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BRUCELLOSIS IN ELK III. SEROLOGIC EVALUATION

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Abstract: The efficacy of the standard plate agglutination (SPT), buffered *Brucella* antigen rapid card (BBA), rivanol (Riv) and complement fixation (CFT) tests was statistically evaluated and correlated with known brucellosis infections in elk. Low titers on the SPT were detected in artificially exposed mature cow elk 2 weeks postinoculation and other tests began detecting antibodies at 3 weeks. Titers on all tests were detected as long as 4 years postinoculation. Serologic response was similar in artificially and naturally infected cows. Bulls did not maintain serologic titers as long as cows. The SPT at 1:25 or higher most frequently detected *Brucella* antibodies in infected elk, while the SPT at 1:100 or more least frequently detected antibodies. The percent of elk reacting at 1:100 or greater on the SPT declined rapidly after 6 months postinoculation. Combinations of any 2 of the 4 tests used had close agreement in concurrently identifying infected elk. The CFT correctly identified the greatest number (93%) of elk which were culture positive at necropsy and CFT titers persisted longer than those of the other tests. A CFT reaction persisted longer (average 10.7 weeks) than that of any other test in calves that demonstrated postnatal titers. The serologic responses of calves which acquired active infections were similar to adults. Criteria for identifying seropositive elk are discussed.

INTRODUCTION

Brucellosis was first detected serologically in elk at the National Elk Refuge, Jackson, Wyoming, in 1930 following reports of abortions.[8] Since that time serologic tests on the National Elk Refuge and Greys River Feedground have indicated a high prevalence of the disease in elk.[15] *Brucella abortus* type 1, the organism primarily responsible for bovine brucellosis, has been isolated from aborted fetuses or nonviable calves from the National Elk Refuge, Greys River, Green River, Dog Creek, Horse Creek, Fish Creek and South Park feedgrounds which are all in western Wyoming.[15,16] Studies conducted from 1971 to 1976 at the Wyoming Game and Fish Department's Sybille Wildlife Research Unit demonstrated the effects of brucellosis in elk.[14] This paper reports the serologic results of that study.

MATERIALS AND METHODS

Most elk introduced into the study were trapped wild as adults on the National Elk Refuge, Teton County, Wyoming during the winter and were screened for *Brucella* antibodies at the trap site using the BBA test. Young, sexually mature, seronegative cows and bulls were selected and transported to the Sybille Wildlife Research Unit. Sera from blood collected at the trap were later tested by the SPT, BBA, CFT and Riv tests in the laboratory. A few elk used were selected from the research unit resident herd. All elk tested serologically negative 2 to 4 times over a 2- to 8-week period prior to introduction into the study.

Facilities used and elk groups have been described.[14]

Artificial infections in 24 cows and 3 bulls were induced by inoculating 7.5×10^6 colony forming units of *Brucella*

abortus type 1 strain 2308 in 0.1 ml normal saline into the conjunctival sac. During the course of the study an additional 23 cows and 6 bulls were introduced as uninoculated controls. Twenty-one of these cows and all 6 bulls became infected through natural contact exposure. Fifty-two calves, including first and second generation offspring, were born into the study.

Periodically, jugular blood samples were aseptically obtained from all elk for serologic testing and hemoculture. Blood samples were taken from calves as soon as possible following birth. A total of 2,826 sera were collected and tested.

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.[17,18] The CFT using 3 ml total volume and overnight cold fixation[3] was used from 1971 through 1973. In 1974 the CFT was modified for microtiter[4] using a total volume of 0.125 ml and cold fixation. *Brucella* tube test antigen diluted 1:500 was used, and doubling dilutions of serum were made from 1:10 to 1:640. Tests were read according to the degree of fixation (i.e., 1+. 2+, 3+, 4+ corresponding to 25, 50, 75 and 100% fixation). A notation of 14 represented a 4+ reaction at 1:10.

Bacteriologic samples were collected for hemoculture and at necropsy as previously described.[14]

The chi-square test was used for statistical analyses.

RESULTS

Low serologic reactions (I 1:25 to +1:50) were detected within 2 weeks postinoculation (PI) on the SPT in a few (12%) of 24 artificially infected mature cow elk. Reactions developed at ≥+100 on the SPT and on the BBA, Riv and CFT by 3 weeks PI. The number of sera positive at ≥ 1:100 on the SPT declined fairly rapidly after 6 months, while the number of positive reactions on the SPT at ≥1:25, CFT, BBA and Riv tests decreased slowly after 6 months (Figs. 1, 2, 3 and 4).

Although it was not possible to accurately determine the date of exposure of mature cows which acquired natural-contact infections, their serologic response did not differ greatly from those of artificially exposed females. Higher

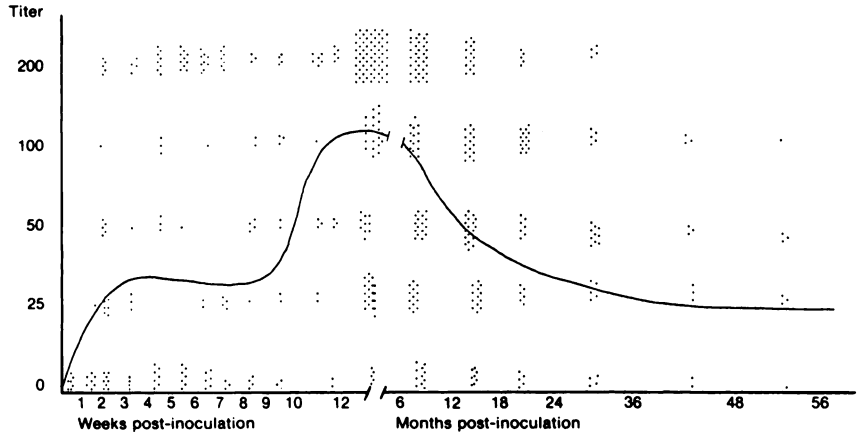

FIGURE 1. Standard plate agglutination titers of mature cow elk artificially exposed to *Brucella abortus* type 1 strain 2308. The solid line represents the geometric mean titer.

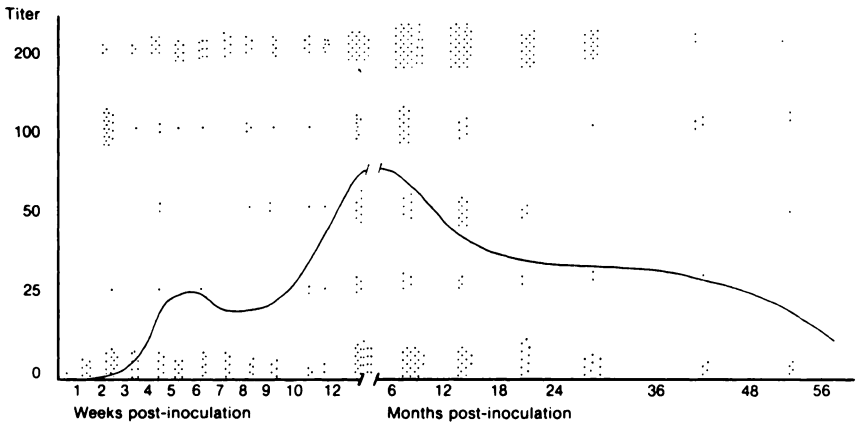

FIGURE 2. Rivanol test titers of mature cow elk artificially exposed to *Brucella abortus* type 1 strain 2308. The solid line represents the geometric mean titer.

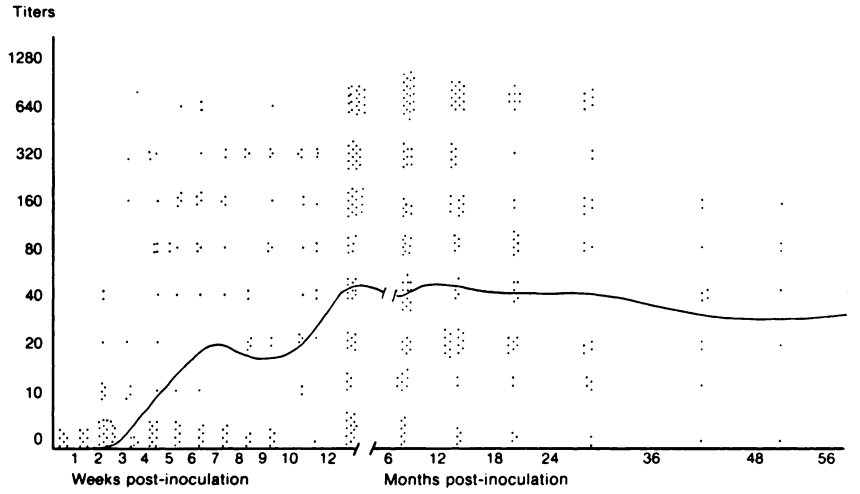

FIGURE 3. Complement fixation titers of mature cow elk artificialy exposed to *Brucella abortus* type 1 strain 2308. The solid line represents the geometric mean titer.

titers were detected on more tests during the first 6 months of infection in naturally exposed than in artificially exposed elk, but most of the differences were not statistically significant.

Serotiters at ≥100 on the SPT, by the BBA and Riv tests and at ≥10 on the CFT were detected by 3 weeks postinoculation in all 3 bull elk artificially exposed to brucellosis. The percent of positive reactions on all tests decreased after 6 months. The only tests detecting reactions after 18 months were the SPT at 1:25 or 1:50, the BBA and the CFT at 1:10 or 1:20; and only the SPT at 1:25 or 1:50 was positive after 24 months.

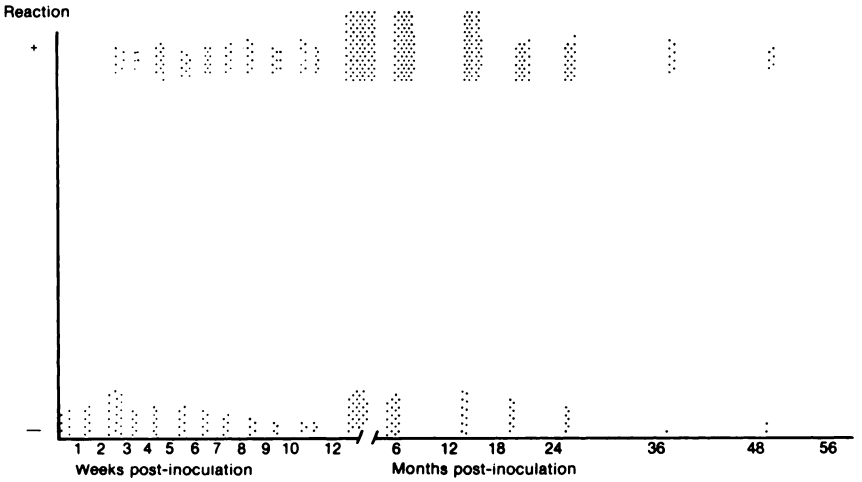

FIGURE 4. Buffered *Brucella* rapid card test results of mature cow elk artificially exposed to *Brucella abortus* type 1 strain 2308.

Nine sexually mature males (including 3 yearlings) became infected through natural-contact exposure. Titer development was the same as among artificially exposed males. The percent of positive reactions at any level on all tests decreased after 6 months, but titers persisted longer than in artificially exposed bulls. At the end of 36 months, 34% of the tests were positive at ≥1:100 on the SPT, 83% were positive on the BBA and Riv tests and all the CFT titers were positive at ≥40.

Eleven cow elk were artificially infected at 5 months gestation and 13 at about 6½ months gestation. The average times from inoculation to detection of the first titer (4.7 and 5.3 weeks, respectively) for the 2 groups were not significantly different ($p = .05$).

Serologic results were evaluated on 23 artificially and 22 natural-contact elk that were confirmed to be infected bacteriologically (Table 1). Isolation of *Brucella abortus* type 1 strain 2308, either by hemoculture or at necropsy, was considered to be a valid indication of infection. Positive serologic reactions on each test during the time of infection were considered correct. Negative reactions were considered incorrect.

The four serologic tests used were equally accurate for detecting artificially and naturally infected cow elk. The frequency of each test being correct was significantly higher for artificially infected cows than for artificially infected bulls ($p = .01$). Every test except the SPT ≥ 25 more frequently identified positive samples from naturally than from artificially exposed bulls ($p = .05$).

Among artificially infected cows, the SPT at ≥1:25 or greater was more frequently correct (93%) than any other test ($p = .01$) except the CFT at ≥1:10 (91%). Reactions on the SPT ≥100 were detected significantly less often (56%) than any other tests ($p = .01$). The frequency of correctness of the SPT ≥1:50 (80%) was significantly higher than by the SPT ≥1:100 ($p = .01$). There were no differences among values for the BBA, Riv or CFT ≥1:10 tests ($p = .05$).

Serologic tests are ranked in Table 2 by decreasing accuracy at different time periods among artificially infected mature females. During the first 3 months of infection, the frequency of

TABLE 1. Percent of sera from known infected elk correctly identified by various serologic tests and dilutions.

Serologic tests	Artificially infected mature females (515 tests)	Naturally infected mature females (345 tests)	Artificially infected mature males (83 tests)	Naturally infected mature males (101 tests)
SPT ≥ 25	93	94	76	83
SPT ≥ 50	80	81	53	73
SPT ≥ 100	56	59	41	73
BBA	89	86	52	80
Riv	86	88	61	88
CFT ≥ 14	91	96	72	94
CFT ≥ 44	83	83	59	92

titers of ≥25 on the SPT correctly identifying infected elk was significantly greater than for any other test ($p = .05$). Titers of ≥100 on the SPT were significantly less accurate at identifying infected elk than any other test, except the Riv test (81%) and the CFT ≥44 (77%) from 0-3 months ($p = .01$).

Table 3 presents frequency of agreement among various levels of two different serologic tests that concurrently identified artificially infected mature cow elk. For example, 86% of the times there were reactions on the SPT at 1:25 or greater, the BBA test was also positive. All tests showed fairly close agreement with the exception of the SPT at 1:100 or greater in combination with any other test indicating sera did not react at dilutions of 1:100 or more on the SPT as often as they did on the other tests.

The CFT ≥1:10 was positive more often than any other test on 45 cow elk culture positive on the day of necropsy (Table 4). As shown previously, sera were positive significantly less often on the SPT at dilutions ≥1:100 than on any other test.

Serotiters detected shortly after birth in 29 of 37 calves born to seropositive cows were considered to be due to passive maternal antibodies. The SPT ≥ 1:25 remained positive an average of 3.5 weeks (range 0-15 weeks), the BBA test 5.5 weeks (range 0-17 weeks), the Riv test 5.7 weeks (range 0-19 weeks) and the CFT 10.7 weeks (range 3-19 weeks) in 23 calves for which the duration of the serotiter could be determined.

In general, mothers of calves with high serotiters also had high serotiters. Low serotiters were detected in calves born to cows with low serotiters. Serology of the

TABLE 2. Percent of 498 serologic tests ranked in decreasing order of frequency for correctly identifying sera from bacteriologically positive mature cow elk at different time periods postinoculation with *Brucella abortus* strain 2308.

Months postinoculation							
0-3 months		3-6 months		6-12 months		12-36 months	
SPT ≥ 25	98%	SPT ≥ 25	93%	CFT ≥ 14	91%	CFT ≥ 14	94%
SPT ≥ 50	88%	BBA	90%	SPT ≥ 25	89%	SPT ≥ 25	91%
BBA	88%	Riv	90%	BBA	88%	BBA	87%
CFT ≥ 14	86%	CFT ≥ 14	88%	Riv	86%	Riv	86%
Riv	81%	SPT ≥ 50	86%	CFT ≥ 44	83%	CFT ≥ 44	82%
CFT ≥ 44	77%	CFT ≥ 44	85%	SPT ≥ 50	75%	SPT ≥ 50	77%
SPT ≥ 100	72%	SPT ≥ 100	68%	SPT ≥ 100	48%	SPT ≥ 100	45%

TABLE 3. Frequency of agreement between pairs of serologic tests correctly identifying 515 sera from known artificially infected mature cow elk.

	BBA	Riv	CFT ≥ 14	CFT ≥ 44
SPT ≥ 25	86%	83%	86%	78%
SPT ≥ 50	66%	77%	77%	72%
SPT ≥ 100	56%	55%	55%	53%
BBA		85%	87%	81%
Riv			85%	81%

calf closely paralleled that of its mother for about the first 3 or 4 weeks after birth. The CFT titer of the calf was occasionally higher than that of the cow 1 or 2 weeks after birth. One cow was positive on the CFT alone at the time of parturition, and the first serum sample of her calf was also positive only on the CFT and at one dilution higher than the mother's.

Nine calves born to seropositive cows acquired active infections between 4 months and 3 years of age as indicated by serologic results which were considered to represent active rather than passive antibodies. Eight of these lost postnatal serotiters, and one was seronegative at birth. Titers due to active antibodies tended to be detected first on the SPT followed by the BBA test an average of 5.25 (range 0-19) weeks later, the Riv test 5.6 (range 0-19) weeks later and the CFT 6.6 (range 0-19) weeks later.

Serotiters were detected from 4 weeks to 1 year of age in 8 calves born to seronegative cows. Again titers were detected first on the SPT ≥ 1:25 followed by the BBA test an average of 1.75 (range 0-6) weeks later, the CFT at 2.5 (range 0-11) weeks and the Riv at 2.62 (range 0-11) weeks later.

TABLE 4. Percent of serologic tests correctly identifying 45 culture positive mature elk at necropsy.

Serologic test	% of tests correct
CFT ≥ 10	98
CFT ≥ 40	93
Riv	89
SPT ≥ 25	84
BBA	80
SPT ≥ 50	78
SPT ≥ 100	56

Serum from the fetus of a cow culture positive for *Brucella* at necropsy reacted at 1:50 on the Riv test and at 1:40 on the CFT. *Brucella* was not isolated from this fetus. No antibodies were detected in 2 calves born to seropositive cows sampled prior to nursing, but both were seropositive a short time later.

Eight calves born to seropositive cows remained seronegative throughout their lives. However, *Brucella* was isolated from one of these when it was examined at necropsy at 10 weeks of age. Two other calves born to infected cows demonstrated serologic titers for 2 and 8 weeks, became seronegative and were culture positive when sacrificed 5 and 13 months later, respectively. *Brucella* was also isolated from a fourth calf that remained seronegative from the first time it was tested at 6 weeks until necropsy at 10 weeks.

DISCUSSION

Serologic test procedures to detect brucellosis in elk are the same as for domestic cattle. Agglutination reactions on the SPT are read as positive or incomplete at dilutions corresponding to 1:25, 1:50, 1:100 and 1:200. A titer of 200 does not necessarily represent an end point. Supplemental tests, including the BBA and Riv, were introduced to distinguish specific (IgG) and nonspecific (IgM) agglutinins. The BBA rapid card test was approved as an official diagnostic test in 1966.[11] The CFT is more laborious, but its value in diagnos-

ing bovine brucellosis is widely accepted.[1,3,5,10]

The serologic response of mature cow elk artificially challenged with *Brucella abortus* type 1 strain 2308 was similar to that seen in domestic cattle.[11] Although it was not possible to make a direct comparison of the serologic response of elk with artificially or naturally acquired brucellosis, titers appeared to be slightly higher during the first 6 months of infection in naturally exposed cows. Possibly, naturally infected cows were exposed to a larger number of organisms. Earlier work with cow elk at Sybille indicated a weaker serologic response occurred in cows exposed to a smaller challenge inoculum.[14] After the first 6 months of infection, titers in both artificially and naturally exposed elk were more comparable.

Although most of the differences in serologic response between artificially infected elk cows and bulls were not statistically significant (Table 1), the higher percentage of bulls reacting 3 weeks postinoculation could have been due, in part, to a smaller sample size of bulls (3) than cows (24). Also, because the bulls were in the rut when they were exposed (September), they may have been physiologically more susceptible to infection. Longer persistence of higher titers in naturally exposed bulls could have been due to exposure to a larger dose of organisms.

Persistence of higher titers longer on the CFT than on the SPT or Riv tests in elk also occurs in the bovine.[3,5,6,7] The higher correlation of CFT results with positive bacteriology in elk is also in agreement with reports for domestic cattle.[1,9,10]

The SPT at 1:25 or greater correctly identified more infected animals than did other tests. However, the difference occurred primarily during the early stages of the disease (Figs. 1-4 and Table 3), probably because the SPT was the first test to detect infection.

Efficacy of all tests in correctly identifying positive sera was similar, except with the SPT at levels ≥ 100 (Table 4). Elk did not maintain titers ≥ 100 with the SPT very long. Thus, this dilution was often negative when other tests were positive. At necropsy only 56% of the infected mature cow elk were positive on the SPT at ≥ 100 (Table 4). In domestic cattle, 52% of culture positive cows were positive at 1:100 on the SPT.[10]

Serologic responses of some elk which did not follow the general pattern of most elk merit individual attention. Early in the course of brucellosis, 2 cows and 1 bull had high titers on all tests which subsequently dropped to low levels. *Brucella* was isolated from all 3 at necropsy when 1 cow and the bull were negative on all except the CFT (4+ at 1:40) and the other cow was negative on the BBA test, positive at 1:25 on the SPT, incomplete at 1:25 on the Riv test, and 4+ at 1:80 on the CFT. Another cow with high titers on the SPT, Riv test and CFT but negative on the BBA test was culture positive at necropsy. A cow introduced into the study as a control had low titers on all tests 1 month prior to when she was killed. *Brucella* was isolated from numerous tissues even though the reaction on the SPT was incomplete at 1:25, the BBA and Riv tests were negative and the CFT was 4+ at 1:20. A bull, introduced as a control, was seropositive on all tests at 7 months following introduction but became seronegative on all tests 5 months later. Titers of all tests, except the SPT, went up 3 months later, but only the BBA and CFT were positive at necropsy when *Brucella* was recovered. Another cow introduced as a control was sampled 52 times in 4 years. Only four times the SPT and CFT reacted at minimal levels and *Brucella* was isolated from the popliteal lymph node at necropsy.

Previously we have considered an elk seropositive at any level on any two tests as positive, and that if only one test was positive, criteria for nonvaccinated

domestic cattle should be used (+1:100 SPT, +BBA test, +≥1:25 Riv test), with a 4+ at 1:40 on the CFT considered positive.[15] However, the results of this serologic evaluation indicate a reaction at ≥1:100 on the SPT for elk is too high to be used as the diagnostic criteria. Many infected elk do not react at that level. We now recommend a reaction at 1:50 or greater on the SPT be considered positive, and the other criteria remain the same.

Various reactions on individual elk, especially those that were seronegative but culture positive at necropsy, emphasize the importance of conducting a battery of tests on each serum sample.[12] The SPT, in particular, should be used in conjunction with another test. Although the SPT more often detects the early stages of the disease, it frequently becomes negative when the animal is still harboring the organism.

A low titer (25 or 50) on the SPT with a negative BBA, negative Riv and negative or low (10 or 20) CFT titer probably indicates a very recent exposure. When there are high titers on all four tests, the infection likely was acquired within the past 6 months. High titers on the Riv (100 or 200) and CFT (40) with a positive BBA and low or negative SPT titer would probably occur in an infection that was established 8 to 12 months previously. A titer on the CFT alone would probably be indicative of a chronic infection.

Latent infections, or infections acquired early in calfhood, that remain undetected until puberty have been implied in domestic cattle.[2,13] Previously we reported 2 female elk which were seronegative until 3 weeks before they aborted their first calves at 2 years of age and a bull which lost a postnatal titer and was culture positive at 15 months of age.[4] Isolation of *Brucella* from 4 calves seronegative at necropsy, two of which had demonstrated postnatal titers, supports the possibility of latent infections in elk.

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LITERATURE CITED

1. ALTON, G.G., J. MAU, B.A. ROGERSON and G.G. MCPHERSON. 1975. The serological diagnosis of bovine brucellosis: an evaluation of the complement fixation, serum agglutination and rose bengal tests. Aust. Vet. J. 51: 57-63.
2. CUNNINGHAM, B. 1977. A difficult disease called brucellosis. Pp. 11-20. In: *Bovine Brucellosis: An International Symposium.* R.P. Crawford and R.J. Hidalgo, eds. Texas A&M University Press, College Station. 421 pp.

3. JONES, L.M., J.B. HENDRICKS and D.T. 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.

4. 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.

5. ——. 1977. The diagnosis of *Brucella abortus* infection in Britain. Pp. 21-39. In: *Bovine Brucellosis: An International Symposium.* R.P. Crawford and R.J. Hidalgo, eds. Texas A&M University Press, College Station. 421 pp.

6. ——, D.J. MACKINNON and G.A. CULLEN. 1969. The rose bengal plate agglutination test in the diagnosis of brucellosis. Vet. Rec. 85: 636-641.

7. —— and R.A. RICHARDS. 1974. The diagnosis, control and eradication of bovine brucellosis in Great Britain. Vet. Rec. 94: 510-517.

8. MURIE, O.J. 1951. *The Elk of North America.* The Stackpole Co., Harrisburg, Pa. and the Wildlife Management Institute, Washington, D.C. 376 pp.

9. MYLREA, P.J. 1972. The diagnosis of brucellosis in dairy herds. Aust. Vet. J. 48: 369-375.

10. NICOLLETTI, P. 1969. Further evaluations of serologic test procedures used to diagnose brucellosis. Am. J. Vet. Res. 30: 1811-1816.

11. PIETZ, D.E. 1970. Brucellosis serology. Brucellosis epidemiology course for veterinarians, Ames, Iowa. 48 pp.

12. PIETZ, D. 1977. Brucellosis antigens and serologic test results. Pp. 49-60. In: *Bovine Brucellosis: An International Symposium.* R.P. Crawford and R.J. Hidalgo, eds. Texas A&M University Press, College Station. 4521 pp.

13. STABLEFORTH, A.W. and I.A. GALLOWAY. 1959. *Infectious Diseases of Animals.* Vol. 1. Academic Press, Inc., New York. 396 pp.

14. THORNE, E.T., J.K. MORTON, F.M. BLUNT and H.A. DAWSON. 1978. Brucellosis in elk II. Clinical effects and means of transmission as determined through artificial infections. J. Wildl. Dis. 14: 280-291.

15. ——, —— and G.M. THOMAS. 1978. Brucellosis in elk 1. Serologic and bacteriologic survey in Wyoming. J. Wildl. Dis. 14: 78-81.

16. ——, —— and W.C. Ray. 1979. Brucellosis, its effect and impact on elk in western Wyoming. Pp. 212-220. In: *North American Elk: Ecology, Behavior and Management.* M. Boyce and L. Hayden-Wing, eds., Univ. of Wyoming. 294 pp.

17. U.S. Department of Agriculture (not dated). Standard agglutination test procedures for the diagnosis of brucellosis. National Animal Disease Laboratory Diagnostic Reagents Manual 65D. ARS, ANH, National Animal Disease Laboratory, Ames, Iowa.

18. 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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