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## Safety of *Brucella abortus* Strain RB51 in Deer Mice

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**ABSTRACT:** *Brucella abortus* strain RB51 is an approved brucellosis vaccine for use in cattle that may have potential as an oral vaccine for use in elk (*Cervus elaphus*) and/or bison (*Bison bison*). This study was designed to determine effects of strain RB51 on deer mice (*Peromyscus maniculatus*), a nontarget species that could have access to treated baits in a field situation. In February 1994, 90 mice were orally dosed or intraperitoneally injected with  $1 \times 10^8$  colony forming units strain RB51 and 77 controls were similarly dosed with sterile saline. At weekly intervals through early April 1994, 4 to 6 mice from each group were euthanized, gross necropsies performed, spleens and uteruses cultured, and tissues examined histologically. All orally inoculated mice cleared the infection by 6 wk post-inoculation (PI). While most of the injected mice cleared the infection by 7 wk PI, a few required 9 wk. There were minimal adverse effects attributable to strain RB51. Apparently, strain RB51 would not negatively impact *P. maniculatus* populations if it were used in a field situation. Also, deer mice appear to be able to clear the vaccine in 6 to 9 wk, thus the probability of these mice transmitting the vaccine to other animals is low.

**Key words:** *Brucella abortus*, brucellosis, deer mice, experimental infection, RB51, *Peromyscus maniculatus*.

Bovine brucellosis is an important disease caused by *Brucella abortus*. The disease typically affects cattle often causing abortion of the first fetus following infection (Nicoletti and Gilsdorf, 1997). Some of the elk (*Cervus elaphus*) and bison (*Bison bison*) in and around the Greater Yellowstone Area (GYA) of Wyoming, Montana, and Idaho (USA) have brucellosis (Cheville et al., 1998). There is concern that these species could transmit *B. abortus* to cattle grazing in the area. If transmission occurred, the state in which it happened could lose its Brucellosis Free Classification from the United States Department of Agriculture, Animal and Plant

Health Inspection Service (Washington, D.C., USA). Losing this status would be an economic hardship to the state's cattle industry because of increased cattle testing requirements, and indirectly through other states' actions related to cattle movements (Cheville et al., 1998). If herds of elk and bison in the GYA were brucellosis free, management would be more efficient and flexible.

Potential methods to reduce or eliminate brucellosis in elk and bison are being investigated. One technique is vaccination; elk on state feedgrounds in Wyoming are currently being vaccinated with *B. abortus* strain 19 (S19) via biobullet (Smith et al., 1997). One problem associated with S19 vaccination is that it induces antibodies which are difficult to differentiate from those caused by field strain infections (Cheville et al., 1998). Additionally, biobullet inoculation is labor intensive and difficult to achieve with animals not accustomed to close human presence. It is generally agreed that administering an oral vaccine would be easier, would probably be less expensive, would cause less stress to the animals, wouldn't require an acclimation period, would likely lead to a greater proportion of the adult herd being vaccinated, and would more easily allow for booster vaccinations (Bowersock et al., 1994).

A candidate vaccine for use in wildlife is *B. abortus* strain RB51 (RB51). This vaccine does not induce false positive serologic reactions (Schurig et al., 1991) and it has replaced strain 19 as the official vaccine for cattle in the USA (Stevens et al., 1997). Preliminary studies of elk and bison have shown that RB51 is safe and may of-

fer protection from infection with virulent *B. abortus* particularly in bison (Olsen et al., 1998; Cook, 1999). In cattle, oral vaccination with RB51 offers 80% protection against *B. abortus* induced abortion (Elzer et al., 1998). Additionally, oral administration of RB51 in elk offered protection to 50% of elk in a small study (Elzer and Davis, 1997); injection studies using RB51 in elk have shown less promise (Cook, 1999). Still, it may be possible to develop RB51 into an oral vaccine for use in wildlife.

If an oral vaccine were used in a field situation many nontarget wildlife species would also likely contact and consume the baits. Before the baits were applied, it would be necessary to insure that the vaccine would not have negative impacts on populations of these nontarget species. Deer mice (*Peromyscus maniculatus*) are common in northwestern Wyoming (Burt and Grossenheider, 1980), and would probably consume the bait if available in the environment. This study was conducted to ensure that RB51 would have no harmful effects on populations of deer mice if it were applied in a field situation.

One hundred eighty captive raised 3–5 mo-old deer mice were purchased from the *Peromyscus* Stock Center at the University of South Carolina (Charleston, South Carolina, USA) and shipped to the University of Wyoming Department of Veterinary Science (Laramie, Wyoming, USA). Mice were randomly divided into four groups of 45 each containing approximately equal numbers of males and non-pregnant females. Mice were given a 2 wk acclimation period to beginning the experiment. They were fed Manna Pro Lab Cube (Manna Pro Corp., St. Louis Missouri, USA) and water ad libitum.

To allow comparison of oral inoculation with intraperitoneal inoculation (IP), the standard test method in laboratory mice, both IP and oral experimental groups were established. Because some mortality occurred during the acclimation period, some groups had fewer than 45 mice remaining. In February 1994, one group ( $n$

$= 37$ ) of mice was injected IP with 0.2 ml of 0.85% sterile saline pH 7.0 (as per Tabatabai et al., 1992). Another group of mice ( $n = 40$ ) was orally dosed with 0.2 ml of 0.85% saline via a tuberculin syringe (without a needle). A third group of mice ( $n = 45$ ) was injected IP with 0.2 ml of 85% sterile saline pH 7.0 containing  $1 \times 10^8$  colony forming units (cfu) of *B. abortus* strain RB51 (Schurig et al., 1991). The last group of mice ( $n = 45$ ) was orally dosed with 0.2 ml of 0.85% sterile saline pH 7.0 containing  $1 \times 10^8$  colony forming units (cfu) of *B. abortus* strain RB51 using the same technique described for oral inoculation of the controls.

Every week for 8 wk beginning 1 wk post inoculation (PI) four to six mice from each group were euthanized via cervical dislocation and necropsied. Any gross abnormalities were noted and samples of tissues (usually including lung, liver, kidney, spleen, small intestine, cecum, colon, skeletal muscle, heart, tongue, pancreas, urinary bladder, uterus or testis and epididymis, medulla oblongata, cerebellum, thalamus, and cerebral cortex) were fixed in 10% buffered neutral formalin. Sections were prepared by routine paraffin embedding and sectioning, stained with haematoxylin and eosin, and examined under a light microscope. Spleens were weighed and approximately half of the spleen was fixed in 10% neutral buffered formalin, the other half was added to 10 parts sterile 0.85% saline in a sterile plastic bag, digitally macerated, further diluted with saline. Dilutions of  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ , and  $10^{-4}$  were plated in duplicate on *Brucella* medium (Alton et al., 1988) and incubated at 37 C with 5% CO<sub>2</sub> for 7 days. The number of colonies per plate were counted at least twice in that 7-day period. The colony forming units (cfu)/spleen were estimated by using the data from those dilutions which had counts in the 10–100 range. The average number of cfu for this dilution was calculated, using the formula #CFU/TISSUE = (average cfu counted)  $\times$  (dilution factor)  $\times$  (total amount of tissue/

TABLE 1. Isolation of *Brucella abortus* strain RB51 from orally inoculated deer mice.<sup>a</sup>

Week post-inoculation	Spleen		Uterus	
	Number positive/number cultured	Mean cfu <sup>b</sup> /spleen (SD) <sup>c</sup>	Number positive/number cultured	Mean cfu/uterus (SD)
1	2/5	76 (147)	0/2	0 (0)
2	4/5	326 (167)	1/3	24 (41)
3	3/5	1,092 (2,259)	0/3	0 (0)
4	1/5	40 (89)	0/3	0 (0)
5	1/5	34 (76)	0/3	0 (0)
6	0/4	0 (0)	0/3	0 (0)
7	0/6	0 (0)	0/2	0 (0)
8	0/5	0 (0)	0/3	0 (0)
9	0/5	0 (0)	0/3	0 (0)

<sup>a</sup> Mice were orally inoculated with  $1 \times 10^8$  CFU *B. abortus* strain RB51.

<sup>b</sup> CFU = Colony forming units of *B. abortus* strain RB51.

<sup>c</sup> SD = Standard deviation.

amount of tissue cultured). In a similar manner, the uteruses of female mice were cultured and cfu/uterus calculated.

With one possible exception (see below), no lesions attributable to brucellosis were noted on gross necropsy. Culture results from the orally and intraperitoneally RB51 inoculated groups are summarized in Table 1 and 2, respectively. Splenic and uterine cultures from the control groups were negative. Mice orally inoculated with strain RB51 developed low level splenic infections with strain RB51, but all were negative by 6 wk PI. RB51 (390 cfu) was isolated from the uterus of one orally inoculated mouse necropsied 2 wk PI; no other orally inoculated mouse had a uterine infection. Intraperitoneally inoculated mice had a higher rate of splenic and uterine infections and took longer to clear the infections than orally inoculated mice with some remaining positive at 8 wk PI.

Nine of the 20 (45%) orally inoculated RB51 mice necropsied in the first 4 wk PI had mild multifocal pleocellular inflammatory infiltrates in the liver; 3 of the 25 (12%) orally inoculated RB51 mice necropsied after wk 4 PI had similar lesions. All ten of the RB51 IP inoculated mice necropsied in the first 2 wk PI had mul-

TABLE 2. Isolation of *Brucella abortus* strain RB51 from intraperitoneally inoculated deer mice.<sup>a</sup>

Week post-inoculation	Spleen		Uterus	
	Number positive/number cultured	Mean cfu <sup>b</sup> /spleen (SD) <sup>c</sup>	Number positive/number cultured	Mean cfu/uterus (SD)
1	6/6	2,731,305 (6,544,525)	2/2	328 (92)
2	5/5	13,006 (27,356)	2/2	15 (7)
3	4/5	25 (35)	1/3	2 (4)
4	1/5	5 (10)	0/2	0 (0)
5	2/5	32 (51)	0/2	0 (0)
6	1/5	3 (7)	0/3	0 (0)
7	1/5	22 (48)	0/2	0 (0)
8	1/5	7 (15)	0/3	0 (0)
9	0/4	0 (0)	0/2	0 (0)

<sup>a</sup> Mice were inoculated with  $1 \times 10^8$  CFU *B. abortus* strain RB51.

<sup>b</sup> CFU = Colony forming units of *B. abortus* strain RB51.

<sup>c</sup> SD = Standard deviation.

tifocal pleocellular inflammatory infiltrates in the liver; two of ten (20%) RB51 IP mice necropsied wk 3 to 4 PI had similar lesions, and only 1 mouse necropsied after wk 4 PI from this group had these lesions. Generally, the lesions in the IP group were more severe (had more cellular infiltrates) than in the RB51 orally inoculated group. Lesions were milder in mice at longer times PI. One orally RB51 inoculated male mouse necropsied 5 wk PI had a mild lymphocytic infiltrate in the interstitium of the seminal vesicle but no RB51 was recovered from the spleen. Culture results were usually correlated with histologic lesions. The mice with the highest number of colonies of RB51 isolated were those with the most severe lesions. Mice that were culture negative tended to be void of lesions and visa versa. Incidental findings found in some mice in all groups included: mild myocarditis (19% of controls; 30% of RB51 inoculates), mild metritis (10% of controls; 5% of RB51 inoculates), mild multifocal pyogranulomatous pneumonia (8% of controls; 5% of RB51 inoculates), and nematodes in the small intestines and/or cecum (54% of controls; 33% of RB51 inoculates).

One male RB51 IP inoculated mouse died 5 days PI. Grossly the liver was pale yellow. Histologic lesions included acute multifocal suppurative hepatitis with lymphocytic infiltration and scattered hepatocellular degeneration and necrosis. There also was a mild interstitial pneumonia. We estimated that this mouse had  $1.6 \times 10^7$  cfu in the spleen at the time of death. Another male RB51 IP mouse died 57 days PI. No bacteria were isolated from this mouse's spleen. This mouse was very thin with an enlarged heart. The only histologic lesions were myocarditis and nematodes in the cecum. No other mice died prior to being euthanized.

Clearance of strain RB51 in deer mice was longer than expected. Laboratory mice generally clear the organism within 4 wk following IP injection (Schurig et al., 1991). Additionally, there was greater variation among deer mice in the time it took to clear infections as compared to laboratory mice.

In a field situation, deer mice would only be exposed to the vaccine orally and not IP. All the orally exposed animals remained clinically healthy with no evidence of illness. Orally exposed mice had fewer cfu cultured from their spleens and uteruses, cleared the vaccine more quickly, and had milder lesions than mice inoculated IP. Strain RB51, with the possible exception of one IP inoculated animal, did not cause significant morbidity or mortality. Thus, RB51 would be unlikely to have appreciable effects on populations of deer mice should they consume RB51 baits in a field situation.

Deer mice could serve as a source of RB51 for predators and scavengers for a short period following exposure, but, it is unlikely that they would be a significant source of RB51 because they appear to clear the vaccine within 7 wk. Studies are ongoing to determine the safety of RB51 in coyotes (*Canis latrans*) and other predators and scavengers (T. Kreeger, unpubl. data).

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