. 1970. Age determination of buffalo by teeth eruption and wear. Plains Anthropolgy 50: 46–50.

FULLER, W. A. 1959. The horns and teeth as indicators of age in bison. The Journal of Wildlife Management 23: 342–344.

GATES, C. 1993. Biopolitics and pathobiology: Diseased bison in northern Canada. *In* Proceedings, North American Public Bison Herds Symposium, Custer State Park, Custer, South Dakota, Archives, Yellowstone National Park, pp. 271–281.

———, T. CHOWNS, AND H. REYNOLDS. 1992. Wood buffalo at the crossroads. *In* Buffalo, J. E. Foster, D. Harrison, and I. S. MacLaren (eds.). Alberta Nature and Culture Series, University of Alberta Press, Edmonton, Alberta, pp. 139–165.

GENERAL ACCOUNTING OFFICE. 1992. Wildlife management. Many issues unresolved in Yellowstone bison-cattle brucellosis conflict. Report to the Honorable Alan Cranston, U.S. Senate. 93-2 Wildlife Management, General Accounting Office/Resources Community and Economic Development Devision, Gaithersburg, Maryland, 35 pp.

GILMAN, H. L, AND W. C. WAGNER. 1959. The evaluation of brucellosis vaccination at 4 and 8 months of age. Cornell Veterinarian 49: 399–408.

GRAND TETON NATIONAL PARK. 1994. The Jackson bison herd. Long term management plan and environmental assessment. Public Review Draft, September 1994. Prepared in cooperation with the National Elk Refuge, Wyoming Game and Fish Department, National Wildlife Health Center, and Bridger-Teton National Forest. Report 1994-577-049/05172, U.S. Government Printing Office, Washington, D.C., 111 pp.

HERR, S., D. ROUX, AND P. M. PIETERSON. 1982. The reproducibility of results in bovine brucellosis serology and their correlation with isolation of *Brucella abortus*. Onderstepoort Journal of Veterinary Research 49: 79–83.

JAMES, D. O. 1985. Laboratory reports on serology on Yellowstone bison entering Montana. Archives, Yellowstone National Park, Wyoming, 24 pp.

———. 1992. Laboratory reports on serology and bacteriology on Yellowstone bison entering Montana. Archives, Yellowstone National Park, Wyoming, 19 pp.

JOINT FOOD AND AGRICULTURE ORGANIZATION/ WORLD HEALTH ORGANIZATION EXPERT COMMITTEE ON BRUCELLOSIS. 1964. Technical Report Service Number 289, World Health Organization, Geneva, Switzerland, pp. 9–17.

JUBB, K. V. F., P. C. KENNEDY, AND N. PALMER. 1985. Pathology of domestic animals, 3rd ed., Vol. 1. Academic Press, New York, New York, 527 pp.

KEITER, R. B., AND P. H. FROELICHER. 1993. Bison, brucellosis, and law in the greater Yellowstone ecosystem. University of Wyoming College of Law, Laramie, Wyoming. Land and Water Review 28(1) :1–75.

KING, N. B. 1957. The survival of *Brucella abortus* (U.S.D.A. strain 2308) in manure. Journal of the American Veterinary Medical Association 131: 349–352.

———, AND N. A. FRANK. 1961. Effect of age on resistance and retention of titer in cattle vaccinated with strain 19 *Brucella abortus* vaccine. Journal of the American Veterinary Medical Association 139: 100–103.

LAMBERT, G., T. E. AMERAULT, C. A. MANTHEI, AND E. R. GOODE, JR. 1960. Further studies on the persistence of *Brucella abortus* infection in cattle. Proceedings of the United States Livestock Sanitary Association 64: 109–117.

———, ———, ———, AND ———. 1961. Immunogenic response of calves vaccinated at different ages with *Brucella abortus*, strain 19. Proceedings of the United States Livestock Sanitary Association 65: 93–99.

LANOUE, F. D. 1932. Bang's disease in the Yellowstone National Park buffalo herd. Supplement to Rush's 1932a report. Archives, Yellowstone National Park, Wyoming, 5 pp.

MANTHEI, C. A. 1959. Summary of controlled research with Strain 19. Proceedings of the United States Livestock Sanitary Association 63: 91–97.

———, AND R. W. CARTER. 1950. Persistence of *Brucella abortus* infection in cattle. American Journal of Veterinary Research 11: 173–180.

———, AND B. L. DEYOE. 1970. Brucellosis. *In* Bovine medicine and surgery and herd management, W. J. Gibbons, E. J. Catcott, and J. F. Smithcors (eds.). American Veterinary Publishers, Inc., Wheaton, Illinois, pp. 104–121.

MAYFIELD, J. E., J. A. BANTLE, D. R. EWALT, V. P. MEADOR, AND L. B. TABATABAI. 1990. Detection of *Brucella* cells and cell components. *In* Animal brucellosis, K. Nielsen and J. R. Duncan (eds.). CRC Press, Boca Raton, Florida, pp. 97–120.

MCCORQUODALE, S. M., AND R. F. DIGIACOMO. 1985. The role of wild North American ungulates in the epidemiology of bovine brucellosis: A review. Journal of Wildlife Diseases 21: 351–357.

MCDERMOTT, J. J., K. A. DENG, T. N. JAYATILEKA, AND M. A. ELJACK. 1987. A cross-sectional cattle disease study in Kongor Rural Council, southern Sudan. II. Brucellosis in cows: Associated factors, impact on production, and disease control considerations. Preventive Veterinary Medicine 5: 125–132.

MCDIARMID, A. 1951. The vaccination of pregnant cattle with strain 19 *B. abortus* vaccine during an outbreak of brucellosis in a dairy herd. The Veterinary Record 68: 265–268.

MEAGHER M. 1973a. The bison of Yellowstone National Park. Scientific Monograph Series Number

One, National Park Service, U.S. Government Printing Office, Washington, D.C., 161 pp.

———. 1973b. The Department of Agriculture, the Department of the Interior, brucellosis, and the farmer. Bioscience 23: 311–312.

———. 1986. *Bison bison*. Mammalian Species 266. American Society of Mammalogists, Provo, Utah, 8 pp.

———. 1989. Range expansion by bison of Yellowstone National Park. Journal of Mammalogy 70: 670–675.

———. 1993. Winter recreation-induced changes in bison numbers and distribution in Yellowstone National Park. Draft report. Archives, Yellowstone National Park, Wyoming. 48 pp.

MEDITERRANEAN FEVER COMMISSION. 1907. Reports of the commission for the investigation of Mediterranean fever Part VI. London. Harrison and Sons, St. Martens Lane; England, pp. 4–68.

MEYER, M. E. 1965. The epizootiology of brucellosis and its relationship to the identification of *Brucella* organisms. American Journal of Veterinary Research 25: 553–557.

———. 1966a. Host parasite relationships in brucellosis. Reservoirs of infection and interhost transmissibility of the parasite. Proceedings of the United States Livestock Sanitary Association 70: 129–134.

———. 1966b. Identification of *Brucella* organisms by immunofluorescence. American Journal of Veterinary Research 27: 424–429.

———. 1978. Brucella. *In* Standardized methods for veterinary bacteriology, G. Cottral (ed.). Comstock Publishing Company, Ithaca, New York, pp. 395–403.

———. 1981. The genus *Brucella*. *In* The prokaryotes. A handbook of habitats, isolation, and identification of bacteria, M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (eds.). Springer Verlag, New York, New York, pp. 1063–1074.

———. 1984. Brucella. *In* Diagnostic procedures in veterinary bacteriology and mycology, G. R. Carter (ed.). Charles C. Thomas, Springfield, Illinois, pp. 85–91.

———, AND H. S. CAMERON. 1961. Metabolic characterization of the genus *Brucella*. I. Statistical evaluation of the oxidative rates by which type 1 of each species can be identified. Journal of Bacteriology 82: 387–395.

MOHLER, J. R. 1917. Abortion disease. *In* Annual reports of the Department of Agriculture. Washington, D.C., U.S. Government Printing Office, Washington, D.C., pp. 105–106.

MORTELMANS, J., AND P. KAGERUKA. 1970. A propos de la brucellose et de souches de *Brucella* en Africa centrale. *In* International symposium on brucellosis. Development in biological standardization. International Biological Standardization Council (eds.). S. Karger, New York, New York, pp. 207–210.

MORTON, J. R., E. T. THORNE, AND G. M. THOMAS. 1981. Brucellosis in elk. III. Serologic examination. Journal of Wildlife Diseases 17: 23–31.

MOULTON, J., AND M. E. MEYER. 1957. The pathogenesis of *Brucella suis* infection in guinea pigs. Lesions of the spleen, liver, testes, and articulations. Cornell Veterinarian 48: 165–195.

NAGY, L. K., AND P. G. HIGNETT. 1967. The long term effects of *Brucella* infection of newly-born calves. Research in Veterinary Science 8: 247–255.

NATIONAL VETERINARY SERVICES LABORATORY. 1992. Laboratory reports, Yellowstone bison data. U.S. Department of Agriculture, Ames, Iowa. Archives, Yellowstone National Park, Wyoming, 7 pp.

NICOLETTI, P. 1977. Adult vaccination. *In* Bovine brucellosis, an international symposium, R. P. Crawford and R. J. Hildago (eds.). Texas A and M University Press, College Station, Texas, pp. 201–208.

———. 1980. The epidemiology of bovine brucellosis. *In* Advances in veterinary science and comparative medicine, Vol. 24, C. A. Brandley and C. E. Cornelius (eds.). Academic Press, New York, New York, pp. 69–98.

———. 1981a. The efficacy of Strain 19 vaccination in reducing brucellosis in large dairy herds. California Veterinarian 9: 35–36.

———. 1981b. Prevalence and persistence of *Brucella abortus*, strain 19 infections and prevalence of other biotypes in vaccinated adult dairy cattle. Journal of the American Veterinary Medical Association 178: 143–145.

———, AND F. W. MILWARD. 1983. Protection by oral administration of *Brucella abortus* strain 19 against an oral challenge exposure with a pathogenic strain of *Brucella*. American Journal of Veterinary Research 44: 1641–1643.

PAC, H. I., AND K. FREY. 1991. Some population characteristics of the northern Yellowstone bison herd during the winter of 1988–1989. Montana Department of Fish, Wildlife, and Parks, Bozeman, Montana, 29 pp.

PHILLIP, C. B. 1952. *Brucella abortus* agglutinations in Yellowstone Park bison serum collected in January, 1952. Archives, Yellowstone National Park, Wyoming, 1 p.

QUORTRUP, E. R. 1944. A report on brucellosis investigations in Yellowstone National Park, December 1 to 20, 1944. Archives, Yellowstone National Park, Wyoming. 44 pp.

RANNEY, A. F. 1934. The elimination of *Brucella abortus* from the feces of calves taking infected milk. Cornell Veterinarian 24: 244–253.

RAY, W. C. 1977. The epidemiology of *Brucella abortus*. *In* Bovine brucellosis, an international symposium, R. C. Crawford and R. J. Hildago

(eds.). Texas A and M University Press, College Station, Texas, pp. 103–115.

———. 1979. Brucellosis due to *Brucella abortus* and *B. suis*. *In* Handbook series on zoonoses, J. H. Steele (ed.). CRC Press, Boca Raton, Florida, pp. 99–127.

RENOUX, G. 1957. Brucellosis in sheep and goats. Advances in Veterinary Science 3: 241–273.

RHYAN, J. C., W. J. QUINN, L. S. STACKHOUSE, J. J. HENDERSON, D. R. EWALT, J. B. PAYEUR, M. JOHNSON, AND M. MEAGHER. 1994. Abortion caused by *Brucella abortus* Biovar 1 in a free-ranging bison from Yellowstone National Park. Journal of Wildlife Diseases 30: 445–446.

ROGERS, E. B. 1950. Bison reports. Lamar bison herd reduction. Memorandum to the Regional Director, 28 March 1950. Archives, Yellowstone National Park, Wyoming, 6 pp.

RUSH, W. M. 1932a. Bang's disease in the Yellowstone National Park buffalo and elk herds. Journal of Mammalogy 13: 371–372.

———. 1932b. Northern Yellowstone elk study. Montana Fish and Game Commission, Helena, Montana, 131 pp.

SKINNER, C. K. 1941. Special report on Yellowstone National Park bison: Summary of investigations made and statistics collected in connection with the 1941 reduction operations. Archives, Yellowstone National Park, Wyoming, 33 pp.

———, AND W. B. ALCORN. 1942. History of the bison in Yellowstone Park. Archives, Yellowstone National Park, Wyoming, 57 pp.

STABLEFORTH, A. W. 1959. Brucellosis. *In* Diseases due to bacteria, Vol. I, A. W. Stableforth and I. W. Galloway (eds.). Butterworths Scientific Publications, London, England, pp. 53–159.

———, AND L. M. JONES. 1963. Report of the subcommittee on taxonomy of the genus *Brucella*. International Bulletin of Bacterial Nomenclature 13: 145–158.

STEELE, J. 1965. Human brucellosis in the United States. *In* Proceedings of the National Brucellosis Committee 1965–1968, United States Department of Agriculture, Washington, D. C., pp. 27–36.

STUART, J. E., C. B. BILLS, J. D. DEMATTEI, AND D. L. MACE. 1959. The results of eleven years vaccinating with strain 19. Proceedings of the United States Livestock Sanitary Association 63: 83–90.

TESSARO, S. V. 1986. The existing and potential importance of brucellosis and tuberculosis in Canadian wildlife: A review. Canadian Veterinary Journal 27: 119–124.

———. 1987. A descriptive and epizootiologic study of brucellosis and tuberculosis in northern Canada. Ph.D. Dissertation. University of Saskatchewan, Saskatoon, Saskatchewan, Canada, 320 pp.

———. 1992. Bovine tuberculosis and brucellosis in animals, including man. *In* Buffalo, J. E. Foster, D. Harrison, and J. S. MacLaren (eds.). Nature and Culture Series, The University of Alberta Press, Edmonton, Alberta, Canada, pp. 207–224.

———, L. B. FORBES, AND C. TURCOTTE. 1990. A survey of brucellosis and tuberculosis in bison in and around Wood Buffalo National Park, Canada. Canadian Veterinary Journal 31: 174–180.

———, C. G. GATES, AND L. B. FORBES. 1993. The brucellosis and tuberculosis status of wood bison in the Mackenzie Bison Sanctuary, Northwest Territories, Canada. Canadian Journal of Veterinary Research 57: 231–235.

THIENPONT, D., M. VANDERVELDEN, P. FAGARD, AND J. MORTELMANS. 1961. L'hygroma brucellique: l'aspect clinique characteristique de la brucellose bovine au Rwanda-Burundi. Revue D'Glevage et de Medicine Veterinaire Des Pays Tropicaux 14: 257–266.

THIMM, B., AND G. NAUWERK. 1974. Bovine brucellosis in Guinea and West Africa. Zentralblatt für Veterinärmedizin B 21: 692–705.

THORNE, E. T., J. K. MORTON, AND G. M. THOMAS. 1978. Brucellosis in elk. I. Serologic and bacteriologic survey in Wyoming. Journal of Wildlife Diseases 14: 74–79.

———, J. K. MORTON, AND W. C. RAY. 1979. Brucellosis, its effect and impact on elk in western Wyoming. *In* North American elk: Ecology, behavior and management, M. S. Boyce and L. D. Hayden-Wing (eds.). The University of Wyoming, Laramie, Wyoming, pp. 212–220.

TUNNICLIFF, E. A., AND H. MARSH. 1935. Bang's disease in bison and elk in the Yellowstone National Park and on the National Bison Range. Journal of the American Veterinary Medical Association 86: 745–752.

U.S. DEPARTMENT OF AGRICULTURE. 1992. Brucellosis eradication, Uniform methods and rules. APHIS 91-45-002, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, U.S. Government Printing Office, Washington, D.C., 100 pp.

WELCH, H., AND H. MARSH. 1935. A whole blood field agglutination test for Bang's disease in range cattle. Journal of the American Veterinary Medical Association 86: 493–507.

WILLIAMS, E. S., E. T. THORNE, S. L. ANDERSON, AND J. D. HERRIGES, JR. 1993. Brucellosis in free-ranging bison (*Bison bison*) from Teton County, Wyoming. Journal of Wildlife Diseases 29: 118–122.

WINSHIP, M. I. 1989. Brucellosis in rural practice. *In* Brucellosis: Clinical and laboratory aspects, E. I. Young and M. J. Corbel (eds.). CRC Press, Boca Raton, Florida, pp. 143–149.

WIXOM, H. G., AND L. C. VANDERWAGON. 1965. Calfhood vaccination: Its relation to brucellosis eradication. Proceedings of the United States Livestock Sanitary Association 69: 141–148.

WOBESER, G. 1992. Disease in northern bison: What to do. *In* Buffalo, J. E. Foster, D. Harrison, and I. S. MacLaren (eds.). Alberta Nature and Culture Series, University of Alberta Press, Edmonton, Alberta, Canada, pp. 179–188.

WORTHINGTON, R. W. 1982. Serology as an aid to diagnosis: Uses and abuses. New Zealand Veterinary Journal 30: 93–97.

XIN, X. 1986. Orally administrable brucellosis vaccine: *Brucella suis* strain 2 vaccine. Vaccine 4: 212–216.

Received for publication 15 February 1993.

Margaret E. Meyer[1] **and Mary Meagher,**[2,3] [1] Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, California 95616, USA; [2] National Biological Service, Midcontinent Ecological Science Center, Greater Yellowstone Field Station, Yellowstone National Park, Box 168, Mammoth, Wyoming 82190, USA. [3] Author for reprints.