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# BRUCELLOSIS IN ELK I. SEROLOGIC AND BACTERIOLOGIC SURVEY IN WYOMING [1]

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*Abstract:* Incidence of brucellosis in elk *(Cervus canadensis)* on two winter feedgrounds in Wyoming was examined over a 5-year period by testing serum samples using the standard plate agglutination (SPT) buffered *Brucella* antigen (BBA), rivanol (Riv) and complement fixation (CFT) tests. Thirty-one percent of 1,165 elk were positive by defined criteria. Considering each test individually, only 29% (106) of 370 positive sera would have been classified as reactors by the SPT, 83% (307) by the BBA test and 86% (314) by the Riv test. The CFT would have identified 85% (267) of 332 positive samples on which it was used. *Brucella abortus*, type 1, was isolated from 17 of 45 elk necropsied. The SPT identified 59% (10) of these as reactors, the BBA test 94% (16) and the Riv test 88% (15). The CFT identified nine of nine (100%) on which it was used. Prevalence of seropositive animals increased with age. Brucellosis has been present in one of the two elk herds since at least 1930, and the incidence of infection among mature females in both herds was approximately 50% during this study. No single serologic test should be relied upon to diagnose brucellosis in elk.

## INTRODUCTION

Brucellosis is a highly contagious bacterial disease affecting many species of mammals. *Brucella abortus*, type 1, typically infects cattle, causing abortions during the latter half of gestation, sterility in the cow, and pathologic changes in the genital tract of bulls.

Tunnicliff and Marsh[18] reported that brucellosis was probably first detected in North American wild ungulates in 1917 when Mohler reported the disease in bison *(Bison bison)* in Yellowstone National Park. From 1931 to 1933, 8% of 105 elk tested from Yellowstone National Park were positive at 1:50, and 14% were suspicious at 1:25 on the standard tube test (STT) and the standard plate test (SPT)[18], the principle serologic tests for diagnosing bovine brucellosis. Since then numerous surveys of wildlife populations have been conducted. Sera from thousands of deer *(Odocoileus virginianus* and *O. hemionus)* across the United States have been tested and the prevalence of infection has been less than 1%. These surveys relied almost entirely upon the standard tube and plate tests using bovine standards to detect reactors.[1,2] Unvaccinated domestic cattle are diagnosed on the basis of the seroagglutination reactions as follows: N1:50-negative; I1:50 to I1:100-suspect; +1:100 or greater-reacter.[12]

Serum samples from elk were first tested at the National Elk Refuge, Jackson, Wyoming, in 1930 and three

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of nine were considered positive. The Wyoming State Veterinary Office found serologic evidence of the disease in 21% of 132 elk from the National Elk Refuge in the winter of 1935-36.[11,14] Between 1947 and 1970, 23 of 335 elk blood samples tested from the National Elk Refuge and the Greys River Feedground, Alpine, Wyoming, were positive for brucellosis.[3,4,5,13,14,15,16] *B. abortus*, type 1, has been recovered from aborted elk fetuses and a nonviable calf from the National Elk Refuge[15] and Greys River Feedground.[17] A total of 1,483 game animals (609 antelope, [*Antilocapra americana*]; 336 mule deer; 183 white-tailed deer; 23 bighorn sheep, [*Ovis canadensis canadensis*]; 18 moose, [*Alces alces shirasi*] and 497 elk) from other areas of Wyoming were tested between 1930 and 1970 and six (3 antelope, 1 mule deer, 1 white-tailed deer and 1 elk) were considered positive. Thorne[15] concluded that any significant wildlife brucellosis was confined to the large winter feedground elk herds of western Wyoming.

Detection of brucellosis in wildlife initially depended on results of the STT and the SPT which measure the total level of agglutinins in serum. The Bang's Disease Laboratory, University of Wyoming, found poor agreement between STT and SPT results on 19 of 143 elk sera in 1935.[8] However, since that time, the two tests have been standardized to give comparable results in cattle.[12]

Since observations in cattle suggested the presence of "nonspecific" agglutinins, supplemental tests were devised to distinguish between specific and nonspecific agglutination and to clarify the status of suspect animals and problem herds. One of these, the buffered *Brucella* antigen (BBA or rapid card test) uses an antigen stained with rose bengal and buffered at pH 3.65 $\pm$ 0.05 to inhibit nonspecific agglutinins. The test can be conducted in the field on plasma using micro blood plasma collectors or in the laboratory using serum. It is interpreted as either positive or negative. In 1966 it was officially approved for use in the National Brucellosis Eradication Program, the only supplemental test to be so classified.[12] The rivanol precipitation-plate agglutination test (Riv) is based on mixing and centrifuging equal quantities of serum and 1% rivanol to precipitate nonspecific agglutinins. The Riv test, like the STT and SPT, is read as negative, incomplete and positive at serum dilutions equivalent to 1:25, 1:50, 1:100 and 1:200. In cattle any reaction to this test is considered significant.[12] The complement fixation test (CFT) is a useful, but unofficial supplemental test for sera with suspect titers to the seroagglutination tests. In cattle CFT titers may develop sooner and persist longer than agglutination titers.[6]

The purpose of this study was to determine the incidence of brucellosis in two Wyoming elk herds and to evaluate the efficacy of the SPT, CFT, BBA and Riv tests for diagnosing brucellosis in elk.

## MATERIALS AND METHODS

During the winters of 1970-71 through 1975-76 elk were live trapped on the Greys River Feedground and National Elk Refuge (except no elk were trapped on Greys River Feedground in 1971-72). Blood samples were aseptically collected by jugular venapuncture. Trapped elk were classed by sex and into calf, yearling and mature age groups using dental incisor replacement as age indication.

### Serology

Standard procedures of the U.S. Department of Agriculture, Animal and Plant Health Inspection Service, were used for conducting the SPT, BBA and Riv tests.[19,20]

On several occasions the BBA card test was performed at the trap site on blood plasma. Freshly collected elk blood was transferred from collecting vials to bovine micro collectors, bovine kit no. 305[2], containing heparin to initiate plasma separation. Collectors were centrifuged for 30 seconds at high speed in a portable 12-volt centrifuge.

Whole blood was allowed to clot at room temperature for approximately 24 h. The tubes were refrigerated and serum drawn off from 2 to 6 days following collection.

A complement fixation test, patterned after that described by Jones *et al.*[6] was used during 1971 through 1973. A 3 ml total volume was used and fixation was permitted to occur at 4 C overnight. Beginning in 1974 the CFT was modified and patterned after the microtiter technique for the diagnosis of bovine anaplasmosis.[9] *Brucella* tube test antigen diluted 1:500 was used. Doubling dilutions of serum were made from 1:10 to 1:640. The test system containing diluted serum, complement and antigen was held at 4 C overnight. Sensitized sheep red blood cells and hemolysin were added the following morning and the microtiter plates incubated in a water bath at 37C for 30 min. Each reagent constituted 0.025 ml with the total volume of the test system being 0.125 ml. Tests were read according to the degree of fixation (i.e., 1+, 2+, 3+, 4+; corresponding to 25, 50, 75 and 100% fixation).

The elk tested in this study had not been exposed to a vaccine and cross reactions between antibodies to *Brucella* and other organisms were not examined. Therefore, serum producing any degree of reaction to two or more tests was considered positive. In the event that all four tests were not conducted on a serum sample or if a sample reacted to only one of the four tests, standards established for nonvaccinated cattle were used for the SPT, BBA and Riv tests. Complete fixation (4+) at the 1:40 dilution or higher was considered positive on the CFT. There was no "suspect" category.

### Bacteriology

On several occasions elk trapped on the National Elk Refuge were transported to the Wyoming Game and Fish Department's Sybille Game Research Unit, Wheatland, Wyoming, for unrelated research studies. Subsequent serologic tests revealed that some of these elk were naturally infected with brucellosis. Forty-five elk, most of which were serologically positive for brucellosis, were killed and numerous samples collected for *Brucella* cultures. Aseptic techniques were used to collect some or all of the following tissues: mandibular, suprapharyngeal, retropharyngeal, prescapular, prefemoral, popliteal, external and internal iliac, mesenteric, gastrohepatic, ileocecal, mediastinal, bronchial, supramammary and supraficial inguinal lymph nodes; spleen, liver, biceps femoris muscle, uterus, udder, cervix, testes, epididymides, seminal vesicles and ampullae; and fetal fluids, stomach contents, lung, liver and spleen. Both of paired structures were collected. Tissues were immediately inoculated on duplicate plates of serum-enriched trypticase soy agar[3] with 30 mg cycloheximide[4] and 1:800,000 crystal violet added per liter. One set of plates was incubated under 10% $CO_2$ and the other under atmospheric conditions to differentiate the field strain of *B. abor-*

[2] Animal, Plant and Health Inspection Service, USDA

[3] Trypticase Soy Agar®, Baltimore Biological Laboratory, Cockeysville, Maryland 21030

[4] Actidione®, The Upjohn Company, Kalamazoo, Michigan 49001

*tus,* type 1, from the research strain being used in other studies at the Unit. Representative cultures were submitted for confirmation as *B. abortus,* type 1, to the Biologic Reagents Section, Veterinary Services Diagnostic Laboratory, Ames, Iowa 50010.

## RESULTS

### Incidence

A total of 1,165 serum samples was collected. The yearly and accumulated incidence of brucellosis for the two elk herds is presented in Table 1 by sex and age class. Among these elk the prevalence of brucellosis significantly increased with age (yearlings>calves at p=.01, mature females > yearling females at p=.01). Year-to year changes in incidence in the mature females of the herd were not significant (p=.05).

### Serology

Three hundred seventy samples produced a positive reaction to brucellosis according to the criteria used. This large number of *Brucella*-positive wild elk sera provided an opportunity to compare the relative efficiency of the four serologic tests used as a means of diagnosing brucellosis.

Thirty-one percent of the positive sera failed to produce a reaction at the 1:25 dilution on the SPT. In addition to these 113 sera, another 79 produced less than a + 1:50 reaction. Thus 52% (192) of the positive sera would have been considered negative for brucellosis if only the SPT using bovine criteria had been employed. Nineteen percent (72) of the 370 positive sera would have been classified as suspect by bovine standards and only 29% (106) would have been considered brucellosis reactors. There were 44 sera which reacted at less than +1:100 on the SPT and did not react to any of the other tests used. Since we established no suspect classification, these sera were considered negative.

Seventeen percent (63) of the positive sera failed to react to the BBA test. The BBA test alone identified only four of 370 positive sera.

The BBA test was conducted under field conditions on plasma and later in the laboratory on 336 serum samples. Two hundred fifty of these sera were identified as brucellosis negative and 76 as positive. The BBA test on plasma and serum failed to agree on only 3% (10) of the samples. Eight of these termed negative in the field were later called positive in the laboratory. One or more of the other three tests agreed with the BBA test on seven of the eight serum samples. Field use of micro blood collector USDA no. 302[2], containing heparin and lectin as specific for the bovine, did not give sufficient separation of the cellular constituents of elk blood to conduct a reliable test. Therefore, bovine kit no. 305[2] was adopted.

The Riv test was not conducted on 39 sera, four of which were considered as positive. Fourteen percent (52) of the positive sera failed to produce any Riv test reaction. One serum sample was considered as brucellosis positive solely on the basis on a Riv reaction.

The CFT was conducted on 933 sera, 332 of which were considered positive. Seven percent (23) of the 332 positive sera produced no CFT reaction, while 8% (26) reacted but at less than 4+ 1:40. Thus a total of 15% (49) of the positive sera would not have been identified by the CFT alone.

Using various combinations of two tests, the Riv and CFT, Riv and BBA, and BBA and CFT agreed most fre-

[2] Animal, Plant and Health Inspection Service, USDA

TABLE 1. Prevalence of brucellosis in elk tested from the National Elk Refuge (NER) and Greys River Feedground (GR) herds during the winters of 1970-71 through 1975-76.

| | 1970-71 | | 1971-72 | | 1972-73 | | 1973-74 | | 1974-75 | | 1975-76 | | Accumulated total | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age and sex | No. | % Pos. | No. | % Pos. | No. | % Pos. | No. | % Pos. | No. | % Pos. | No. | % Pos. | No. | % Pos. |
| *NER* | | | | | | | | | | | | | | |
| Mat. fe.[1] | 82 | 33 | 37 | 46 | 70 | 53 | 106 | 50 | 82 | 52 | 102 | 35 | 479 | 43[2] |
| Mat. ma. | 8 | 50 | 2 | 50 | 4 | 50 | | | 1 | 100 | 2 | 50 | 17 | 53 |
| 1 Yr. fe. | 9 | 56 | 11 | 45 | 7 | 29 | 11 | 18 | 9 | 0 | 4 | 0 | 51 | 27 [2,3] |
| 1 Yr. ma. | 12 | 0 | 3 | 33 | 2 | 0 | 8 | 0 | 10 | 20 | 4 | 0 | 39 | 5[3] |
| Calf | 60 | 0 | 12 | 0 | 11 | 0 | 18 | 22 | 6 | 0 | 48 | 6 | 155 | 5[3] |
| Total | 171 | 20 | 92[4] | 32[4] | 94 | 43 | 143 | 40 | 108 | 40 | 160 | 25 | 768[4] | 32[4] |
| *GR* | | | | | | | | | | | | | | |
| Mat. fe.[1] | 29 | 41 | | | 56 | 54 | 45 | 51 | 2 | 100 | 29 | 72 | 161 | 53[2] |
| Mat. ma. | 3 | 67 | | | 3 | 67 | | | | | | | 6 | 75 |
| 1 Yr. fe. | 17 | 0 | | | 15 | 27 | 5 | 20 | 2 | 50 | 7 | 43 | 46 | 20 [2,3] |
| 1 Yr. ma. | 9 | 33 | | | 10 | 20 | 6 | 17 | 4 | 50 | | | 29 | 21[3] |
| Calf | 39 | 5 | | | 11 | 9 | 47 | 4 | 11 | 9 | 47 | 19 | 155 | 10[3] |
| Total | 97 | 37 | | | 95 | 40 | 103 | 26 | 19 | 26 | 83 | 39 | 397 | 30 |

[1]The incidence among mature females did not change significantly from year to year.

[2]The incidence in mature females is significantly greater than in yearling females (p=.01).

[3]The incidence in yearlings is significantly greater than in calves (p=.01).

[4]Total, tested and percent positive do not agree with totals by sex and age due to inclusion of serum samples for which sex and age were not recorded.

quently in properly identifying positive sera (Table 2).

### Bacteriology

*B. abortus* type 1 was isolated from 38% (17) of 45 elk necropsied. Considering all four serologic tests, only one of these was classified as negative, but the CFT was not run on that sample. The most frequent sites of isolation were the seminal vesicles (43%), ampullae (33%) and mandibular (24%), suprapharyngeal (24%), popliteal (21%), and retropharyngeal (18%) lymph nodes.

The SPT correctly identified 59% (10 of 17) of the culture positive elk as sero-positive. Seven (41%) of the 17 culture positive elk had a SPT reaction of less than +1:100, two of which were negative at 1:25. Ninety-four percent (16 of 17) were positive on the BBA test and the Riv test classified 88% (15) as positive. The CFT correctly identified 100% of nine culture positive elk tested. The BBA test and CFT in combination correctly identified all culture positive elk on which both were used (Table 3).

## DISCUSSION

Brucellosis has been present in the National Elk Refuge herd since at least 1930 and the Greys River Feedground herd since at least 1960. The prevalence of the disease has been high since the winter of 1970-71.

The serologically detected prevalence of brucellosis in wild elk could be markedly influenced by the number of calves and immature animals tested (Table 1). Mature elk of both sexes should be tested to determine if brucellosis is present and to obtain a realistic indication of the prevalence of the disease.

TABLE 2. Percent positive agreement by combinations of two serologic tests on sera from wild elk identified as brucellosis positive.[1]

| Test and dilution | Test and dilution | | | |
|---|---|---|---|---|
| | BBA | Riv, ≥ I 1:25 | CFT, ≥ 1+1:10 | CFT, ≥ 4+1:40 |
| SPT, ≥ I 1:25 | 58 | 58 | 56 | 50 |
| SPT, ≥ +1:100 | 21 | 22 | 22 | 20 |
| BBA | | 69 | 66 | 59 |
| Riv, ≥ I 1:25 | | | 74 | 68 |

[1]The SPT and BBA test were run on 370 positive sera, the Riv test on 366 positive sera and the CFT on 316 positive sera.

TABLE 3. Percent positive agreement by combinations of two serologic tests on sera from wild elk *Brucella* culture positive at necropsy.[1]

| Test and dilution | Test and dilution | | | |
|---|---|---|---|---|
| | BBA | Riv, ≥ I 1:25 | CFT, ≥ 1+1:10 | CFT, ≥ 4+1:40 |
| SPT, ≥ I 1:25 | 76 | 76 | 78 | 78 |
| SPT, ≥ +1:100 | 59 | 59 | 67 | 67 |
| BBA | | 88 | 100 | 100 |
| Riv, ≥ I 1:25 | | | 89 | 89 |

[1]The SPT, BBA and Riv tests were run on 17 sera and the CFT on 9 sera.

Recovery of *Brucella* organisms from elk confirmed infections indicated by serologic tests. The tissues from which *Brucella* were most frequently isolated parallel those in cattle with the possible exception of the supramammary lymph nodes and the udder which are more often infected in cattle.[7,10] Failure to recover *Brucella* from all seropositive elk was not unexpected as long-term infections may be limited to a few localized areas which may not have been included in the tissues cultured. Positive cultures may not be obtained from a lymph node if the infected portion of the node is not sectioned and plated.[10]

No single serologic test should be relied on to diagnose brucellosis in elk. Furthermore, based on bacteriologic results, diagnostic criteria of the SPT for cattle appear too high for use with elk and should be lowered. The SPT used either singly or in combination with another test was the least reliable of the four tests evaluated in this study. The CFT was most efficient in detecting brucellosis. However, it is the most difficult and least practical of the four tests.

A suspect classification is useful in diagnosing bovine brucellosis where a vaccinal titer may produce a serologic reaction and the animal may be retested later. We feel the suspect classification should not be used in the diagnosis of brucellosis in elk. There is no opportunity for retesting when blood smples are acquired through trapping or from hunter-killed animals.

The evaluation of the serologic tests used here and the recovery of *Brucella* from wild elk confirm the need to use a battery of serologic tests and to eliminate the suspect classification to more accurately determine the prevalence of brucellosis in elk.

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## LITERATURE CITED

1. FAY, L. D. 1961. The current status of brucellosis in white-tailed and mule deer in the United States. Trans. N. Am. Wildl. and Nat. Res. Conf. 26: 203-211.
2. FERRIS, D. H., L. E. HANSON, H. E. RHOADES and J. O. ALBERTS. 1961. Bacteriologic and serologic investigations of brucellosis and leptospirosis in Illinois deer. J. Am. vet. med. Ass. 139: 892-896.
3. HEPWORTH, W. G. 1961. Diagnosis of diseases in mammals and birds. P-R Job Compl. Rep., Proj. FW-3-R-8, Work Plan No. 1, Job No. 1W. Wyo. Game and Fish Commission, Cheyenne.
4. HONESS, R. F. and K. B. WINTER. 1956. Diseases of wildlife in Wyoming. Bull. 9, Wyo. Game and Fish Commission, Cheyenne. 279 pp.
5. HOWE, D. L. and W. G. HEPWORTH. 1964. Diagnosis of diseases in mammals and birds. P-R Job Compl. Rep., Proj. FW-3-R-11, Work Plan No. 1, Job No. 1W. Wyo. Game and Fish Commission, Cheyenne.

6. JONES, L. M., J. B. HENDRICKS and D. J. BERMAN. 1963. The standardization and use of the complement-fixation test for the diagnosis of bovine brucellosis, with a review of the literature. Am. J. vet. Res. 24: 1143-1151.
7. LAMBERT, G., T. E. AMERAULT, C. A. MANTHEI and E. R. GOODE. 1964. Further studies on the persistence of *Brucella abortus* infection in cattle. Proc. U.S. Livestock Sanit. Ass. pp. 109-117.
8. LEE, A. M. and M. E. TURNER. 1937. A comparison of the tube and plate methods of testing Bang's disease in elk. J. Am. vet. med. Ass. 90: 637-640.
9. MARTIN, W. H. and W. H. RITCHIE. 1973. The microtiter technique for the diagnosis of bovine anaplasmosis. Proc. 6th Nat'l Anaplasmosis Conf., Las Vegas, Nevada. pp. 141-142.
10. McCULLOUGH, N. B., C. W. EISELE and A. F. BYRNE. 1951. Incidence and distribution of *Brucella abortus* in slaughtered Bang's reactor cattle. Public Health Rep., Washington, D.C. 66: 341-345.
11. MURIE, O. 1951. *The Elk of North America.* The Stackpole Co., Harrisburg, Pa. and Wildl. Manage. Inst., Washington, D.C. 376 pp.
12. PIETZ, D. E. 1970. Brucellosis serology. Brucellosis Epidemiology Course for Veterinarians. Ames, Iowa. 48 pp.
13. POST, G. 1960. Diagnosis of diseases in mammals and birds. P-R Job Compl. Rep., Proj. FW-3-R-7, Work Plan No. 1, Job No. 1W. Wyo. Game and Fish Commission, Cheyenne.
14. RYFF, J. F. 1951. Brucellosis absent in Wyoming game. Wyo. Wildl. 15: 13, 38.
15. THORNE, E. T. 1969. Diagnosis of diseases in wildlife. P-R Job Prog. Rep., Proj. FW-3-R-16, Work Plan No. 1, Job No. 1W. Wyo. Game and Fish Commission, Cheyenne.
16. ———. 1970. Diagnosis of diseases in wildlife. P-R Job Prog. Rep., Proj. FW-3-R-17, Work Plan No. 1, Job No. 1W. Wyo. Game and Fish Commission, Cheyenne.
17. ——— and J. K. MORTON. 1975. The incidence and importance of brucellosis in elk in northwestern Wyoming. P-R Job Prog. Rep., Proj. FW-3-R-21, Work Plan No. 1, Job No. 8W. Wyo. Game and Fish Department, Cheyenne.
18. 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. J. Am. vet. med. Ass. 86: 745-752.
19. U.S. Department of Agriculture (not dated). Standard agglutination test procedures for the diagnosis of brucellosis. National Animal Disease Laboratory, Ames, Iowa.
20. U.S. Department of Agriculture (not dated). Supplemental test procedures for the diagnosis of brucellosis. National Animal Disease Laboratory Diagnostic Reagents Manual 65E. ARS, ANH, National Animal Disease Laboratory, Ames, Iowa.

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