The *Brucella abortus* S19 ΔvjbR Live Vaccine Candidate Is Safer than S19 and Confers Protection against Wild-Type Challenge in BALB/c Mice When Delivered in a Sustained-Release Vehicle

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**Brucellosis is an important zoonotic disease of nearly worldwide distribution.** Despite the availability of live vaccine strains for bovine (S19, RB51) and small ruminants (Rev-1), these vaccines have several drawbacks, including residual virulence for animals and humans. Safe and efficacious immunization systems are therefore needed to overcome these disadvantages. A vjbR knockout was generated in the S19 vaccine and investigated for its potential use as an improved vaccine candidate. Vaccination with a sustained-release vehicle to enhance vaccination efficacy was evaluated utilizing the live S19 ΔvjbR::Kan in encapsulated alginate microspheres containing a non-immunogenic eggshell precursor protein of the parasite *Fasciola hepatica* (vitelline protein B, BALB/c mice were immunized intraperitoneally with either encapsulated or nonencapsulated S19 ΔvjbR::Kan at a dose of 1 × 10^6 CFU per animal to evaluate immunogenicity, safety, and protective efficacy. Humoral responses postvaccination indicate that the vaccine candidate was able to elicit an anti-*Brucella*-specific immunoglobulin G response even when the vaccine was administered in an encapsulated format. The safety was revealed by the absence of splenomegaly in mice that were inoculated with the mutant. Finally, a single dose with the encapsulated mutant conferred higher levels of protection compared to the nonencapsulated vaccine. These results suggest that S19 ΔvjbR::Kan is safer than S19, induces protection in mice, and should be considered as a vaccine candidate when administered in a sustained-release manner.

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*Brucella abortus,* a gram-negative, facultative, intracellular bacterium, is a causative agent of brucellosis, a zoonosis of nearly worldwide distribution (5). In animals, brucellosis is a major cause of abortions and infertility (3, 19, 28). In humans, infection can cause a serious debilitating disease manifested as undulant fever, endocarditis, arthritis, and osteomyelitis (23). Due to serious economic losses and public health risk, extensive efforts have been conducted to prevent the disease in animals through vaccination programs (22). Live attenuated vaccines have been developed and successfully used worldwide against bovine brucellosis (26). Currently, no effective vaccines are available for the prevention of human brucellosis.

*B. abortus* S19 live vaccine has been extensively used to prevent bovine brucellosis (22). The S19 vaccine strain was first isolated from the milk of a Jersey cow in 1923 and, while stored in the laboratory at room temperature, developed an attenuated phenotype (22). Numerous efficacy studies conducted with cattle for this vaccine have demonstrated that 70% of the vaccinated cattle are protected from a wild-type exposure (22). The effectiveness depended on a series of variables, including the age of the vaccinated animal, the prevalence of the disease in vaccinated herds, and the dose and route of the vaccination (22, 26). Although S19 typically exhibits low virulence in cattle, the vaccine can cause abortions when administered to pregnant animals at rates between 1 to 2.5% (26). A less-frequent adverse effect of S19 vaccination is the development of an arthropathy associated with *Brucella* antigen-containing immune complexes (22, 26).

In many developing nations, immunizations derived from the S19 vaccine have been evaluated in humans. In the former Soviet Union, the administration of live S19 preparations were immunogenic, and protection was achieved and considered to last 1 year but caused a modest but notable incidence of clinical cases, as well as a hypersensitivity reaction (6, 22). As such, S19 is not a safe vaccine candidate for human use. Previous research in our lab has identified *Brucella* genes required for virulence and survival via transposon mutagenesis (2, 13). Among these, *vjbR* (BMEI1116), encoding the luxR-like quorum sensing-related transcriptional regulator is required for *virB* expression, virulence in mice, and survival in macrophages (8). BALB/c mice immunized with the *vjbR* mutant were protected against wild-type challenge without exhibiting any local or adverse reactions, making such mutants ideal vaccine candidates for future consideration (4). In the present study, the effects of eliminating the *vjbR* gene from *B. abortus* S19 were evaluated in vitro and in BALB/c mice. When *vjbR* is deleted in S19, two positive effects are observed: diminished inflammation (reduced splenomegaly) and reduced persistence. Taken together, these effects lead to an increased safety of the vaccine strain, since S19 alone elicits splenomegaly, an undesirable side effect of vaccination. The capacity of the *B. abortus* S19 ΔvjbR::Kan mutant to elicit *Brucella*-specific immune responses was also evaluated. In an effort to enhance the vaccination efficacy, the knockout was encapsulated into alginate microspheres containing a nonimmunogenic eggshell precursor protein of the parasite *Fasciola hepatica* (vitelline protein B [VpB]) as previously described (4) that has been used to alter the release properties of the microcapsules.
with the aim of producing vaccines that are safer while retaining protective efficacy.

**MATERIALS AND METHODS**

**Mice.** One hundred fifty 8- to 10-week-old female BALB/c mice were obtained from the Jackson Laboratory (Bar Harbor, ME). All experimental procedures and animal care were performed in compliance with institutional animal care regulations.

**Bacterial strains.** Bacterial strains used in these experiments include *B. abortus* S19 (NVSL, Ames, IA), *B. abortus* S19 ΔvjbR::Kan (engineered for this study), and *B. abortus* virulent strain 2308 (originally obtained from Billy Deyoe). The bacteria were routinely grown on tryptic soy agar (TSA) at 37°C with 5% (vol/vol) CO2. For *B. abortus* S19 ΔvjbR::Kan, the medium was supplemented with kanamycin (100 μg/ml). Following 3 days of incubation, the bacteria were harvested from the surface of the plates into phosphate-buffered saline (PBS).

The bacteria were pelleted, washed twice by resuspension in MOPS (morpholinepropanesulfonic acid) buffer (10 mM MOPS, 0.85% NaCl [pH 7.4]), and resuspended to a final concentration of 1 × 10^8 CFU/ml (for encapsulation) or 1 × 10^9 CFU/ml in 100 μl of PBS (for nonencapsulated bacteria) based upon optical density readings using a Klett meter and a standardized curve. The actual viable counts were confirmed by serial dilution and plating of portions onto TSA plates with or without antibiotic. To inoculate the mice, the bacteria were harvested the same day of challenge. Inoculum doses were plated retrospectively to determine the number of organisms inoculated.

*Escherichia coli* cultures utilized during the knockout construction were grown on Luria-Bertani (Difco, Becton Dickinson) plates or in broth overnight at 37°C with or without kanamycin (100 μg/ml), carbenicillin (100 μg/ml), or chloramphenicol (50 μg/ml).

**Construction of the S19 ΔvjbR::Kan deletion mutant.** The marked S19 ΔvjbR::Kan deletion mutant was constructed in S19 as previously described with some modifications (15). Specifically for this mutant, the sequence downstream of the vjbR gene was amplified from *B. abortus* 2308 with the primer pair 5′-GTCTTCGAAGGATGCAATTGCGC and 5′-CAGTTGGAAAAGGGCTTTTCA. The sequence of downstream of vjbR was amplified with the primers 5′-GAGGCGCAGGATGTCG and 5′-ACAGTTGGAAAAGGGCTTTTCA. These two products were ligated to one another via overlapping PCR with an Ascl site (New England Biolabs) engineered between the two sequences.

The bacteria were routinely grown on tryptic soy agar (TSA) at 37°C with 5% (vol/vol) CO2. The bacteria were pelleted, washed twice by resuspension in MOPS (morpholinepropanesulfonic acid) buffer (10 mM MOPS, 0.85% NaCl [pH 7.4]), and resuspended to a final concentration of 1 × 10^8 CFU/ml (for encapsulation) or 1 × 10^9 CFU/ml in 100 μl of PBS (for nonencapsulated bacteria) based upon optical density readings using a Klett meter and a standardized curve. The actual viable counts were confirmed by serial dilution and plating of portions onto TSA plates with or without antibiotic. To inoculate the mice, the bacteria were harvested the same day of challenge. Inoculum doses were plated retrospectively to determine the number of organisms inoculated.

**Evaluation of Brucella-specific antibody.** To determine the effect of encapsulation in the production of anti-Brucella-specific antibody in sera from inoculated mice, 100 μl of blood was taken from each mouse after 0, 3, and 7 weeks postvaccination as well as 1 week postchallenge. The serum was separated and used for immunoglobulin G1 (IgG1) and IgG2a determination by enzyme-linked immunosorbent assay (ELISA). Heat-killed and sonicated *B. abortus* whole-cell antigen was used to coat 96-well plates (Nunc-Immu-no plates) at a concentration of 25 μg total protein/well. Following overnight incubation at 4°C, the plates were washed, blocked with 0.5 ml of 0.5 M NaOH and the absorbance measured at 450 nm. All the assays were performed in triplicate and repeated at least three times.

**Efficacy of vaccination.** At selected times postvaccination, the mice (*n* = 5 per group) were challenged i.p. using 1 × 10^7 CFU/mouse of *B. abortus* wild-type 2308. One week postchallenge, the mice were euthanized by CO2 asphyxiation, and their spleens were removed, weighed, and homogenized in 1 ml of peptone saline. Serial dilutions were prepared, and 100-μl portions were plated onto TSA plates. In some instances, 200 to 1,000 μl of spleen homogenate was plated to determine organism recovery. To differentiate between the vaccine candidate and the challenge strain, each dilution was also plated on TSA with kanamycin to identify any residual kanamycin-resistant strain present. The levels of infection were expressed as the mean ± standard error of the mean (SEM) of individual log CFU/spleen.

**Histopathology.** Twelve 8- to 10-week-old female BALB/c mice were distributed into groups of three mice and inoculated with 1 × 10^9 CFU per mouse of S19, S19 ΔvjbR::Kan, *B. abortus* 2308, or PBS. At 3 weeks postinoculation, the animals were euthanized by CO2 asphyxiation, and the spleens, lungs, livers, kidneys, and hearts were harvested, fixed in 10% buffered formalin, paraffin embedded, and stained. Histological changes were assessed between the treatment groups.

**Preparation of *B. abortus* antigen-loaded microspheres.** Alginic beads were prepared as previously described with some modifications (1). Briefly, 6 × 10^9 CFU of live *B. abortus* S19 ΔvjbR::Kan or *B. abortus* S19 was resuspended in 1 ml of MOPS buffer (10 mM MOPS, 0.85% NaCl [pH 7.4]) and mixed with 5 ml of alginate solution (1.5% sodium alginate, 10 mM MOPS, 0.85% NaCl [pH 7.3]). VpB was added as a component of the alginate core by the addition of 1 mg of VpB to the bacterium- alginate suspension described above. Spheres were obtained by extruding the suspension through a 200-micron nozzle into a 100 mM calcium chloride solution and stirring for 15 min using the Intotech encapsulator I50 (Intotech Biosystems International, Rockville, MD). After the bacterium- alginate mixture was extruded into the CaCl2, the capsules were washed twice with MOPS for 5 min and further cross-linked with 0.05% poly-L-lysine (molecular weight, 22,000; Sigma) for 10 min. Following two successive washes, the beads were stirred in a solution of 0.03% (wt/vol) alginate for 5 min to apply a final outer shell and washed twice with MOPS before storage at 4°C. To determine the bacterial viability postencapsulation, 1 ml of capsules was removed from the encapsulator prior to permanent cross-linking with poly-L-lysine. The capsules were allowed to settle and were washed twice with MOPS buffer, and particles were dissolved using 1 ml of depolymerization solution (50 mM Na2 citrate, 0.45% NaCl, 10 mM MOPS [pH 7.2]) with stirring for 10 min. The bacterial number (CFU/ml) in each ml of capsules was determined by plating onto TSA plates.

**Immunization of mice.** Twenty 8- to 10-week-old female BALB/c mice were randomly distributed into groups of five mice for i.p. vaccination. Initial preliminary vaccination studies indicated no difference in vaccination efficacy when animals were given either 1 × 10^5 CFU or 1 × 10^6 CFU. The treated animals were given a single dose of vaccine containing 1 × 10^6 CFU of either encapsulated *B. abortus* S19 ΔvjbR::Kan in alginate with VpB inside the capsule’s shell or nonencapsulated *B. abortus* S19 ΔvjbR::Kan. The control groups received 1 × 10^6 CFU of either nonencapsulated S19 or empty capsules (no bacteria entrapped) resuspended in 100 μl of MOPS buffer.

**Statistical procedures.** Macrophage infection and survival were expressed as the mean log CFU ± standard deviation for each group and analyzed by analysis of variance. Post hoc comparisons were made by Tukey’s test.
of variance (ANOVA) followed by a Tukey’s posttest comparing all groups to one another at the same time point. The intensity of infection (bacterial clearance) at each time point was expressed as the mean log CFU ± SEM for each group and analyzed by a two-tailed Student’s t test. The efficacy of the vaccination and the differences in spleen weight postchallenge were expressed as the mean log CFU ± SEM for each treatment group and analyzed by ANOVA followed by Tukey’s posttest comparing all groups to one another. IgG production was expressed as the mean absorbance ± SEM. The significance of differences between the groups was determined by ANOVA followed by a Tukey’s posttest comparing all groups to one another. P values of <0.05 were considered statistically significant.

RESULTS

*B. abortus* S19 ΔvjbR::Kan is more attenuated for survival in macrophages and in mice than S19. To determine the role of the deletion of the vjbR gene in virulence in S19, J774A.1 macrophages were infected with the marked deletion mutant and compared to the parental strain and to the wild-type *B. abortus* 2308. Using an MOI of 100, the ability of the bacteria to enter and survive within this cell line was evaluated. At 1 h postinfection (t = 0), there was no difference (P > 0.05) between S19, *B. abortus* 2308, or S19 ΔvjbR::Kan in the number of bacteria infecting the cell. By 48 h postinfection, there was a 0.96-log difference (P < 0.001) in the number of organisms infecting the cell for S19 versus that for wild-type 2308 or a 1.47-log difference between the number for S19 ΔvjbR::Kan versus that for wild-type 2308 (P < 0.001). When the marked mutant was compared to the parental strain (S19), there was a 0.51-log reduction in the number of bacteria surviving inside the macrophage (P < 0.05) (Fig. 1). These results indicate that S19 ΔvjbR::Kan is attenuated in macrophages.

To determine the virulence of S19 ΔvjbR::Kan in vivo, mice were inoculated i.p. with 1 × 10⁶ CFU/mouse of S19 ΔvjbR::Kan or the parental strain S19. Compared to that of S19, the colonization of S19 ΔvjbR::Kan in the spleen did not differ (P > 0.05) at 1, 3, 5, or 7 weeks postinfection, and at only 9 weeks postinfection, bacterial colonization of S19 ΔvjbR::Kan was significantly reduced (P < 0.013) compared to that of S19 (Fig. 2A). Interestingly, inflammation in the spleen was diminished in animals that received S19 ΔvjbR::Kan at the majority of the time points compared to that in the S19-infected animals, as was evident by the lack of splenomegaly (Fig. 2B). Gross morphology was evaluated and again demonstrated the significant reduction in the spleen sizes of mice receiving the S19 ΔvjbR::Kan vaccine compared to those of the S19 controls (Fig. 3). The spleens of the S19 ΔvjbR::Kan-vaccinated mice appeared similar in size to those of the PBS-treated controls, whereas the spleens from the S19-vaccinated mice were comparable to those of the wild-type-treated mice.

**Evaluation of histological changes in mice inoculated with S19 ΔvjbR::Kan.** Due to the significant differences between the spleen sizes of the animals inoculated with S19 ΔvjbR::Kan and
that received PBS (Fig. 4H), one mouse vaccinated with S19, and one mouse that received S19 ΔvjbR::Kan.

Multifocal aggregates of mineralization with accompanying mild fibrosis and small numbers of lymphocytes, histiocytes, and rare neutrophils were observed in the epicardial surfaces of almost all of the mice and were interpreted as unassociated with the inflammatory process (data not shown).

Changes in the lungs were generally unremarkable in all the animals, with the exception of two animals infected with 2308. Changes included minimal edema and a mild-to-moderate perivascular and peribronchiolar cellular infiltrate composed of lymphocytes, histiocytes, and neutrophils. There were no changes observed in any of the kidneys in any group (data not shown).

Evaluation of immune protection provided by S19 ΔvjbR::Kan. In order to determine the efficacy of the S19 ΔvjbR::Kan mutant as a vaccine, the level of protection provided by equal numbers of either S19 ΔvjbR::Kan or S19 was evaluated against B. abortus wild-type challenge at 20 weeks postvaccination. To try to enhance the efficacy of the S19 ΔvjbR::Kan mutant, the strain was also encapsulated into alginate VpB microcapsules at the same dose. At 21 weeks postvaccination (1 week postchallenge), there was a statistically significant decrease in the splenic bacterial loads from the mice vaccinated with S19 ΔvjbR::Kan relative to those of the naïve mice, with a 3.06-log-unit (P < 0.001) reduction for the nonencapsulated mutant. When administered in a microencapsulated format, the efficacy of the S19 ΔvjbR::Kan vaccine was enhanced by 0.8 log unit compared to that of the nonencapsulated S19 ΔvjbR::Kan vaccine (P < 0.05 encapsulated to nonencapsulated; P < 0.001 naïve to encapsulated) (Fig. 5A). The S19-vaccinated mice exhibited a 5.02-log-unit (P < 0.001) reduction compared to that of the naïve animals.

Postchallenge safety was increased in the mice vaccinated with the novel mutant strain, and splenomegaly was significantly reduced in the mice vaccinated with either the encapsulated or the nonencapsulated S19 ΔvjbR::Kan strain compared to the S19-vaccinated mice postchallenge (P < 0.001) (Fig. 5B). Taken together with the efficacy results, these data suggest that the encapsulation of this novel vaccine strain is highly effective, with the improved benefit of diminished splenomegaly and improved safety pre- and postchallenge.

Encapsulated S19 ΔvjbR::Kan elicited humoral responses that the nonencapsulated vaccine failed to induce. Serum collected at 0, 3, 7, and 21 weeks postvaccination (1 week postchallenge) was assayed for the presence of Brucella-specific IgG1 versus IgG2a antibodies by ELISA. Immunization with encapsulated S19 ΔvjbR::Kan elicited stronger IgG1 and IgG2a responses than the nonencapsulated mutant (P < 0.01) (Fig. 6A and B). During the vaccination stages, both isotypes were induced at similar levels (1:1 ratio), but after challenge, a higher IgG1 (2:1) subtype was seen. In this case, an induction of higher and sustained antibody levels coincides with increased protection from encapsulated S19 ΔvjbR::Kan.

**DISCUSSION**

The development of vaccines to control brucellosis has proven to be a challenge for years. Extensive use of the S19 vaccine has played an enormous role in reducing the disease in cattle, but it...
became clear that this vaccine in its existing form is of limited use in controlling the disease for humans and in wildlife populations (7). Years of investigation have led to a better understanding of *Brucella* virulence and the correlates of protective immunity so that vaccines superior to S19 can be developed. The observation that the highest levels of protection are obtained when the host is immunized with live vaccines indicates that persistence and vaccine viability are key aspects required for an efficacious antibrucellosis vaccine (15, 33).

Previous studies in our laboratory have identified genes related to survival and virulence using transposon-based mutagenesis strategies. Among the genes identified, *Brucella*
in this study, S19 ΔvjbR::Kan was defective for survival in macrophages and cleared faster than S19 in BALB/c mice. During the initial weeks postinoculation in mice, the mutant and the parental strain did not clear at statistically different rates until 9 weeks postinfection, when S19 ΔvjbR::Kan was undetectable (below the level of detection) in spleens. In contrast, the S19-inoculated mice had 1.36 log units of recoverable bacteria remaining at this time. The increased safety of S19 ΔvjbR::Kan was further revealed by the lack of splenomegaly in inoculated mice. Even at the initial weeks postinoculation (1, 3, and 5 weeks) when the bacterial loads in the spleens were similar to those of the S19-vaccinated mice, the mean spleen weight of the S19 ΔvjbR::Kan-vaccinated mice was decreased compared to that of the S19-vaccinated mice. A histological analysis supported this finding by indicating that the animals that receive the S19 ΔvjbR::Kan mutant did not elicit the degree of inflammatory response observed in the S19-vaccinated animals. Furthermore, inflammatory changes observed in other organs, including the liver, were also significantly diminished with the mutant. This difference in inflammatory response exhibited by the S19 ΔvjbR::Kan-vaccinated animals provides an opportunity to evaluate the use of S19 ΔvjbR::Kan as an improved vaccine candidate.

In vitro studies using antigen-presenting cells have demonstrated that microencapsulated antigens are taken up and processed differently than nonencapsulated materials (12). Similarly, in vivo data has shown that microencapsulation serves to modify the uptake, trafficking, and processing of antigens (29). Additionally, recent reports demonstrate that the persistence of the vaccine strain in the host is needed for the development of suitable and long-term immunity (15). Consistent with this, the S19 vaccine exhibits only modest attenuation, meaning it survives longer in the host but also produces unwanted side effects, such as the severe inflammation reported here. To enhance the immunization efficacy, we investigated the vaccine potential of S19 ΔvjbR::Kan when delivered in a controlled-release vehicle. For this purpose, alginate, a polysaccharide extracted from algae, was used in combination with VpB, derived from the parasite Fasciola hepatica, as the capsular material used to entrap the S19 ΔvjbR::Kan mutant (25, 30, 32). By encapsulating the organism, we attempted to increase its persistence without causing inflammation.

Protection studies against wild-type challenge with either the S19 or S19 ΔvjbR::Kan strains protected mice significantly, but the efficacy of the vaccine was reduced in the nonencapsulated S19 ΔvjbR::Kan mutant. This indicated that the vjb gene in S19 is necessary to induce a complete immunity toward Brucella infections. The reduced efficacy was successfully compensated by delivering the mutant vaccine in a sustained, microencapsulated format, corroborating the observation of persistence as a function of vaccine efficacy. The encapsulation of live attenuated organisms is an interesting approach to improve immunization efficacy in potential vaccine candidates. Using alginate-VpB microencapsulation, bacteria are exposed to mild conditions in which bacterial viability is not compromised; this approach permits the development of live vaccines in contrast to previously published encapsulation procedures in which the viability of the bacteria is compromised due to the extreme conditions (17). It is important to mention that the microencapsulation of vaccines has been previously docu-

FIG. 5. Immunization efficacy and safety of B. abortus S19 ΔvjbR::Kan vaccine formulations. BALB/c mice were immunized i.p. with 1 × 10⁵ of either nonencapsulated or encapsulated S19 ΔvjbR::Kan. Control groups received empty capsules or S19. After 20 weeks, the mice were challenged i.p. with 1 × 10⁵ CFU wild-type 2308. At 1 week postchallenge, the mice were euthanized, their spleens harvested, and the bacterial loads (A) and spleen weights (B) determined. (A) Values are reported as the mean log₁₀ recovery of the 2308 challenge organism recovered from the spleens. Differences in colonization between all the groups were determined by ANOVA followed by a Tukey’s posttest (*, P < 0.05; **, P < 0.001). (B) Spleen weights were measured in mg and were compared and analyzed by ANOVA followed by a Tukey’s posttest comparing all groups to one another (*, P < 0.01; **, P < 0.001). For the statistical representation on both graphs, “a” symbolizes naïve animals, “b” S19-vaccinated animals, “c” encapsulated S19 ΔvjbR::Kan-vaccinated animals, and “d” nonencapsulated S19 ΔvjbR::Kan-vaccinated animals.

mellitensis vjbR mutants have been evaluated for survival in macrophages and the mouse model to confirm attenuation and immune potential (4). We have previously demonstrated that B. mellitensis vjbR mutants are suitable vaccine candidates due to their ability to generate protection in BALB/c mice. Also, by using this mutant, we were able to increase safety by preventing splenomegaly in inoculated mice, a significant improvement over current vaccine strains S19 and Rev-1 that induce splenomegaly in mice (27). In this study, we generated a marked deletion mutant in the S19 vaccine strain with the aim of increasing safety and the possible use of this vaccine in other populations.

The S19 ΔvjbR::Kan mutant was evaluated for survival and attenuation in the macrophage and mouse models. As shown
mented as an alternative method to enhance the efficacy of DNA, protein-based, and killed vaccines, but to our knowledge, a microencapsulated live Brucella vaccine has been described only by us (9–11, 14, 16, 20, 21, 31, 34).

The degree of protection (assessed by splenic bacterial burden) conferred by either the encapsulated or nonencapsulated S19 ΔvjbR::Kan mutant was compared to humoral profiles. Immunization with encapsulated S19 ΔvjbR::Kan induced higher IgG1 and IgG2a levels compared to the nonencapsulated S19 ΔvjbR::Kan mutant. The animals that received the encapsulated vaccine mounted a stronger IgG1 response at 1 week postchallenge.

In this study, a vjbR knockout was successfully generated in the S19 vaccine and investigated for its potential use as an improved vaccine candidate. Vaccination with a sustained-release vehicle to enhance the vaccination efficacy was evaluated utilizing live S19 ΔvjbR::Kan to immunize BALB/c mice i.p. with either encapsulated or nonencapsulated S19 ΔvjbR to evaluate immunogenicity, safety, and protective efficacy. Enhanced safety was revealed by the absence of splenomegaly in mice that were inoculated with the mutant. Humoral responses postvaccination indicate that the vaccine candidate was able to elicit an anti-Brucella-specific IgG response even when the vaccine was administered in an encapsulated format. Finally, a single dose with the encapsulated mutant conferred higher levels of protection compared to those of the nonencapsulated vaccine. These results suggest that S19 ΔvjbR is safer than S19, induces protection in mice, and should be considered as a vaccine candidate when administered in a sustained-release manner.

FIG. 6. IgG1 and IgG2 anti-Brucella antibodies in serum from mice immunized with S19 ΔvjbR::Kan. BALB/c mice were inoculated i.p. with 1 × 10⁵ CFU of either nonencapsulated S19 ΔvjbR::Kan or encapsulated S19 ΔvjbR::Kan. Mice within the control group received empty capsules in lieu of vaccine. At 0, 3, 7, and 21 weeks postvaccination (1 week postchallenge), serum samples were collected for IgG1 (A) and IgG2 (B) determination by ELISA. Results are shown as the means ± SEM of absorbance at 450 nm. For the statistical representation on both graphs, “a” symbolizes naïve animals, “b” encapsulated S19 ΔvjbR::Kan-vaccinated animals, and “c” nonencapsulated S19 ΔvjbR::Kan-vaccinated animals.

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