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Source: Journal of Wildlife Diseases, 38(3) : 552-557

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-38.3.552

Journal of Wildlife Diseases, 38(3), 2002, pp. 552–557

SAFETY OF *BRUCELLA ABORTUS* STRAIN RB51 VACCINE IN NON-TARGET UNGULATES AND COYOTES

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ABSTRACT: Brucellosis is endemic in free-ranging elk (*Cervus elaphus*) and bison (*Bison bison*) in the Greater Yellowstone Area (GYA; USA). It is possible that an oral brucellosis vaccine could be developed and disseminated in the GYA to reduce disease transmission. Should this occur, non-target species other than elk and bison may come in contact with the vaccine resulting in morbidity or mortality. To assess biosafety, bighorn sheep (*Ovis canadensis*; $n=10$), pronghorn (*Antilocapra americana*; $n=9$), mule deer (*Odocoileus hemionus*; $n=11$), moose (*Alces alces shirasi*; $n=10$), and coyotes (*Canis latrans*; $n=24$) were given a single oral dose of at least 1.0×10^{10} colony-forming units of *Brucella abortus* strain RB51 vaccine (RB51). Animals were randomly divided into vaccinated and control groups. Ungulates were captured, blood sampled, and swabs taken from the nares, rectum, and vagina for bacterial culture on day 0, 42, and 84 post-inoculation (PI). On day 42, the vaccinated group became a control group and vice versa in a crossover design. Blood and swab samples were taken from coyotes on days 0, 14, 28, and 42 PI. There was no crossover for the coyote study. Two coyotes from each group were also euthanized and cultured for RB51 on days 42, 84, 168, and 336 PI. Blood samples were analyzed for hematologic changes and antibodies to RB51 using a modified dot-blot assay. No morbidity or mortality as a result of vaccination was observed in any animal. There were no differences in hematologic parameters at any time for ungulate species; vaccinated coyotes had higher hematocrit, hemoglobin, and eosinophil counts ($P\leq0.006$). All individuals, except some moose, seroconverted to RB51. Strain RB51 was cultured from oropharyngeal lymph nodes from one coyote 42 days PI and from a moose 117 days PI. This study suggested that a single oral dose of RB51 was safe in these species.

Key words: *Alces alces,* *Antilocapra americana,* bighorn sheep, *Brucella abortus,* *Canis latrans,* coyote, moose, mule deer, *Odocoileus hemionus,* *Ovis canadensis,* pronghorn, strain RB51, vaccination.

INTRODUCTION

Brucellosis is a zoonotic disease causing abortion in domestic and wild ungulates and undulant fever in humans (Hunter and Kreeger, 1998). A federal/state Cooperative Brucellosis Eradication Program began in 1934 with the goal of eliminating brucellosis in cattle from the United States. This goal is close to being accomplished (Cheville et al., 1998). However, brucellosis is endemic in elk (*Cervus elaphus*) and bison (*Bison bison*) in the Greater Yellowstone Area (GYA), an ecosystem encompassing Yellowstone and Grand Teton National Parks and surrounding areas in Wyoming, Montana, and Idaho (Tunnicliff and Marsh, 1935).

Brucellosis can be controlled through vaccination (Cheville et al., 1998). Brucellosis vaccines are comprised of living, mutant *Brucella* organisms that infect the host and induce protective immunity, but are less pathogenic than the parent strains. Two licensed brucellosis vaccines are *B. abortus* strain 19 and RB51. Strain 19 has been used for decades in cattle and free-ranging elk (Herriges et al., 1989). However, strain 19 induces production of antibodies to the lipopolysaccharide (LPS) O-side chain of *B. abortus* that are detected in most brucellosis serologic tests (Stevens et al., 1995) which makes differentiation between infection and vaccination difficult. Strain RB51 is a laboratory-derived rough mutant of virulent *B. abor-*

tus strain 2308 that lacks most of the antigenic LPS O-side chain (Schurig et al., 1991). Because RB51 does not induce positive responses on brucellosis serologic tests (Stevens et al., 1994), it has become the preferred vaccine for cattle and it may become the preferred vaccine for wildlife.

Currently, several thousand elk are vaccinated annually by the Wyoming Game and Fish Department with strain 19 as they congregate on winter feedgrounds (S. Smith, unpubl. data). Vaccine is delivered via a biodegradable ballistic implant or "biobullet" (Herriges et al., 1989). However, ballistic delivery may limit widespread application to free-ranging animals. Animals not on feedgrounds could be vaccinated if an oral vaccine was developed and deployed. Oral delivery of RB51 has been investigated and results indicated that this could be a safe and efficacious route (Elzer et al., 1998).

If an oral brucellosis vaccine were disseminated in bait in the GYA, species other than elk and bison would probably consume the vaccine. Non-target species could also be exposed to the vaccine by contacting abortions from vaccinated dams. It is important for wildlife managers to evaluate the safety of such vaccines in these non-target species. Herein, we report on the safety of a single oral dose of RB51 in bighorn sheep (*Ovis canadensis*), pronghorn (*Antilocapra americana*), mule deer (*Odocoileus hemionus*), moose (*Alces alces*), and coyotes (*Canis latrans*).

MATERIALS AND METHODS

The ungulate study took place from November 1997 to February 1999 at the Wyoming Game and Fish Department's Sybille Wildlife Research and Conservation Education Unit (Sybille), Wheatland, Wyoming (USA, 41°45′778″N, 105°22′605″W). The coyote study took place from May 1998 to May 1999 at the National Wildlife Research Center (NWRC), Logan, Utah (USA, 41°39′000″N, 105°111′300″W). The following adult animals were used in the study: 10 Shira's moose (two males, eight females), 10 Rocky Mountain bighorn sheep (one male, nine females), nine pronghorn (four males, five females), 11 mule deer (seven males, four females), and 24 coyotes (16 males, eight females). Pronghorn and sheep were residents at Sybille; the moose and mule deer were captured in Wyoming via chemical immobilization and transported to Sybille. Moose were housed in large 90-ha semi-natural enclosures; other ungulates were housed in 0.5 ha pens. All ungulates were fed alfalfa hay supplemented with a high-energy pelleted supplement. Water and a trace mineral block were provided ad libitum. Coyotes were residents at the NWRC and housed in 1.2×3.7 m pens. Coyotes were fed a commercial canine diet (Mazuri Feeds, Purina Mills, Inc., St. Louis, Missouri, USA).

The experimental design was the same for all ungulate species. Animals were randomly and equally divided into vaccinated and control groups. On day 0 of an experiment, animals were captured (chemically or physically), blood sampled, and swabs taken from the nares, rectum, and vagina for bacterial culture. Swabs were placed on ice for transport and cultured (Alton et al., 1988) within 24 hr of sampling.

The vaccinated group was given a single standard cattle dose of RB51 orally. This was accomplished by reconstituting commercial vaccine (Colorado Serum Co., Denver, Colorado, USA) and injecting 2 ml of the vaccine into the buccal cavity via syringe. The cheek was then rubbed for about 1 min against the molars to excoriate the mucosa to enhance vaccine uptake. The vaccine was sampled for potency on the day of vaccination (Alton et al., 1988). The control group was treated similarly except given only physiologic saline.

On day 42, ungulates were again captured, blood sampled, and cultured as before. Animals in the previous vaccinated group then became the new control group and animals in the previous control group were given oral RB51 vaccine and became the new vaccinated group in a crossover design. Vaccine was again sampled for potency. On day 84, animals were recaptured, blood sampled, and cultured as before. All animals were observed daily for morbidity or mortality during the test and monitored for several months thereafter.

Coyotes were randomly (except for sex) and evenly divided into a vaccinated (eight males, four females) and a control group (eight males, four females). The control group received saline orally. Blood and swab samples were taken as for ungulates except samples were taken on days 0, 14, 28, and 42 post-inoculation (PI). There was no crossover for the coyote study.

Two coyotes from each group were euthanized and cultured for RB51 on days 42, 84, 168, and 336 PI as part of this study. Of the ungulates, only moose were euthanized at the end of the study (day 84) secondary to man-

agement considerations and other research. If any other ungulate died, it was subjected to a full necropsy with selected tissues cultured for *Brucella.* Aseptic techniques were used to collect the following tissues: mandibular, medial and lateral retropharyngeal, parotid, prescapular, prefemoral, external and internal iliac, popliteal, mediastinal, mesenteric, hepatic, supramammary and bronchial lymph nodes; mammary gland; uterus; cervix; ovaries; testes; kidney; biceps femoris; spleen; and liver. When applicable, both paired structures were collected. Tissue samples were macerated, cultured (Alton et al., 1988), and bacterial isolates identified (Quinn et al., 1994).

Blood samples were analyzed for hematologic changes (Schalm et al., 1975) and antibodies to RB51 using a modified dot-blot assay (Kreeger et al., 2000). For ungulates, differences in hematologic parameters and RB51 antibody titers (unvaccinated versus 42 days post-vaccination only) between vaccinated and control groups were compared by one-way ANOVA at a significance level of $P \leq 0.05$. If there were no differences between groups at day 42, then the vaccinated group was considered a suitable control group for the crossover design. If there were differences at day 42, then groups were compared only for the initial 6 wk period. For coyotes, differences in parameters between groups were compared by ANOVA for repeated measures at a significance level of $P \leq 0.05$. Means are reported with standard errors.

RESULTS

On day 0, the vaccinated groups of bighorn sheep, pronghorn, and mule deer received 2.0×10^{10} colony-forming units (CFU) RB51; on day 42, the vaccinated groups were given 1.4×10^{10} CFU RB51. No bighorn sheep or pronghorn died during the study, nor was any morbidity observed. However, three mule deer (two male, one female) vaccinated on day 0 died between 6–12 wk PI. No *Brucella* were cultured from any tissues. Two of the three deaths were diagnosed as pneumonia; the other cause of death was undetermined. There were no differences in hematologic parameters between any of the groups at any time ($P \geq 0.14$). No swab cultures analyzed for RB51 shedding resulted in growth of *Brucella* for any of the groups. All bighorn sheep, pronghorn, and mule deer seroconverted post-vaccination ($P \leq 0.017$; Table 1).

The moose study was started a few weeks after the other ungulate groups. On day 0, the vaccinated group received 1.0×10^{10} CFU RB51; on day 42, the vaccinated group was given 8.0×10^{9} CFU RB51. No morbidity was observed during the study. One unvaccinated bull died from acute pneumonia 1 day after handling. There were no differences in hematologic parameters between groups at any time ($P \geq 0.46$). No swab cultures resulted in growth of *Brucella.* All female moose were eventually euthanized several weeks after termination of the study. One cow, euthanized 117 days PI, was culture positive for RB51 in the parotid, retropharyngeal, and submandibular lymph nodes. Not all moose seroconverted ($P = 0.07$; Table 1).

On day 0, vaccinated coyotes received 1.0×10^{10} CFU RB51. No morbidity or mortality was observed during the study. During week 6 of the study, vaccinated coyotes had higher hematocrit ($P = 0.007$), hemoglobin ($P = 0.006$), and eosinophil ($P = 0.0001$) values than did controls; however, all hematologic values were within the range of normal values established for coyotes at this facility (T. Deliberto, unpubl. data). All vaccinated coyotes seroconverted to RB51 ($P \leq 0.05$; Table 2). No swabs were culture positive for *B. abortus.* One male, euthanized 42 days PI, was culture positive for RB51 in the parotid, submandibular, and medial retropharyngeal lymph nodes. Strain RB51 was not cultured from any other tissues at any time.

DISCUSSION

This study demonstrated that oral inoculation of RB51 resulted in infection and, thus, seroconversion in these species. The results also suggested that RB51 was neither shed during the period of this study nor resulted in clinical disease in these species, including moose. Other than moose, these results were anticipated because there was no evidence that the more

TABLE 1. Immunologic responses of ungulates measured 6 wk post-oral vaccination with *Brucella abortus* strain RB51 vaccine.

	Bighorn sheep		Pronghorn		Mule deer		Moose	
Parameter	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate
Sample size	10	10	9	9	8	11	9	9
Mean RB51 antibody titer[a]	138.8 ± 27.3	10,813.3 ± 4,018.6	62.5 ± 36.9	830.0 ± 278.7	107.5 ± 36.0	1,060 ± 258.2	196.4 ± 109.6	2,538.2 ± 1,231.8

[a] Reciprocal of dot-blot titer ± SE.

TABLE 2. Immunologic responses of coyotes orally vaccinated with *Brucella abortus* strain RB51 vaccine (n = 12) and coyotes given saline orally (n = 12).

	Day 0		Day 14		Day 28		Day 42	
Parameter	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate	Control	Vaccinate
RB51 antibody titer[a,b]	28.3 ± 12.4	45.4 ± 12.7	16.7 ± 6.4	538.2 ± 54.0	5.7 ± 3.7	5325.7 ± 75.8	10.9 ± 4.1	432.0 ± 58.7

[a] Significant from control values at $P \leq 0.05$ (ANOVA for repeated measures).
[b] Reciprocal of dot-blot titer ± SE.

virulent field strain *B. abortus* was a significant pathogen of bighorn sheep, pronghorn, mule deer, or coyote.

In Arizona (USA) and Alberta (Canada), 52 bighorn sheep were tested for *B. abortus* antibodies and none were found positive (Zarnke and Yuill, 1981; Davis, 1990). Pronghorn also do not appear to be susceptible to brucellosis. Only one of 6,046 pronghorn from Colorado, Wyoming, North Dakota, Arizona, Idaho, Nebraska, Alberta, and Saskatchewan had antibodies against *B. abortus* (Adrian and Keiss, 1977; Davis, 1990). Mule and white-tailed deer (*Odocoileus virginianus*) have been intensively surveyed. Only 46 of >25,000 deer in 29 states reacted positively on a variety of serologic tests (Davis, 1990).

Moose, however, have long been thought to be highly susceptible to brucellosis. *Brucella abortus* was isolated from moribund moose in Minnesota (Fenstermacher and Olson, 1942) and Montana (Jellison et al., 1953) and two sick moose from Alberta were highly seropositive (Corner and Connell, 1958). Serologic evidence of brucellosis was found in nine of 44 moose in Montana (Jellison et al., 1953), 0 of 124 moose in Alberta, 0 of 104 moose in British Columbia (Canada; Corner and Connell, 1958), 0 of 44 moose in Alberta (Zarnke and Yuill, 1981), one of 39 moose in Alaska (Zarnke, 1983), and 0 of 208 moose from Quebec (Canada; Bourque and Higgins, 1984). Low seropositivity in these surveys plus observed morbidity suggested that brucellosis in moose was usually fatal (Thorne et al., 1978). In this study, RB51 infected moose but caused no morbidity. This lack of pathogenicity could be due to either a low inoculation dose or reduced virulence of RB51 (Palmer et al., 1996).

Coyotes can become infected with *B. abortus* in the wild (Davis et al., 1979) and can transmit brucellosis to cattle under artificial conditions (Davis et al., 1988). Transmission to cattle was thought to have occurred by coyotes shedding organisms in feces. Domestic canids can shed *Brucella* in uterine discharges, urine, and feces (Morse et al., 1951). However, we did not detect coyotes shedding RB51 in this study.

The coyote results were consistent with a previous study where beagles were orally inoculated with a single, higher dose (10^{12} CFU) of RB51 (Palmer and Cheville, 1997). Beagles did not show clinical signs after inoculation nor was shedding observed. Oropharyngeal lymph nodes were culture positive in beagles necropsied 49 days PI. No coyotes in this study were pregnant, but oral RB51 did not cause abortion in pregnant beagles, although placentitis was observed (Palmer and Cheville, 1997).

Differences in hematologic parameters between vaccinates and control coyotes cannot be explained at this time. It is unknown how or why infection with RB51 could affect these values, particularly red blood cell indices. Since all coyotes were housed and fed similarly, it is not thought that husbandry practices caused these results. Although these differences were statistically significant, these parameters are within normal ranges for coyotes and they are not thought to be biologically significant.

A single oral inoculation of RB51 was safe in bighorn sheep, pronghorn, mule deer, moose, and coyotes. It would have been valuable to assess multiple doses because this may occur in the field. However, it is difficult to obtain these species for research, particularly moose and bighorn sheep, and they are expensive to maintain.

ACKNOWLEDGMENTS

This study was supported by a grant from the U.S. Department of Interior, National Park Service, Yellowstone National Park. Special thanks to D. Zeiler, N. Perischini, and P. Bleicher for their assistance.

LITERATURE CITED

ADRIAN, W. J., AND R. KEISS. 1977. Survey of Colorado's wild ruminants for serologic titers to brucellosis and leptospirosis. Journal of Wildlife Diseases 13: 429–431.

ALTON, G. G., L. M. JONES, R. D. ANGUS, AND J. M. VERGER. 1988. Techniques for the brucellosis laboratory. Institut National de la Recherche Agronomique, Paris, France, 190 pp.

BOURQUE, M., AND R. HIGGINS. 1984. Serologic studies on brucellosis, leptospirosis and tularemia in moose (*Alces alces*) in Quebec. Journal of Wildlife Diseases 20: 95–99.

CHEVILLE, N. F., D. R. MCCULLOUGH, AND L. R. PAULSON. 1998. Brucellosis in the Greater Yellowstone Area. National Academy Press, Washington, D.C., 186 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: 9–20.

DAVIS, D. S. 1990. Brucellosis in wildlife. *In* Animal brucellosis, K. Nielsen and J. R. Duncan (eds.). CRC Press, Boca Raton, Florida, pp. 321–334.

———, W. J. BOEER, J. P. MIMS, F. C. HECK, AND L. G. ADAMS. 1979. *Brucella abortus* in coyotes. I. Serologic and bacteriologic survey in eastern Texas. Journal of Wildlife Disease 15: 367–372.

———, F. C. HECK, J. D. WILLIAMS, T. R. SIMPSON, AND L. G. ADAMS. 1988. Interspecific transmission of *Brucella abortus* from experimentally infected coyotes (*Canis latrans*) to parturient cattle. Journal of Wildlife Diseases 24: 533–537.

ELZER, P. H., T. J. KREEGER, G. G. SCHURIG, AND D. S. DAVIS. 1998. Oral vaccination of North American elk with *Brucella abortus* strain RB51. Proceedings of ARC-Onderstepoort OIE International Congress on Anthrax, Brucellosis, CBPP, Clostridial, and Mycobacterial Diseases. Bergen-Dal, Kruger National Park, South Africa, pp. 91–95.

FENSTERMACHER, R., AND O. W. OLSON. 1942. Further studies of diseases affecting moose. Cornell Veterinarian 32: 241–254.

HERRIGES, J. D., E. T. THORNE, S. L. ANDERSON, AND H. A. DAWSON. 1989. Vaccination of elk in Wyoming with reduced dose strain 19 *Brucella*: Controlled studies and ballistic implant field trials. Proceedings of the United States Animal Health Association 93: 640–655.

HUNTER, D., AND T. J. KREEGER. 1998. Brucellosis in wildlife. *In* Zoo and wild animal medicine: Current therapy 4, M. Fowler and R. E. Miller (eds.). W. B. Saunders, Orlando, Florida, pp. 621–626.

JELLISON, W. L., C. W. FISHEL, AND E. L. CHEATUM. 1953. Brucellosis in a moose, *Alces americanus.* Journal of Wildlife Management 17: 217–218.

KREEGER, T. J., M. W. MILLER, M. A. WILD, P. H. ELZER, AND S. C. OLSEN. 2000. Safety and efficacy of *Brucella abortus* strain RB51 vaccine in captive pregnant elk. Journal of Wildlife Diseases 36: 477–483.

MORSE, E. V., T. KOWALCZYK, AND B. A. BEACH. 1951. The bacteriologic aspects of experimental brucellosis in dogs following oral exposure. I. Effects of feeding aborted fetuses and placentas to adult dogs. American Journal of Veterinary Research 12: 210–233.

PALMER, M. V., AND N. F. CHEVILLE. 1997. Effects of oral or intravenous inoculation with *Brucella abortus* strain RB51 vaccine in beagles. American Journal of Veterinary Research 58: 851–856.

———, S. C. OLSEN, M. J. GILSDORF, L. M. PHILO, P. R. CLARKE, AND N. F. CHEVILLE. 1996. Abortion and placentitis in pregnant bison (*Bison bison*) induced by the vaccine candidate, *Brucella abortus* strain RB51. American Journal of Veterinary Research 57: 1604–1607.

QUINN, P. J., M. E. CARTER, B. K. MARKEY, AND G. R. CARTER. 1994. Clinical veterinary microbiology. Mosby International, London, England, 648 pp.

SCHALM, O. W., N. C. JAIN, AND E. J. CARROL. 1975. Veterinary hematology. Lea and Febiger, Philadelphia, 807 pp.

SCHURIG, G. G., R. M. ROOP, T. BAGCHI, S. BOYLE, D. BUHRMAN, AND N. SRIRANGANATHAN. 1991. Biological properties of RB51; A stable rough strain of *Brucella abortus.* Veterinary Microbiology 28: 171–188.

STEVENS, M. G., S. G. HENNAGER, S. C. OLSEN, AND N. F. CHEVILLE. 1994. Serologic responses in diagnostic tests for brucellosis in cattle vaccinated with *Brucella abortus* 19 or RB51. Journal of Clinical Microbiology 32: 1065–1066.

———, S. C. OLSEN, AND N. F. CHEVILLE. 1995. Comparative analysis of immune responses in cattle vaccinated with *Brucella abortus* strain 19 or strain RB51. Veterinary Immunology and Immunopathology 44: 223–235.

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: 74–81.

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

ZARNKE, R. L. 1983. Serologic survey for selected microbial pathogens in Alaskan wildlife. Journal of Wildlife Diseases 19: 324–329.

———, AND T. M. YUILL. 1981. Serologic survey for selected microbial agents in mammals from Alberta, 1976. Journal of Wildlife Diseases 17: 453–461.

Received for publication 29 November 2000.