

Post-exposure serological and bacteriological responses of water buffalo (*Bubalus bubalis*) to *Brucella abortus* biovar 1 following vaccination with *Brucella abortus* strain RB51

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Summary

Serological and bacteriological responses to *Brucella abortus* biovar 1 following vaccination with *B. abortus* strain RB51 (RB51) were evaluated in thirty domestic water buffalo (*Bubalus bubalis*) randomly divided into five treatment groups. Groups I to V received, respectively, the recommended dose (RD) of RB51 vaccine once, RD twice 4 weeks apart, double RD once, double RD twice 4 weeks apart, and saline once (control). Vaccination did not result in a serological response. Experimental animals released 27 weeks post initial inoculation (27 PIIW) into a brucellosis-positive herd failed to seroconvert after 29 weeks. Experimental challenge commenced at 57 PIIW. All animals received *B. abortus* biovar 1 intraconjunctivally at 0, 5 and 9 weeks post experimental exposure (PEEW). Serum samples collected at 4, 8 and 13 PEEW were negative. At 16 PEEW all animals received *B. abortus* biovar 1 subcutaneously (SC), and all seroconverted by 20 PEEW. Five of twenty-six animals were positive for *Brucella* infection on bacterial culture. *Brucella abortus* biovar 1 was isolated from three animals; *B. abortus* RB51 was isolated from two. Treatment group, age and sex had no effect on the isolation of Brucellae ($P>0.05$).

Keywords

Brucella abortus RB51 – *Bubalus bubalis* – Challenge – Vaccine – Water buffalo.

Introduction

The isolation of *Brucella abortus* in Trinidad in 1999 (14) resulted in the loss of brucellosis free status as classified by the World Organisation for Animal Health (OIE). Subsequent testing, conducted by the Ministry of Agriculture, Land and Marine Resources (MALMR), identified two relatively large herds of water buffalo (*Bubalus bubalis*), totalling approximately 3,000 animals, that were serologically positive for brucellosis. Depopulation of these herds was not an option as this

represented 25% of the national water buffalo herd and culling was considered economically and ecologically unacceptable. Rearing of water buffalo in Trinidad is predominantly a small farmer activity; the animals provide power for field preparation and for transport, mainly in sugarcane production. Water buffalo are also used for meat and milk in a limited way. Herd vaccination with the commercially available *B. abortus* RB51 vaccine, administered at the recommended calfhood dose, was instituted by the MALMR in an attempt to reduce losses and prevent the spread of disease.

Brucellae are Gram-negative, facultative, intracellular pathogens (4). They infect mainly cattle, sheep, goats and other ruminants by horizontal transmission, and cause abortions, fetal deaths and genital infections (23). Brucellosis has been reported in bison (*Bison bison*), elk (*Cervus elaphus*), pronghorn (*Antilocapra americana*), mule deer (*Odocoileus hemionus*), moose (*Alces alces shirasi*), and coyotes (*Canis latrans*) (7, 12, 18, 19). Brucellosis is also a disease of public health importance in most of the developed and, more significantly, the developing world (6, 23). The incidence of human brucellosis is closely linked to the prevalence of the infection in livestock and to activities that allow exposure of humans to potentially infected animals or their products (23). Although the reported incidence and prevalence of brucellosis in cattle and water buffalo vary from country to country, the disease caused by *B. abortus* biovar 1 is still the most widespread form (13). Official estimates of the losses due to brucellosis in Latin America are equivalent to approximately US\$ 600 million per annum (6, 17).

Brucella abortus RB51 is a genetically stable, rough mutant strain that is produced by several passages of *B. abortus* smooth strain 2308 in media supplemented with sub-inhibitory concentrations of rifampicin (34). Strain RB51 has a *wboA* gene that is disrupted by an IS711 insertion element, which impairs the synthesis of the lipopolysaccharide O-chain (40). Due to this fact, vaccination with strain RB51 does not induce antibodies that can be detected by routine brucellosis surveillance tests, and any positive reaction to standard tests is considered to indicate an infected animal (36). The RB51 vaccine has proved safe and efficacious against infection and abortion in cattle and American bison (*Bison bison*) (4, 20, 27). Vaccination with RB51 induces a protective cell-mediated immune response against challenge with the virulent strain 2308 in certain animal species (37). The RB51 vaccine is also safe when inoculated into pregnant females at a reduced dose (30) or the recommended calfhoo dose (31), and is protective against infection of the cow and fetus, and against abortions.

Evaluation of the RB51 vaccine in water buffalo indicated that the vaccine was ineffective at preventing infection with *B. abortus* following natural exposure (16). Cell-mediated and antibody responses, safety and clearance following RB51 vaccination have, however, been reported for water buffalo (9, 10). The primary goal of this study was to evaluate the dose–response relationship following RB51 vaccination in domestic water buffalo challenged with *B. abortus* biovar 1. Our objectives were to investigate the ability of the RB51 vaccine to protect against infection and the relationships between seroconversion and the challenge dose and route of administration.

Materials and methods

Source of animals

Thirty domestic water buffalo calves, 6 to 10 months old (18 females and 12 males), were obtained from a brucellosis-free farm (8) in Aripo, Trinidad, for the purposes of this study. All animals on this farm that were more than one year old were subjected to the buffered plate agglutination test (BPAT) for *Brucella* agglutinins and were seronegative for brucellosis on three occasions 12 months apart; none of them had been previously vaccinated with S19. No new animals had been added to this herd in the eight years prior to the study and no brucellosis vaccination was ever performed. Serum samples, collected one month prior to commencement of the study and monthly for the duration of the study period, were evaluated by BPAT and a competitive enzyme-linked immunosorbent assay (c-ELISA) to assess the brucellosis status of all the experimental animals. The study animals were given three doses of ivermectin (Anupco, Suffolk, United Kingdom [UK]) 12 weeks apart (the first dose was administered 7 days prior to the commencement of the study). Forage, supplemental feed, water and a balanced vitamin/mineral additive were provided until 27 weeks after the initial inoculation (PIIW: post initial inoculation week).

The Ethics Committee of the Faculty of Medical Sciences of the University of the West Indies approved the research protocol and monitored the care of the experimental animals. The ‘five freedoms’ (freedom from hunger, thirst and malnutrition; freedom from fear and distress; freedom from physical and thermal discomfort; freedom from pain, injury and disease; and freedom to express normal patterns of behaviour) were employed as indicators of animal welfare (42).

Vaccination of study animals

Brucella abortus strain RB51 (RB51) vaccine (serial # 1615) (Professional Biological Company, Denver, Colorado, United States of America [USA]) was obtained for use in this study. Vaccine viability was assessed by serial 100-fold dilutions, which were plated on tryptose agar (Difco, Michigan, USA) fortified with 2% equine serum and incubated at 37°C in 5% CO₂. The colony-forming units (CFU) per 2 ml reconstituted dose, i.e. the calfhoo recommended dose (RD), was found to be 2.02×10^{10} CFU of RB51. This value was within the labelled specifications of $1.0\text{--}3.4 \times 10^{10}$ CFU (38, 41) of RB51 per calfhoo dose. The reconstituted vaccine was handled with care to avoid accidental injection or eye or skin contamination (41).

Calves were identified with numbered ear tags and were randomly divided into five treatment groups with

stratification according to age and sex. Twenty-four calves (six per group) were inoculated subcutaneously (SC) with RB51 vaccine into the right and left mid-cervical regions. Groups I to IV received, respectively, the RD of RB51 vaccine once, the RD twice 4 weeks apart, and double the RD once, and double the RD twice 4 weeks apart. Six calves (group V; controls) were similarly inoculated with phosphate buffered saline (PBS) (5). Study animals were housed together in a brucellosis-free quarantine station until 27 PIIW. All samples taken post-treatment were assigned random numbers to avoid bias and to ensure that subsequent testing was performed blindly.

Serological detection of brucellosis

Exposure to field strain *B. abortus* was evaluated using the BPAT and c-ELISA with S-1119 antigens from the United States Department of Agriculture (USDA) using an established protocol (24). Exposure to the vaccine-specific antigen, *B. abortus* strain RB51, was evaluated by the dot-blot assay as previously described (29).

Experimental animals were identified by numbered neck straps (Nasco, Forth Atkinson, USA) and released at 27 PIIW into an infected herd of water buffalo that had a 56% seroprevalence of *B. abortus* biovar 1 (16). Blood was obtained at 0 (on the first day of natural exposure), 5, 9, 14, 19 and 29 weeks following the start of the period of natural exposure (PNEW: post natural exposure week). The BPAT and c-ELISA were used to monitor for evidence of seroconversion.

Experimental challenge of animals with *Brucella abortus*

After thirty weeks under conditions of natural exposure, all the study animals were transferred to a confined, isolated facility and experimentally challenged. For the experimental challenge, *B. abortus* strains 24 E and Trinidad 1 were utilised. Strain 24 E was isolated from a lymph node of a naturally infected water buffalo in a previous study (14); Trinidad 1 was isolated 6 months prior from a superficial abscess on a naturally infected water buffalo and was obtained from the Veterinary Diagnostic Laboratory (MALMR, Eric Williams Medical Sciences Complex Mount Hope, Trinidad). Both strains were confirmed as *B. abortus* biovar 1 by the Veterinary Laboratories Agency, Weybridge, UK. *Brucella abortus* challenge cultures were grown on blood agar plates for 72 h at 37°C in 5% CO₂. Bacteria were harvested into PBS using a sterile glass spreader. Bacterial suspensions were adjusted spectrophotometrically (Bausch and Lomb, Rochester, New York, USA) based on the different levels of challenge. Serial dilutions and standard plate counts were

used to determine the actual concentrations of viable bacteria within each inoculum, pre- and post-inoculation.

Experimental challenge commenced at 57 PIIW. Animals were fasted for 24 h and sedated intravenously (IV) with xylazine (0.05 mg/kg) (Bomac, Auckland, New Zealand). Physical restraint (a squeeze shoot and ropes) was used as an adjunct to sedation to ensure the safety of the buffalo and operators. Animals were challenged intraconjunctivally (IC) by placing 50 µl of inoculum in one eye and allowing at least 60 s to elapse before depositing an equal quantity of inoculum in the other eye. Sedation was reversed, 5 min after eye inoculation, with tolazoline (4 mg/kg, IV) (Lloyd Laboratories, Iowa, USA). An initial challenge dose of 1.1×10^7 CFU of strain 24 E per 100 µl of inoculum was used. When seroconversion was not detected by either BPAT or c-ELISA at 4 weeks after experimental exposure (PEEW: post experimental exposure week), animals were challenged at 5 PEEW with 2.2×10^7 CFU of strain Trinidad 1. Failure to seroconvert resulted in re-challenge with strain Trinidad 1 at 9 and 16 PEEW with 1.2×10^8 CFU IC and 1.1×10^{10} CFU SC, respectively. Serum samples were collected at 13 and 20 PEEW to monitor for seroconversion to experimental exposure (Table 1). Protective clothing (including gloves, eye shields, masks, and oronasal respirators) was employed on all occasions by personnel during the challenge experiment, and suspensions were handled under appropriate conditions of biohazard containment because both RB51 and *B. abortus* biovar 1 can infect humans (41).

Isolation of *Brucella abortus* (strains RB51 and biovar 1) from tissues

Experimental animals were slaughtered at the government-approved abattoir on the island of Trinidad by trained public health officials. Parotid, retropharyngeal, bronchial, mesenteric, pre-femoral, supramammary, internal iliac and hepatic lymph node specimens were collected in sterile Whirl-Pack® bags (Nasco, Fort Atkinson, Wisconsin, USA) and stored at -20°C until processed at the veterinary public health laboratory of the School of Veterinary Medicine, University of the West Indies, Champs Fleurs, Trinidad.

Bacteriological isolation procedures for Brucellae were based on a modification of published recommendations (2, 32, 42) and the standard operating protocols for National Veterinary Services Laboratories (NVSL) of the USDA (kindly provided by Drs J. Payeur and B. Martin, NVSL, Ames, Iowa, USA). Selective media for primary isolation were prepared using a tryptose agar base fortified with 2% equine serum that contained antibiotic concentrations recommended by NVSL. The antibiotics included were cyclohexamide (30 mg/l of media),

Table 1
Natural and experimental exposure of water buffalo (*Bubalus bubalis*) to field strains of *Brucella abortus* biovar 1

Type of challenge	Date of challenge	Source of challenge	Route of exposure	Exposure dose (CFU)	Sample ^(c) dates (results) ^(d)
Natural	8 October 2002 to 15 May 2003	Uterine fluids / membranes	Oral	Unknown	13 November 2002 (–) 17 December 2002 (–) 28 January 2003 (–) 4 March 2003 (–) 21 May 2003 (–)
Experimental	21 May 2003	Lymph node ^(a)	IC ^(e)	1.1×10^7	18 June 2003 (–) 16 July 2003 (–)
Experimental	24 July 2003	Skin abscess ^(b)	IC	2.2×10^7	27 August 2003 (–)
Experimental	5 September 2003	Skin abscess	IC	1.2×10^8	2 October 2003 (–)
Experimental	21 October 2003	Skin abscess	SC ^(f)	1.1×10^{10}	17 November 2003 (+)

a) Isolated from lymph node samples obtained from a naturally infected buffalo

b) Isolated from a superficial skin abscess from a naturally infected buffalo

c) BPAT (buffered plate agglutination test) and c-ELISA performed on indicated dates

d) All animals were BPAT and c-ELISA negative (–) or positive (+)

e) IC – intraconjunctival (total dose in 100 µl; 50 µl per eye)

f) SC – subcutaneous (total dose in 1 ml given just above the elbow)

bacitracin (7,500 units/l), and polymixin B sulphate (1,800 units/l) (Azupharma, Gerlingen, Stuttgart, Germany). Blood agar plates, without serum or antimicrobial agents, were used for secondary isolation.

Lymph nodes were allowed to thaw at room temperature before processing. Excess fat was trimmed from the nodes and they were dipped in 95% ethanol and flamed. The lymph nodes from each animal were pooled and minced in a sterilised laboratory blender with an equal volume of PBS (pH 6.4) and then serially diluted ten-fold with PBS prior to plating. A volume of 100 µl of each of the 10-fold serial dilutions of lymph node extract from each animal was plated on selective media and incubated at 37°C in an atmosphere containing 5% CO₂. Plates were examined at 3, 5, 7, 9 and 11 days of incubation, after which primary isolation plates were discarded. Suspect *Brucella* colonies were sub-cultured on blood agar plates, without serum and antimicrobial agents, before identification procedures were performed (2, 41).

Suspect isolates were identified as *Brucella* based on colony morphology, Gram stain evaluation, biochemical test results and agglutination with *B. abortus* positive control sera. Biochemical tests included determination of catalase, oxidase, and urease production. Presumptive *Brucellae* were non-haemolytic, and were catalase, oxidase and urease positive (2, 41). Isolates with biochemical test results typical of *Brucella* were stored in skimmed milk at –40°C and later sent on tryptose slants to the Veterinary Laboratories Agency, Weybridge, UK for confirmation and biovar determination. Cultures and suspensions were handled under appropriate conditions of biohazard containment (Class II safety cabinets) (41).

Statistical analysis

The mean age and sex of animals in the five treatment groups were compared using one-way analysis of variance (ANOVA) to investigate the possibility of age and sex as confounding variables. The proportion of animals in each treatment group that were positive for *B. abortus* biovar 1 infection was compared using one-way ANOVA. Four experimental animals that were lost to follow-up, for reasons unrelated to brucellosis challenge (10), were not included in the analyses because determination of outcome was not possible. Animals were classified as positive for *Brucella* infection if persistent brucellosis titres were present or if *Brucella* was isolated from lymph node specimens collected at slaughter. Persistent titres were defined as positive results on both serological tests used in one sampling period or consecutive positive results on any single test in two different sampling periods.

Results

All animals tested negative on both the BPAT and the c-ELISA for field strain *B. abortus* prior to inoculation and at all sampling intervals following vaccination and before exposure. Vaccinates and controls that were exposed to a brucellosis-infected herd of water buffalo also failed to seroconvert on BPAT and c-ELISA (Table 1). Both treatment groups failed to seroconvert on BPAT after experimental exposure to three doses of *B. abortus* biovar 1 inoculated IC on three separate occasions. However, all experimental animals (vaccinates and controls) seroconverted after SC exposure to *B. abortus* biovar 1.

Table II
Isolation of *Brucella abortus* from regional lymph nodes of water buffalo (*Bubalus bubalis*)

Group/treatment	<i>B. abortus</i> isolates by number, sex and proportion positive	
	Biovar 1	RB51
I – Recommended dose (RD) $1.0\text{--}3.4 \times 10^{10}$ CFU	0, NA, NA	0, NA, NA
II – RD twice, four weeks apart	0, NA, NA	1, F, 0.20
III – Double RD	1, F, 0.20	1, F, 0.20
IV – Double RD & double RD in four weeks	1, F, 0.20	0, NA, NA
V – Phosphate buffered saline (control)	1, F, 0.25	0, NA, NA

F: female (indicates sex of animals from which *Brucella* was isolated)

NA: not applicable

Five (19.2%) of the twenty-six animals that completed the study were classified positive for *Brucella* infection based on isolation of the bacterium. Two vaccinates and one control were infected with *B. abortus* biovar 1. Two vaccinates were persistently infected with *B. abortus* RB51. *Brucella abortus* RB51 was not isolated from any of the controls (Table II). Age and sex had no effect on the isolation of Brucellae from infected animals ($P>0.05$). The proportions of *B. abortus* biovar 1 positive animals in the vaccinated and control groups were not statistically significantly different ($P>0.05$).

Discussion

Brucella abortus RB51 has been approved for use as the official vaccine in the USA as a replacement for S19 (20). Our finding that serological tests following RB51 vaccination and pre-exposure were negative confirms the fact that RB51 vaccination does not interfere with the serological diagnostic tests for brucellosis, as earlier reported in cattle (25). This is a desirable attribute of the RB51 vaccine that allows the differentiation of RB51 vaccinates from animals infected with field strain Brucellae.

The water buffalo is potentially the most important tropical bovine species in areas where rivers and swamps abound (35), yet there is no reportedly effective vaccine against brucellosis in this valuable livestock species (16). Protection in response to brucellosis vaccine is measured by significant decreases in abortions and in colonisation of vaccinated cattle when compared after challenge with non-vaccinated controls (11). Previous studies in cattle have demonstrated that vaccinated heifers have lower rates of infection than non-vaccinated controls (3, 11, 31). Protection against infection results in reduced risk of dissemination of the bacteria to other animals, including humans. In this study, isolation of the challenge strain from lymph nodes was used as the criterion for *B. abortus*

biovar 1 infection. Seroconversion was used as the criterion to quantify an appropriate challenge dose. We hoped that this information would allow us to detect significant differences across treatment groups to give an indirect measure of the vaccine efficacy of *B. abortus* strain RB51.

Vaccine evaluation using standardised virulent challenge protocols in strictly controlled experiments allows for comparison with previous studies (1). The dosage, the strain of *B. abortus*, and the route and time of exposure in relation to challenge studies, particularly in non-cattle species, remain topics of speculation and discussion (27). *Brucella abortus* biovar 1 is a field strain of *B. abortus*, but the strain that has been used under field and experimental conditions to mimic natural infection is *B. abortus* strain 2308 (S2308) (27). We were forced to use *B. abortus* biovar 1 as the challenge strain in this study as a result of local import and foreign export restrictions on obtaining S2308 from the USA. Two different isolates, one from the lymph node of an apparently healthy animal and the other from an active lesion (abscess), were used to increase the likelihood of adequate exposure based on theoretical differences in pathogenicity.

The standard protocol for brucellosis vaccine experiments in cattle has been to administer 1×10^7 CFU of S2308 at around 180 days of gestation (4). A mean challenge dose of 3.14×10^7 CFU of S2308 was used in American bison (27). Experimental challenge with insufficient numbers of pathogenic *B. abortus* can fail to cause infection and seroconversion (21). It has been reported that vaccine-induced protection may be overwhelmed, however, if challenge dosages are too high (5). Some brucellosis vaccines, such as those derived from strains 45 and S19, have been documented to result in abortions when administered IV but in few or no abortions when administered SC (22).

In the current study, the release of experimental animals into a naturally infected herd of 700 water buffalo with a reported *Brucella* seroprevalence of 56% (15) failed to produce seroconversion after 29 weeks (November to May) of exposure. This fact may be explained in part due to the low number of births that occurred during the exposure period. This period of natural exposure was unfortunately restricted mainly to the dry season, which corresponded to the end of the calving season for the water buffalo herd. Calving in water buffalo in Trinidad commences in July and ends in January. Water buffalo are observed to be territorial and tend to stay in groups that are controlled by a dominant male. This behaviour may have contributed to inadequate exposure to infected animals at calving time and hence the lack of seroconversion to *B. abortus* biovar 1 under field conditions.

The RB51 vaccine doses used in this study ($>2 \times 10^{10}$ CFU) were slightly higher than the experimental challenge doses

($\leq 1.1 \times 10^{10}$ CFU). It has been reported that products of conception at the time of abortion may contain up to 10^{10} to 10^{13} Brucellae per gram (2, 23). In this study the dose given by IC challenge was equivalent to or higher than that described as standard methodology for brucellosis experiments in cattle in the USA (27) and Brazil (31), yet failed to produce seroconversion. The overall lack of seroconversion following IC exposure suggests either that water buffalo are highly resistant to infection or that the route of exposure and dosages used in this study were inappropriate. In the current study, no conclusion can be reached on the protective effect of RB51 vaccination against infection after IC challenge because no animals were slaughtered shortly after exposure to ascertain the presence of *B. abortus* biovar 1 in tissue. The fact that SC exposure to 1.1×10^{10} CFU of biovar 1 resulted in seroconversion of all animals (vaccinates and controls) was not unexpected because a similar dose of RB51 vaccine, injected via the same route, elicited a serological response detectable by dot-blot and complement fixation tests (10). Seroconversion as a result of the SC challenge, in this study, may be explained in part by the possibility that the dose and route of challenge overwhelmed any protective effect of vaccination.

Different strains of *Brucella* spp. may display variable pathogenicity or virulence in their host animals and these characteristics will affect the manifestation of brucellosis in exposed herds (5). There are reported differences in susceptibility and clinical manifestations of infection with *B. abortus* in cattle and bison (23). The existence of genetic resistance to brucellosis has also been documented (39) and this may account for some of the species differences. It is also possible that there are innate differences in the immune systems of cattle and water buffalo (8, 9, 10). The RB51 vaccine was reported to be ineffective in elk (*Cervus elaphus*) (18) due to inherent species differences in the immunological response (28). Flow cytometry, along with other techniques, failed to detect significant anamnestic responses to *Brucella* antigens in S19 or SRB51 vaccinates after booster vaccination in elk (26). It is therefore possible that the immune response of water buffalo to RB51 vaccination is similar to that reported for elk (18) and this may account for the apparent lack of efficacy of the RB51 vaccine in water buffalo.

Bacteriological analysis of the samples obtained in the present study indicated that there is no protection offered

by RB51 vaccination to prevent infection by *B. abortus* biovar 1 at a challenge dose of 1.1×10^{10} CFU administered SC. It is interesting to note that two of the vaccinates were culture positive for RB51 at the end of this study (77 weeks following the initial vaccination). The duration of tissue recovery of RB51 post-vaccination in water buffalo is therefore considerably longer than the 12 weeks reported for cattle (25) and the 30 weeks reported for the American bison (33).

Conclusion

There remains no reported effective *Brucella* vaccine, challenge dose, challenge strain or challenge route of administration for the water buffalo (*B. bubalis*). The results of this study indicate that the IC exposure of water buffalo to *B. abortus* biovar 1 at doses equivalent to and higher than those reported for cattle and bison exposed to *B. abortus* S2308 is insufficient to cause seroconversion. This study also indicates that *B. abortus* strain RB51 can persist much longer in the regional lymph nodes of water buffalo compared with those of cattle and bison following vaccination with RB51. More research is necessary to establish the appropriate challenge route and dose of *B. abortus* biovar 1 for seroconversion and to develop an effective brucellosis vaccine protocol for water buffalo.

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Étude de la réponse sérologique et bactériologique chez des buffles d'eau (*Bubalus bubalis*) exposés à *Brucella abortus* biovar 1 après avoir été vaccinés avec la souche RB51 de *Brucella abortus*

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Résumé

Une étude a été réalisée sur trente buffles d'eau domestiques (*Bubalus bubalis*) répartis de manière aléatoire en cinq groupes afin de déterminer les réponses sérologique et bactériologique de ces animaux à une inoculation d'épreuve de *Brucella abortus* biovar 1, après qu'ils aient été vaccinés avec la souche RB51 de *B. abortus*. Les buffles du groupe I ont reçu une injection unique du vaccin à la dose recommandée (DR), ceux du groupe II ont reçu deux injections du vaccin à la DR à quatre semaines d'intervalle, ceux du groupe III ont reçu le double de la DR en une injection unique, ceux du groupe IV ont reçu deux fois le double de la DR à quatre semaines d'intervalle et le dernier groupe (de contrôle) a reçu une dose unique de solution saline. La vaccination n'a pas produit de réponse sérologique. À la 27^e semaine après la première administration de vaccin, les buffles ont été relâchés dans un troupeau atteint de brucellose, mais à la 29^e semaine aucune apparition d'anticorps n'était constatée chez les animaux vaccinés. L'inoculation d'épreuve a commencé à la 57^e semaine après la première vaccination. Tous les animaux ont reçu une inoculation d'épreuve de *B. abortus* biovar 1 administrée par voie conjonctivale au moment de l'exposition expérimentale, puis 5 semaines et 9 semaines après l'exposition expérimentale. Les échantillons de sérum prélevés 4 semaines, 8 semaines et 13 semaines après l'exposition expérimentale ont tous donné des résultats négatifs. A la 16^e semaine après l'exposition expérimentale, les animaux ont reçu une injection de *B. abortus* biovar 1 par voie sous-cutanée, et à la 20^e semaine l'apparition d'anticorps était constatée chez tous les animaux. *Brucella* a été isolée de 5 buffles sur 26 par culture bactérienne. *Brucella abortus* biovar 1 a été isolée de trois de ces buffles et *B. abortus* RB51 des deux autres. Aucune corrélation entre le groupe, l'âge ou le sexe des animaux et l'isolement des *Brucella* n'a été constatée ($P > 0.05$).

Mots-clés

Brucella abortus RB51 – *Bubalus bubalis* – Buffle – Inoculation d'épreuve – Vaccin.



Respuesta serológica y bacteriológica a la exposición al biovar 1 de *Brucella abortus* en búfalos de agua (*Bubalus bubalis*) vacunados con la cepa RB51 de *Brucella abortus*

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Resumen

Los autores describen la evaluación de la respuesta serológica y bacteriológica a la exposición al biovar 1 de *Brucella abortus* de treinta búfalos de agua domésticos (*Bubalus bubalis*) previamente vacunados con la cepa RB51 del microorganismo, divididos al azar en cinco grupos de tratamiento. Los grupos I a V recibieron, respectivamente, la dosis recomendada de vacuna RB51 una vez, la misma dosis dos veces con 4 semanas de intervalo, el doble de la dosis en una sola vez, el doble de la dosis por dos veces con 4 semanas de intervalo y, por último, una solución salina (grupo control). La vacunación no generó respuesta serológica. Los animales introducidos en un rebaño positivo a la brucelosis 27 semanas después de la primera inoculación no habían experimentado seroconversión a la semana 29. A las 57 semanas dio comienzo la exposición experimental. Todos los animales recibieron el biovar 1 por vía intraconjuntival después de 0, 5 y 9 semanas de la exposición experimental. Las muestras séricas extraídas al cabo de 4, 8 y 13 semanas de la exposición experimental resultaron negativas. A las 16 semanas de la exposición experimental se inoculó el biovar 1 de *B. abortus* a todos los animales por vía subcutánea, y en la semana 20 todos ellos mostraban seroconversión. Cinco de veintiséis animales resultaron positivos a la infección por *Brucella* en cultivo bacteriológico. Se aisló el biovar 1 de *Brucella abortus* en tres ejemplares, y en otros dos se aisló la cepa RB51. Ni el grupo de tratamiento, ni la edad ni el sexo tuvieron influencia alguna en el aislamiento de brucelas ($P>0,05$).

Palabras clave

Brucella abortus RB51 – *Bubalus bubalis* – Búfalo de agua – Exposición – Vacuna.



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