Brief Original Article

Diagnostic efficacy of *Brucella abortus* strain RB51 in experimentally inoculated Sprague-Dawley rats using western blot assay

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Abstract

Background: To investigate the diagnosis and efficacy of *Brucella abortus* strain RB51 (SRB51) in experimentally inoculated Sprague-Dawley (SD) rat using western blot assay.

Methodology: Female SD rats were orally administered with 1.0 x 10^7 colony forming unit (cfu) suspension of SRB51 and half of these SD rats were challenged at 4 weeks post inoculation with 1.0 x 10^9 cfu suspension of *B. abortus* biotype 1 isolated in South Korea. Sera of SD rats were monitored at regular intervals by western blot assay using whole cell antigen of *B. abortus* strain 1119-3 (S1119-3). The bacteriological examination of blood and clinical examination of the rats were also performed.

Results: There were several bands at 120, 70, 45, 30, 20 kDa and clear specific bands were found after vaccination (20, 70 kDa) and challenge (15, 20, 45, 70, 120 kDa). The highest immune response was observed in sera 4 weeks post SRB51 vaccination. SRB51 was recovered from the blood of all of SRB51 inoculated rats until one week post vaccination and there were no clinical signs in that inoculated rats.

Conclusions: It is concluded that the SRB51 elicits antigen specific immunity in SD rats based on western blot assay.

Key Words: *B. abortus* strain RB51, *B. abortus* biotype 1, Sprague-Dawley rats, western blot assay, South Korea

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Introduction

Brucellosis, one of the world's major zoonoses, has been recognized as an emerging disease since the discovery of *B. melitensis* as the causative organism of Malta Fever by Bruce in 1887 and the isolation of *B. abortus* from aborted cattle by Bang in 1897 [1,2]. The infection can result from direct contact with infected animals and can be transmitted to humans through the consumption of raw milk and milk products. In humans, symptoms include general weakness, joint and muscle pain, headache, undulant fever, hepatomegaly, splenomegaly, and night sweats [3]. In animals, brucellosis mainly affects reproduction and fertility and reduces the survival of newborns [4].

The introduction of *B. abortus* strain RB51 (SRB51) has been recently recommended to control bovine brucellosis. SRB51 is an alternative to *B. abortus* strain 19 as a vaccine for preventing brucellosis in cattle because, unlike *B. abortus* strain 19, it does not induce antibodies to the *Brucella* LPS O-antigens that are detected by traditional sero-diagnostic tests for brucellosis [5-9]. SRB51 has also been approved as an official calfhood vaccine by the Animal and Plant Health Inspection Service branch of the United States Department of Agriculture [10]. The different aspects of SRB51 have been studied in domestic animals [6,10-15], laboratory animals [16,19], and in wild animals and birds [20]. In this study, the diagnosis and efficacy of SRB51 to induce specific antibodies in experimentally inoculated Sprague-Dawley (SD) rats were conducted by western blot assay using whole cell *B. abortus* strain 1119-3 (S1119-3) as antigen. Bacteriological examination of the blood and clinical examination of the rats were also performed.

Materials and Methods

The study was conducted in the Veterinary Public Health laboratory, College of Veterinary Medicine, Chonbuk National University, Jeonju 561 756, South Korea.

Brucella abortus culture

The seed stock-culture of SRB51 was supplied by the Colorado Serum Company in the USA. SRB51 was

cultured in *Brucella* broth (Difco, USA) for 48 hours at 37°C without carbon dioxide. *B. abortus* biotype 1 isolated in South Korea was also cultured in *Brucella* broth (Difco, USA) for 48 hours at 37°C with 5% carbon dioxide. The bacteria were washed with saline 3 times and suspended in physiological saline before use. The master seed of S1119-3 was cultured on *Brucella* agar (Difco, USA) for 72 hours at 37°C and then culture was performed in *Brucella* broth (Difco, USA) in a shaking incubator for 48 hours at 37°C at 180 rpm.

Experimental rats and inoculation

Healthy disease-free 6-to 10-month-old female SD rats weighing 200 to 250 grams with no history of exposure to *Brucella* were used in this experiment. Using a stomach catheter, 48 SD rats (InoR) were orally administered 1.0×10^7 colony forming unit (cfu) suspension of SRB51 per rat in 1 ml of saline while 48 SD rats (CR) were administered saline following the same procedure to serve as controls. InoR were equally divided into 12 subgroups of 4 each for 0, 1, 2, 3, 4, 5, 6, 8, 12, 16, 20, and 24 weeks. CR were also equally divided following the same procedure (Table 1). The rats were maintained in cages under hygienic conditions and provided with commercial feed and water *ad libitum*.

Table 1. Sprague-Dawley rats used for diagnosis and efficacy of *B. abortus* strain RB51 using western blot assay.

Groups	Number of rats at weeks post inoculation											
	0	1	2	3	4	5	6	8	12	16	20	24
Inoculated rat	4	4	4	4	4	4	4	4	4	4	4	4
Control rat	4	4	4	4	4	4	4	4	4	4	4	4

Challenge with B. abortus biotype 1

Fifty percent of the group InoR (n=24) were challenged at 4 weeks post SRB51 vaccination using 1.0×10^9 cfu suspension of *B. abortus* biotype 1, inoculated subcutaneously to each of InoR in 500 microliter of saline, and marked as the inoculated challenged rat (InoChR) group. Each of CR (n=24) was also inoculated with *B. abortus* biotype 1 following the same procedure and marked as the non-inoculated challenged rat (NInoChR) group.

Clinical examination

All the SD rats of InoR, CR, InoChR and NInoChR were clinically examined to record rectal temperatures and clinical signs, and to determine whether they suffered any adverse reactions resulting from the vaccination and challenge, such as arthritis or anaphylactic shock during the experiment.

Bacteriological examination of blood and collection of sera

One ml of blood was collected from the heart with heparin (100 IU) at 0, 1, 2, 3, 4, 5, 6, 8, 12, 16, 20, and 24 weeks post challenge from each rat of InoR, CR, InoChR and NInoChR after anaesthesia with ketamin (25 mg, I. M.). The blood was cultured at 37°C with 5% CO_2 for 3 days in glass tube containing 3 ml of tryptose soy broth (Difco Co., USA) with 5% bovine serum. From this tube, the cultured content was cultured again at 37°C with 5% CO_2 for 3 days on tryptose soy agar (Difco Co., USA) plate with 5% bovine serum to observe the characteristics of the bacterial colony [19]. A similar procedure was followed for InoR and CR but cultured without CO_2. Additionally, 1 ml of blood was collected following the same procedure without heparin and serum was separated, frozen and stored at -20°C until used.

Western blot assay

Protein electrophoresis was conducted in a mini-gel system (Bio-Rad Co., USA) as described by Stevens *et al.* [21]. Briefly, S1119-3 whole cell was heated at 100°C for 5 minutes in the presence of SDS and mercaptoethanol in Tris-glycine buffer (×5 sample buffer: 0.6 ml 1M Tris-HCl (pH 6.8), 5 ml 50% glycerol, 2 ml 10% SDS, 0.5 ml 2-mercaptoethanol, and 1 ml 1% bromophenol blue, 0.9 ml D.W.). The resultant mixtures were clarified separately by centrifugation at 5,000 rpm prior to loading onto a 10.5% (w/v) acrylamide running gel with a 4% (w/v) stacking gel. Electrophoresis was run at constant 30 volts until the bromophenol blue tracking dye came in the running gel, and then 80 volts until the tracking dye had migrated to the bottom of the gel. Polypeptides were transferred electrophoretically from the acrylamide gel to nitrocellulose paper (0.45 micrometer pore size, Sigma Co., USA) in a Trans-Blot Apparatus (Bio-Rad Co., USA) under conditions essentially similar to that described by Sambrook *et al*. [22]. Immunoblots were blocked by incubation overnight in PBS (pH 7.4: 19 ml 0.2M NaH_2PO_4, 81 ml 0.2M Na_2HPO_4) containing 0.2% Tween-20. The membrane was washed and then treated with sample serum diluted in PBS containing 0.05% Tween-20 for 60 minutes at room temperature. After 3 rinses with PBS containing 0.1% Tween-20 for 5 minutes, alkaline phosphatase conjugated goat anti-rat Ig G (Sigma Co., USA) diluted in PBS at 1:1,000 were

allowed to bind for 60 minutes at room temperature. After 3 rinses as described above, the reactive bands were color developed with BCIP/NBT (Kirkegaard & Perry Laboratories, Inc., USA).

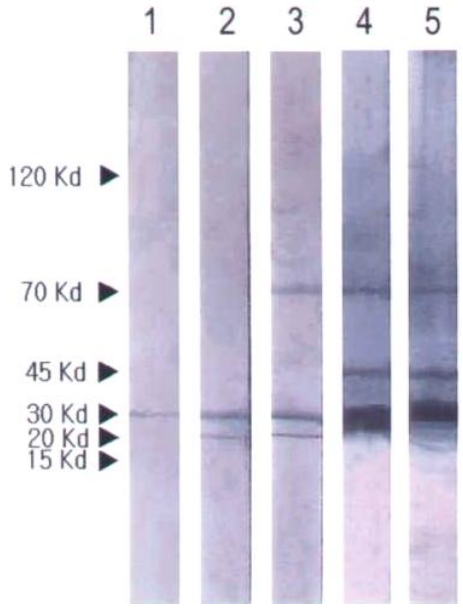
Results

The rectal temperatures were within normal range (35-36°C) in CR, InoR and InoChR. But NInoChR developed lethargic, anorectic conditions and the rectal temperature raised at 38°C. Evidence of arthritis or anaphylactic shock was observed in none.

SRB51 was easily isolated from InoR group until the first week post vaccination. *B. abortus* biotype 1 was easily isolated from the InoChR group rat until 8 weeks post challenge but it was possible to isolate these bacteria from cultured blood until 24 weeks post challenge from NInoChR. *B. abortus* biotype 1 and SRB51 were not easily isolated from blood of any CR.

The result of the western blot assay is shown in Figure 1. It reveals that there were several bands at 120, 70, 45, 30, 20 kDa and clear specific bands were found after vaccination (20, 70 kDa) and challenge (15, 20, 45, 70, 120 kDa). The highest immune response was observed in sera of rats 4 weeks post SRB51 vaccination. The intensity of bands was higher in the InoChR group than in the InoR group.

Figure 1. Western blot analysis in sera of B. abortus strain RB51 inoculated and *B. abortus* strain RB51 inoculated challenged Sprague-Dawley rats using *B. abortus* strain 1119-3 whole cell antigen. 1-3 indicated sera from 0, 1 and 4 weeks post inoculated rat (InoR) and 4-5 indicated sera from 6 and 8 weeks post inoculated challenged rat (InoChR), respectively.



Discussion

Since the first report of the occurrence of bovine brucellosis in South Korea in 1956 [23], sero-diagnostic methods, etiological study, and rapid identification of the organisms by PCRhave been developed [24-30].

Western blot assay has been used to determine the immune response for SRB51 vaccinated as well as challenged animals by other workers [8,16]. Jimenez de Bagues *et al*. [16] prepared whole cell extract (WCE) of *B. abortus* from methanol killed and boiled in Laemmli's sample buffer, LPS extraction of *B. abortus* from the aqueous phase of water-phenol and observed the reaction with SRB51 immunized serum obtained from mice inoculated with SRB51 live cells (3.0×10^8 cfu, I. P.). WCE reacted with immunized mice serum and at least six bands appeared around 80 to <20 kDa, while their specificity to inoculation was not determined. Stevens and Olsen [8] reported that pooled sera from SRB51-vaccinated cattle produced bands sized from 84 to <20kDa. They also reported that of 30 serum samples obtained from SRB51 inoculated cattle, 87% were recognized with 20 kDa proteins, while 77% samples reacted with 20 to 29 kDa, 67% with 29 to 35 kDa, 63% with 53 to 84 kDa, and only 53% were recognized with 35 to 53 kDa.

The western blot assay for the diagnosis of immune response and the efficacy of SRB51 using whole cell antigen of S1119-3 in rats has not been presented earlier. In this study, WCE preparation of S1119-3 from heat-killed whole cell boiled in sample buffer was electrophoresed. There were in total 5 bands at 120, 70, 45, 30, 20 kDa from InoR and InoChR, and clear specific bands were found after vaccination (20, 70 kDa) and challenge (15, 20, 45, 70, 120 kDa). The intensity of the bands was higher in SRB51 inoculated and subsequently challenged rats than in SRB51 inoculated rats; this observation may be due to a higher degree of resemblance in the outer membrane protein composition between strains. [31].

SRB51 was able to induce protective immune reponse against *B. abortus*, *B. melitensis* and *B. ovis* challenge in mice [11, 16, 32]. In rams, the protective efficacy of live SRB51 against a *B. ovis* challenge has been evaluated using the attenuated *B. melitensis* strain Rev 1 as reference [12]. Cheville *et al*. [6] demonstrated that subcutaneous inoculation of heifers with SRB51 was completely protective against virulent *B. abortus* strain 2308. Our study demonstrated the protective immunity of SRB51 in SD rats against *B. abortus* biotype 1 challenge. *B. abortus* biotype 1 was easily isolated from the blood of SRB51

inoculated challenged rats until 8 weeks, but it was also easily isolated until the end of experiment in challenged rats that were not vaccinated with SRB51.

The doses, used for the inoculation and challenge in this study, were standardized [2]. *B. abortus* biotype 1, isolated in South Korea and in other countries, has been reported as a pathogenic strain for natural infection [33-35]. Based on western blot assay, it is concluded that the SRB51 elicits antigen specific immunity in SD rats.

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