Evaluation of a DNA vaccine encoding *Brucella* BvrR in BALB/c mice

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Abstract. Brucellosis is an important neglected zoonotic disease, and the pathogens responsible are *Brucellae*. In order to evaluate the immunogenicity and protective efficacy of a DNA vaccine encoding *Brucella* BvrR, the recombinant plasmid pCDNA-BvrR was constructed by inserting the BvrR gene fragment into a pCDNA3.0 vector. The His_6-tagged BvrR was purified with His-trap FF crude affinity chromatography and verified with an anti-histidine monoclonal antibody by western blot analysis. The specific immunoglobulin antigens and their isotypes were detected by indirect ELISA. The recombinant His_6-BvrR protein was expressed and purified by affinity chromatography. The optical density 450 value of immunoglobulin G (IgG) in the pCDNA-BvrR group was significantly increased compared with the pCDNA3.0 vector or PBS groups ($P<0.05$), and the pCDNA3.0 vector and PBS groups exhibited no significant difference ($P>0.05$). BvrR induced specific antibodies with a dominance of IgG2a over IgG1 and the T cell-proliferative response, in addition to a typical T helper-1 (Th1)-dominated immune response in mice. The splenocytes from mice of the pCDNA-BvrR group demonstrated significant proliferative activity compared with the pCDNA3.0 vector group. The present results indicated that immunization with BvrR induced a specific Th1-type immune response in mice. Subsequent to challenging with *B. abortus* S19, it was identified that the DNA vaccine pCDNA-BvrR induced a significant level of protection in BALB/c mice by evaluating systemic bacterial clearance. These results suggested that BvrR may be a good candidate for a DNA vaccine against brucellosis.

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Introduction

Brucellosis is an important neglected zoonotic disease (1). The causative pathogen of this disease is *Brucella* (a facultative intracellular Gram-negative bacterium). In order to control this disease in domestic animals, few attenuated vaccines, including *B. melitensis* Rev1, *B. abortus* S19 and RB51 have been introduced (2). *Brucellosis* has been reported to exist in wildlife populations since the early part of the 20th Century. At the beginning of this century in the USA, *Brucella abortus* was a problem in elk and bison in the Greater Yellowstone Area (3). *B. suis* is prevalent in millions of feral swine in the majority of the southern states, and caribou and reindeer in Alaska are infected with *B. suis biovar* 4 (3). However, the existing vaccines were considered too virulent or unsafe for humans (4).

To develop safe and efficacious vaccines, a number of different strategies, including the development of subunit vaccines (5), the utilization of bacterial vectors (6) and the overexpression of protective homologous antigens (7,8), have been applied. In addition, another strategy was developed involving immunization with DNA vaccines, which encode a protective antigen (9,10). It was noted that DNA vaccines may be effective vaccines due to their strong cell-mediated immune (CMI) responses, which serve an important role in protection against intracellular pathogens (11). Various animal models demonstrated the protective roles of DNA vaccination against different viral, fungal and parasitic diseases (12-14). With regard to brucellosis, a number of previous studies demonstrated that specific DNA vaccines [for example, the SEN1002 and SEN1395 genes (15), Cu-Zn superoxide dismutase (16) and lumazine synthase (17)] were able to induce a significant level of protection in mice.

As a member of the two-component BvrR/BvrS system, BvrR is necessary for *Brucella* virulence (18,19). Previous studies demonstrated that dysfunction of BvrR may alter the expression of the type IV secretion system and specific principal outer membrane proteins, in addition to the pattern of lipid A acylation (20-22). At present, studies on BvrR have primarily focused on its functions. In the present study, the immunogenicity and protective ability of the BvrR gene were demonstrated to function as a DNA vaccine.

Materials and methods

Bacterial strains and vector. B. abortus S19 and *B. suis S2* were purchased from Tanon Science and Technology, Co., Ltd. (Shanghai, China). These bacterial stains were qualified by standard biochemical tests prior to experimentation. The bacterial cells were cultured in tryptose-soy broth (Qingdao Hope Bio-Technology Co., Ltd, Qingdao, China) for 72 h at 37˚C under aerobic conditions. For the inoculation experiments, the bacterial suspension was adjusted spectrophotometrically to $2x10^8$ colony forming units (CFU). All experiments with live *Brucella* were conducted in biosafety level 2 laboratories.

E. coli strains DH5α and BL21 (DE3; Takara Biotechnology Co., Ltd., Dalian, China) were used for cloning of the various plasmid constructs and recombinant protein expression, respectively. The *E. coli* were cultured at 37˚C in lysogeny broth (Sangon Biotech Co., Ltd., Shanghai, China) with 100 μg ampicillin/ml. The eukaryotic vector pCDNA3.0 (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and prokaryotic vector pET28a (Merck KGaA, Darmstadt, Germany) were used to construct plasmids for the DNA vaccine and recombinant protein expression, respectively.

Animals and grouping. A total of 75 pathogen-free female BALB/c mice (20±2 g, 6-weeks old) were purchased from the Animal Center at the Academy of Military Medical Sciences (Changchun, China), which were fed with commercial mouse chow and water *ad libitum* in clean conditions (18-22˚C, 40-70% relative humidity and 10-14 h light/dark cycle) at the laboratory animal center of Shenyang Agricultural University (Shenyang, China). The mice were randomly divided into three groups (n=25): The pcDNA-BvrR immunization group; pcDNA control group; and the PBS control group. The pcDNA-BvrR immunization group and the pcDNA control group were injected with pcDNA-BvrR plasmid and pcDNA plasmid at a concentration of 1 μg/μl in the hindlimb tibialis anterior muscle, and 100 μl of each was injected into the mice. The PBS control group was injected with 100 μl PBS. The first immunization was performed at day 0, the second immunization was at day 14 and the third immunization was at day 28. Samples were collected 1 week following each immunization. On the 7th day following each immunization, the blood of five mice was taken for serum testing. At the same time, spleens were taken for relevant experiments. Finally, the 10 remaining mice were used for the challenge experiments. All the animal experiments were approved by the Laboratory Animal Welfare and Ethical review committee of Shenyang Agricultural University.

BvrR DNA vaccine construction. The primers for BvrR were designed according to the corresponding genome sequence (GenBank accession no. AF005157.1; http://www.ncbi.nlm.nih.gov/genbank/): BvrR forward, 5'-AAAAGGATCCGCCACCATGAAGGAAGCTTCGGCAACG-3' and BvrR reverse, 5'-AAAACTCGAGTACGCTTCCCGGAAACGATAAC-3'. Kozak sequences and restriction sites for *Eco*RI and *Xho*I were transferred into the oligonucleotides to aid expression and cloning, respectively.

B. suis S2 chromosomal DNA was used as the template for amplifying the coding region of the BvrR gene. The DNA (Takara Biotechnology Co., Ltd.) parameters of the polymerase chain reaction (PCR) were as follows: 30 cycles at 94˚C for 30 sec, 50˚C for 30 sec and 72˚C for 45 sec. A 1.5% agarose gel was used to purify the PCR amplified product, which was digested by *Eco*RI and *Xho*I restriction enzymes (Takara Biotechnology Co., Ltd.) and ligated using T4 DNA ligase (Takara Biotechnology Co., Ltd.) into the pCDNA3.0 vector. The pCDNA-BvrR plasmid was verified by DNA sequencing following purification using the UNIQ-500 Column Endotoxin-Free Plasmid Maxi-Preps kit (Sangon Biotech Co., Ltd.; data not shown).

Protein expression and purification. The BvrR gene was inserted into a pET28a vector between the restriction sites of *Eco*RI and *Xho*I, and DNA sequencing was verified (data not shown). *E. coli* BL21 (DE3) cells harboring pET-BvrR were induced in the auto-induction medium ZYP-5052 (22). The resulting protein contained a His_6-tag in its N-terminus.

The His_6-tagged BvrR was purified by His-trap FF crude (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) affinity chromatography (data not shown) and verified with an anti-histidine monoclonal antibody (cat. no. D199987; Sangon Biotech Co., Ltd.) and Brucella polyclonal antibody (cat. no. Z244; China Veterinary Culture Collection Center, Beijing, China) by western blot analysis. Determination of protein concentration was performed using a Bradford assay. Protein samples (20 μg) were loaded onto a 12% SDS-PAGE gel for separation. Following this, proteins were transferred to nitrocellulose membranes and then blocked with 5% bovine serum albumin (BSA; cat. no. B600036; Sangon Biotech Co., Ltd.) at room temperature for 2 h. Membranes were subsequently incubated with anti-His mouse monoclonal antibodies (1:500; cat. no. D199987; Sangon Biotech Co., Ltd.) with 3% BSA overnight at 4˚C. Following this, membranes were incubated with horseradish peroxidase-conjugated rabbit anti-mouse IgG secondary antibodies (1:1,000 dilution with 1% BSA; cat. no. D110098; Sangon Biotech Co., Ltd.) at room temperature for 2 h. A horseradish catalase 3,3'-diaminobenzidine color kit (cat. no. C520017; Sangon Biotech Co., Ltd.) was used for the visualization of proteins. SDS-PAGE was used to separate recombinant His_6-BvrR protein. Lane 1 was a low molecular weight protein marker (Takara Biotechnology Co., Ltd., Dalian, China and MBI Fermentas, Vilnius, Lithuania), Lane 2 was the recombinant His_6-BvrR protein, and Lane 3 was the negative control. Determination of His6-tagged BvrR protein concentration was performed using a Bradford assay. Finally, Bradford assay was used for analysis of the recombinant BvrR (rBvrR) protein or *in vitro* stimulation of lymphocytes.

Immunization. The immunological studies were performed in three groups. Following anesthetization with inhaled halothane, different groups of experimental mice were inoculated separately in the tibialis anterior muscle with 100 μg pCDNA-BvrR, pCDNA3.0 vector or PBS at 0, 14 and 28 days.

On the 7th day following each vaccination, blood was collected from five mice and the serum samples from each group were kept in sterile microfuge tubes. The final serum was kept at -70˚C until further use.

Measurement of specific immunoglobulin antibodies and their isotypes. Pooled serum collected from five mice of the different groups at 7, 21 and 35 days was used for detecting specific

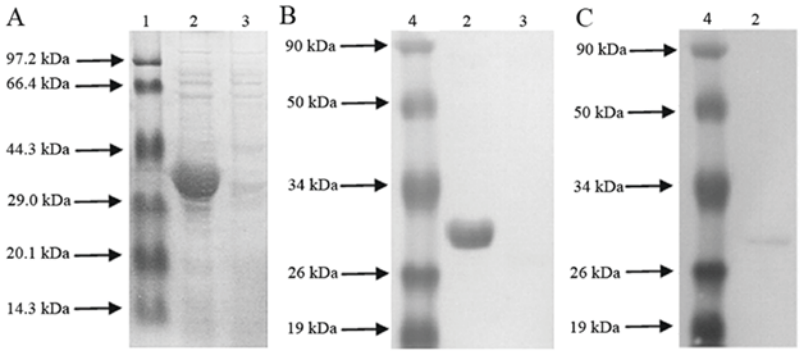

Figure 1. Expression levels and purification of recombinant His_6-BvrR protein. (A) SDS-PAGE analysis of recombinant His_6-BvrR protein. Lane 1 was a low molecular weight protein marker, Lane 2 was the recombinant His6-BvrR protein, and Lane 3 was the negative control. (B) Western blot analysis of the rBvrR protein by anti-histidine antibody. Lane 1 was pre-stained protein marker, Lane 2 was rBvrR protein, and Lane 3 was the negative control. (C) Immunoreactivity test results of purified BvrR protein and *Brucella* polyclonal antibody. Lane 1 was the pre-stained protein marker and Lane 2 was purified BvrR protein.

antibodies with the purified rBvrR proteins by indirect ELISA. Serum at 35 days was used for the determination of the antibody subtypes. A total of 3 μg/ml purified rBvrR proteins diluted with carbonate buffer (0.05 M; pH 9.6) were applied for coating the wells of polystyrene plates at 4˚C overnight. The plates were washed with PBS with 0.05% Tween-20 (PBS-T) buffer three times, and skimmed milk powder (3%) in PBS-T was used to block for 1 h at 37˚C. The plates were subsequently incubated with serial dilutions of serum or the negative control starting from a 1:200 dilution for 3 h at room temperature, followed by washing four times. Horseradish peroxidase-conjugated anti-mouse immunoglobulin G (IgG; D720358), IgG1 (D720359) and IgG2a (D720360, all Sangon Biotech Co., Ltd. at 100 μl/well) was added into the wells and incubated at 37˚C for 1 h. Following washing four times at room temperature for 30 min, 100 μl substrate solution was added, and incubated in the dark at room temperature for 20 min. Finally, 100 μl 0.5 M sulfuric acid per well was added to stop the enzymatic reaction, and the absorbance was measured at 450 nm. The titer was expressed as the optical density (OD).

Splenocyte cultures and lymphocyte proliferation. Under aseptic conditions, mice were sacrificed to obtain their spleens at 7, 21 and 35 days following the first vaccination. The spleen was mixed intensively with chilled PBS to collect the splenocytes. The flushed PBS, including splenocytes and red blood cells was layered slowly onto an equal volume of lymphocyte separation medium and centrifuged at 4˚C, 1,000 x g for 40 min. The interface, including splenocytes, was collected and washed with chilled PBS and finally washed with RPMI-1640 (10% newborn calf serum; 2 mM L-glutamine; 100 μg/ml streptomycin; and 100 IU/ml penicillin; Gibco; Thermo Fisher Scientific, Inc.). In the presence of rBvrR (1 μg/ml), splenocytes at a density of $4x10^5$ viable cells were cultured at 37˚C for 72 h with 5% CO_2 in 96-well plates. Subsequently, 10 μl MTT (5 mg/ml thiazolyl blue in RPMI-1640) was added, and was incubated at 37˚C for 4 h. Later, the frozen crystals were obtained by centrifugation at 4˚C, 1,000 x g for 10 min. A total of 150 μl dimethyl sulfoxide per well was used to dissolve the crystals following pipetting of the supernatant. Finally, the absorbance was measured at 570 nm. The stimulation indices were calculated as the ratio between absorbance values of stimulated cells and unstimulated cells.

Cytokine ELISA. Cytokines in the culture supernatants of spleen cells were determined by mouse interferon (IFN)-γ and interleukin (IL)-4 ELISA kits (cat. no. 558258; BD Biosciences, Franklin Lakes, NJ, USA; and cat. no. D720336; Sangon Biotech Co., Ltd.). All assays were performed in triplicate. The absorbance values of standards were used to obtain a linear regression equation, and concentrations of IFN-γ and IL-4 were calculated.

Protection experiments. The protection experiments were performed by vaccinating mice intramuscularly with *B. abortus S19*. Simultaneously, mice were vaccinated with PBS and 10^8 CFU of *B. suis* S2 as a negative and positive control, respectively. A total of 42 days following the first vaccination, mice were challenged with 10^8 CFU of S19 by intramuscular injection. A total of 2 weeks later, infected mice were sacrificed to obtain their spleens, which were removed aseptically and triturated. A 10 μl dilution of spleen lysate diluted in triplicate was used to measure the CFU of *Brucella*. Colonies were counted subsequent to all the plates being incubated at 37˚C with 5% CO_2 for 3 days. Finally, the protection was obtained by subtracting the mean of log^{10} CFU of the experimental groups from that of the corresponding PBS group.

Statistical analysis. Data are presented as the mean ± standard deviation and evaluated using the SPSS 15.0 program for Windows (SPSS, Inc., Chicago, IL, USA). The data for the antibodies, lymphocyte proliferation and cytokines were analyzed with paired-samples t-test. Multiple groups were compared using one-way analysis of variance, and Newman-Keuls method was subsequently used for pairwise comparison. Tukey's honest significant difference procedure was used for the data for the protection experiments. $P<0.05$ was considered indicate a statistically significant difference.

Results

Expression and purification of the recombinant His_6-BvrR protein. To obtain the rBvrR, *E. coli* harboring the plasmid pET28a-BvrR was induced for expression. The molecular weight (MW) of the expressed protein detected by SDS-PAGE was 31 kDa, which was consistent with the theoretical MW of His_6-BvrR (Fig. 1A). Subsequently, rBvrR protein was confirmed by an anti-histidine monoclonal antibody

in the western blot analysis (Fig. 1B). The appearance of a specific band at ~31 kDa coincided with the expected size, demonstrating that the purified BvrR protein exhibited immunoreactivity with the polyclonal antibodies of *Brucella* (Fig. 1C).

BvrR is involved in humoral immunity. ELISA was used to measure the titers of anti-BvrR antibodies in serum from mice immunized separately with pCDNA-BvrR, pCDNA3.0 vector or PBS as a control. The serum from mice vaccinated with pCDNA-BvrR was reactive to the antibody of BvrR between the first and fifth week post-vaccination and the value of OD_{450} ranged between 0.8 and 1.4 (Table I). The OD_{450} value of IgG in pCDNA-BvrR group was significantly higher compared with the pCDNA3.0 vector or PBS control groups ($P<0.05$). However, the OD_{450} value was not different between the pCDNA3.0 vector and PBS control groups ($P>0.05$; Table I).

Subtype analysis suggested that the anti-BvrR antibody in pCDNA-BvrR-immunized mice was primarily the IgG2a subtype at 35 days of post-vaccination. The OD_{450} value of the specific IgG2a subtype was increased compared with the specific IgG1 subtype in the pCDNA-BvrR group ($P<0.05$); however, not significantly increased in the PBS control group (Fig. 2A).

Role of BvrR in lymphocyte proliferation. To test the CMI response to the *Brucella* rBvrR protein, the proliferation rate of spleen cells from immunized mice was determined. As demonstrated in Fig. 2B, at 1-week post-booster, the splenocytes from mice of the pCDNA-BvrR group demonstrated significant proliferative activity compared with the pCDNA3.0 vector group ($P<0.05$). This phenomenon also existed at 35 days post-vaccination.

Determining the expression levels of IFN-γ and IL-4. The cultured splenocyte supernatant of the mice was assessed to determine the expression levels of IFN-γ and IL-4 following stimulation with rBvrR. An increased expression level of IFN-γ was identified in supernatants of cell cultures from pCDNA-BvrR-immunized animals, which reached a peak (95 pg/ml) at 35 days post-vaccination, compared with the pCDNA3.0 vector and PBS groups ($P<0.05$; Table II). Notably, the levels of IL-4 were not significantly different among the three groups (Table II).

Protection of B. abortus S19 challenge. To test the protective efficacy of BvrR, mice were sacrificed on the 14th day post-challenge. The protection efficacy was calculated as the reduction of bacteria number in the spleens from immunized mice compared with control mice receiving PBS. When the $\log^{10}$ CFU of *B. abortus S19* was measured at 2-weeks post-challenge, it was indicated that the maximum clearance was observed in the positive control group (*B. suis* S2; 1.415) or the pCDNA-BvrR group (0.814), which were significantly different in the pCDNA3.0 vector and PBS groups ($P<0.05$; Table III).

Table I. Optical density 450 values of immunoglobulin G among the three groups on different days.

Groups	7 days	21 days	35 days
pcDNA-BvrR	0.81±0.05	1.05±0.11	1.40±0.57
pcDNA vector	0.50±0.01[a]	0.52±0.08[a]	0.52±0.04[a]
PBS control	0.49±0.04[a]	0.51±0.03[a]	0.50±0.21[a]

[a]$P<0.05$ vs. pcDNA-BvrR group.

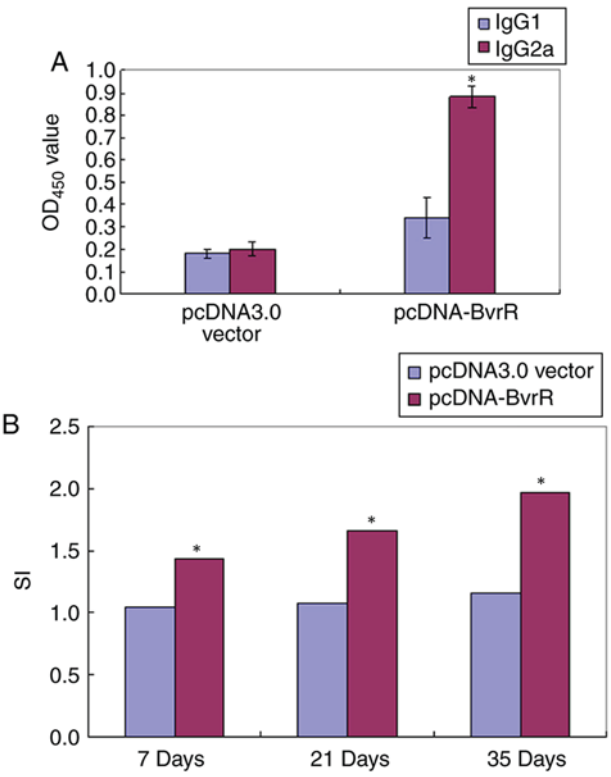

Figure 2. Subtype analysis of anti-BvrR antibodies and the role of BvrR. (A) Subtype analysis of anti-BvrR antibody between pCDNA-BvrR, and pCDNA3.0 vector groups. (B) Role of BvrR in lymphocyte proliferation at 7, 21 and 35 days between pCDNA-BvrR and pCDNA3.0 vector groups. SI, stimulation indices; OD, optical density; IgG, immunoglobulin G. *$P<0.05$.

Discussion

At present, vaccination remains the most successful method of preventing brucellosis in animals from countries with a high incidence (23). However, specific types of live-attenuated vaccines used for controlling animal brucellosis are disadvantageous to humans (4), leading to the development of novel vaccines.

It was suggested that tuberculosis may depend on the T helper-1 (Th1)-type cell-mediated immune response to protect against infection by an intracellular pathogen, including *Brucella* (24,25). A number of studies demonstrated that DNA vaccines acted on the major histocompatibility complex class I and II following naked DNA immunization, inducing a wide range of immune responses, including antibody production, CD8 cytotoxic T cells and CD4 T helper cell activation (10,23,26). DNA vaccines overcame the disadvantages of acellular vaccines, including recombinant proteins and synthetic peptides that were not adequately processed and presented, which resulted in a failure to induce a strong CMI

Table II. Determining expression levels of IFN-γ and IL-4 in immunized mice.

Factor	Group	7 days	21 days	35 days
IFN-γ	pcDNA-BvrR	31.500±1.800	42.000±11.200	95.000±23.000[a]
	pcDNA vector	19.800±1.600	20.000±3.100	25.000±2.000[b]
	PBS control	20.500±3.200	19.600±2.100	22.500±1.500[b]
IL-4	pcDNA-BvrR	5.747±0.046	5.701±0.200	5.697±0.015
	pcDNA vector	5.625±0.109	5.725±0.050	5.700±0.050
	PBS control	5.708±0.095	5.733±0.038	5.750±0.075

IFN, interferon; IL, interleukin. [a]No difference was observed between IFN-γ in pcDNA and PBS control groups, and they all were significant differences from pcDNA-BvrR group ($P<0.05$). [b]Indicated that IFN-γ in pcDNA-BvrR group was significant different from other two groups ($P<0.05$).

Table III. Protection against challenge with *B. abortus* S19 in mice following immunization with the DNA vaccine pCDNA-BvrR.

Group	Log CFU	Log units of protection
PBS	2.916±0.019	0.000[a]
pcDNA	2.760±0.070	0.156[a]
pcDNA-BvrR	2.102±0.144	0.814[b]
B. suis S2	1.557±0.056[c]	1.415[c]

Vaccinated and control mice were challenged by intramuscular inoculation of $1x10^8$ CFU of the *B. abortus* S19. At 2 weeks post-challenge, five mice from each group were sacrificed and the *Brucella* CFU in their spleens were determined. Data are the average of the CFU from five mice. [a]Indicates that there was no difference between the PBS and pcDNA groups, but they both were significantly different from the pcDNA-BvrR and *B. suis* S2 groups ($P<0.05$). [b]Indicates that the pcDNA-BvrR group was significantly different from other three groups ($P<0.05$). [c]Indicates that *B. suis* S2 group was significantly different from other three groups ($P<0.05$). CFU, colony forming units.

response as well as to confer a high degree of protection (6,27). Regarding brucellosis, it was documented that all the genes or specific epitopes of *Brucella*, including Cu/Zn superoxide dismutase (SOD), ribosomal L7/L12 or lumazine synthase, were able to induce significant levels of protection in mice (9).

The pathogenesis of *Brucella* is controlled by the two-component system BvrR/BvrS (TCS BvrRS) and type IV secretion machinery VirB (T4SS VirB) (18,28). Furthermore, the TCS BvrRS and T4SS VirB control the expression of specific outer membrane proteins through direct and indirect mechanisms, respectively (21,22). TCS BvrRS serves an important role in the intracellular replication of *Brucella*.

A previous study suggested that a DNA vaccine encoding *Brucella* Cu/Zn SOD may be a good candidate for vaccination against *Brucella* (29). A wide variety of *Brucella* vaccines have been developed for protection against brucellosis; however, they have had limited acceptance and success. An advantage of DNA vaccines is that multiple antigens may be expressed; however, it was essential to fully evaluate the benefits and risks of these types of *Brucella* vaccines for the prevention of brucellosis in animals and particularly humans, including *B. abortus S19,* Vaccine strain RB51 and outer membrane vesicles (30). Plasmid DNA carrying the BLS gene was additionally a good candidate for vaccination against *Brucella* (17). Another previous study demonstrated a protective immune response induced by a novel double DNA vaccine encoding the *Brucella melitensis* omp31 gene and the *E. coli* eae gene in a mouse model (31). All these results suggested that DNA vaccines demonstrated great immunogenicity and protective efficacy against infection in a mouse model.

The plasmid DNA containing the BvrR gene was injected to induce specific humoral and cellular immunities. A total of 1 week following the first immunization, it was observed that a weak titer of specific IgG was identified in mice, which was twice as high at the end of experiment. The induced antibody titers in pCDNA-BvrR vaccine were lower compared with previous DNA vaccines against *Brucella* (29,32). It was possible that there existed differences in numerous factors, including the addition of adjuvant, and the method and time of detection.

Subsequent to *in vitro* stimulation of splenic cells, lymphocyte proliferation and cytokine production were measured to evaluate the T-cell immunity following DNA immunization. These results demonstrated that rBvrR was able to elicit increased expression levels of IFN-γ compared with IL-4 and a strong T cell-proliferative response. Furthermore, the anti-BvrR antibody was IgG2a-predominant compared with IgG1. Following naked DNA immunization, IgG2a was the predominant antibody subclass in responsive mice, indicating that Th1-$CD4^+$ cellular responses were identified in BALB/c mice (33). Hinkula *et al* (34) documented that full protection of mice vaccinated with the specific DNA vaccine and an extra Th1-specific cellular response were required. Together, it was concluded that immunization with the plasmid pCDNA-BvrR induced a Th1 cellular response.

Subsequently, pCDNA-BvrR vaccines from different strains were investigated in the present study and the protective efficacy of the pCDNA-BvrR vaccine against more virulent *B. abortus* S19 challenge was analyzed. Animals vaccinated with pCDNA-BvrR demonstrated a log protection of 0.814, which was markedly increased compared with PBS or the pCDNA3.0 vector, and lower compared with *B. suis*

S2. Therefore, pCDNA-BvrR vaccines from different strains were studied in the present study, and the protective efficacy of the pCDNA-BvrR vaccine against *B. abortus S19* challenge was analyzed. Animals vaccinated with pCDNA-BvrR demonstrated a log protection of 0.814, which was markedly increased compared with PBS or the pCDNA3.0 vector, and lower compared with *B. suis* S2. All these results demonstrated that BvrR may be used as a powerful candidate for DNA vaccination.

In conclusion, antibody and Th1 cellular responses were elicited following immunization with the plasmid pCDNA-BvrR, and protection against *B. abortus* challenge was obtained. The present results suggested that BvrR is a promising candidate for studies of DNA vaccines against brucellosis in the future.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

BC acquired data obtained from animal experiments, performed statistical analyses, designed and modified experimental protocols, and wrote and revised the manuscript. GW acquired data obtained from animal experiments and was responsible for the redrafting of the manuscript. BL performed statistical analyses, designed and modified experimental protocols, and wrote and revised the manuscript. ZZ analyzed and interpreted data obtained from animal experiments and revised the manuscript.

Ethics approval and consent to participate

The present study was approved by the Laboratory Animal Welfare and Ethical review committee of Shenyang Agricultural University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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