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Short Communication

Diagnosis and Efficacy of *Brucella abortus* Strain RB51 in Experimentally Inoculated Sprague - Dawley Rats Using Dot Blot Assay

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Abstract. Sprague-Dawley (SD) rats were experimentally inoculated with *Brucella abortus* strain RB51 and challenged 4 weeks post inoculation with *B. abortus* biotype 1. Monitoring of the sera by dot-blot assay and clinical examination of the rats revealed the highest immune response. SRB51 was isolated from the blood of all inoculated rats until 1st week post inoculation without any clinical sign. It is concluded that SRB51 produces immunity in SD rats and induces protection against *B. abortus* biotype 1 and that Dot Blot Assay is an easy, less time consuming and correct-result reading test.

Keywords: *B. abortus* strain RB51, *B. abortus* biotype 1, Sprague-Dawley rats, dot blot assay, South Korea

Brucellosis is one of the world's major zoonoses, alongside bovine tuberculosis and rabies (Rahman *et al.*, 2006). In animals, brucellosis mainly affects reproduction, fertility, survival of newborns and milk yield (Sewell and Brocklesby, 1990). *B. abortus* biotype 1, isolated in South Korea and other countries, has been reported as pathogenic strain for natural infection (Park *et al.*, 1998; Chung *et al.*, 1988; Cordes and Carter, 1979).

The introduction of *Brucella abortus* strain RB51 (SRB51) has been recently recommended as an alternative to *B. abortus* strain 19 vaccine for the control of bovine brucellosis. SRB51 has also been approved as a calfhood vaccine by United States Department of Agriculture (Palmer *et al.*, 1997).

The serodiagnostic tests, viz the card test, tube agglutination test, plate agglutination test, Rose Bengal test, Rivanol test, mercaptoethanol particle concentration fluorescence immunoassay and the complement fixation test remain negative after inoculation with live SRB51, (Baek *et al.*, 2002; Stevens *et al.*, 1995a, Jimenez de Bagues *et al.*, 1994; Cheville *et al.*, 1993, 1992). Thus for diagnosis of SRB51 inoculated animals, an easy, simple, less-time consuming and correct-result reading diagnostic test is preferred. To the best of our knowledge, the dot blot assay, a test of such a kind, for the diagnosis of immune response and the efficacy of SRB51 using whole cell antigen of S1119-3 and SRB51 in rats had not been presented before.

In the present study, the diagnosis and efficacy of SRB51 in experimentally inoculated Sprague-Dawley (SD) rats were studied through dot blot assay using whole cell antigen of *Brucella abortus* strain 1119-3 (S1119-3) and SRB51. The doses used for the inoculation and challenge in this study were according to the standard methodology for brucellosis experiment (Nielsen and Duncan, 1990). The seed stocks of SRB51 (without carbon dioxide) and *B. abortus* biotype 1 (with 5% carbon dioxide) were cultured in *Brucella* broth for 48 h at 37 °C. The bacteria were washed and suspended in physiological saline before use. The master seed of S1119-3 was cultured on *Brucella* agar for 72 h and then in *Brucella* broth for 48 h. Suspension of SRB51 (1.0×10^7 cfu) was administered to 48 healthy disease free 6~10 months old female SD rats, orally and only saline was administered to 48 control SD rats; these will be hereafter referred to as innoculated and control rats or groups, respectively. Both the types were divided into 12 groups of 4 rats each and reared in cages under hygienic conditions. Half of the control and the inoculated rats each (n=24) were challenged with 1.0×10^9 cfu suspension of *B. abortus* biotype 1 on 4 weeks post inoculation of SRB51, referred to control-challenged and challenged rats, respectively, hereafter.

One ml of blood was collected from the hearts of all of the rats with heparin (100 IU) at 0, 1, 2, 3, 4, 5, 6, 8, 12, 16, 20, 24 weeks post and cultured at 37 °C–control-challenged and challenged rats, with 5% CO_2 and control and inoculated rats, without CO_2–in tryptose soy broth with 5% bovine serum for 3 days and re-cultured for further 3 days on the same media. Another 1 ml of blood was collected without heparin and the serum was separated, frozen and stored at -20 °C, for further use.

Dot-blot assay, for the detection of sera antibody in rats of all of the groups, were performed basically by the procedure of Cheville *et al.* (1993). For reciprocal antibody titre, the inten-

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sity of colour was measured by executing the molecular analyst software (Utsugisawa *et al.*, 1999).

All rats were examined carefully for any adverse reaction at suitable intervals. The rectal temperature of all the rats was observed to be within the normal range (35-36 °C) except control-challenged rats which developed lethargic, anorectic conditions and the temperature rose to 38 °C. Evidence of arthritis or anaphylactic shock was not seen in any of the rats of either group. SRB51 was isolated from the blood of all inoculated rats until 1st week post inoculation. *B. abortus* biotype 1 was isolated from the blood of challenged rats until 8 weeks post but from that of control-challenged, until 24 weeks post. *B. abortus* biotype 1 and SRB51 could not be isolated from the blood of any control rat.

The highest reciprocal antibody titres in sera by dot-blot assay using SRB51 whole cell antigen was observed in 4 weeks post in control-challenged rats (Fig. 1) and none, before inoculation and challenge. The results of dot-blot assay (Fig. 2) revealed the darkest colour density reaction on 4 weeks post in inoculated rats using SRB51 whole cell antigen and on 8 weeks post in challenged and control challenged rats using S1119-3 whole cell antigen, but no reaction in inoculated rats or before inoculation and in control rats using S1119-3 and SRB51 whole cell antigens.

In the present study, inoculated rats reacted strongly against SRB51 whole cell antigen rather than against S1119-3 whole cell antigen. However, when they were exposed to *B. abortus* biotype 1, the antibody response increased against S1119-3 in comparison to against SRB51. The humoral immune responses were different between inoculated and challenged rats, probably due to differences in outer membrane protein of the strains in the existence of LPS O-side chain or otherwise and corresponding different proportion of antibodies against LPS or proteins induced in naturally infected animals, whereas antibody against protein is induced at very low level in these animals.

It has been noted that IgG can nonspecifically bind to intact *B. abortus* strain 2308 bacteria by attachment to a cell surface protein (Bricker *et al.*, 1991). Stevens and Olsen (1996) observed low and short persistence of antibody titre by dot-blot assay using *B. abortus* strain 2308 to SRB51 antisera. Evidence indicates that vaccination of mice with SRB51 induces immunity to infection with pathogenic strain by cell-mediated but not by antibody responses (Stevens *et al.*, 1995a, b; Jimenez de Bagues *et al.*, 1994). In this study, the antibody responses were detected through dot-blot assay using SRB51 and S1119-3 whole cell antigen, both in SRB51 inoculated and challenged SD rats.

SRB51 was able to induce protective immunity against *B. abortus*, *B. melitensis* and *B. ovis* challenge in mice (Stevens *et al.*, 1995a; Jimenez de Bagues *et al.*, 1994; Schurig *et al.*, 1991). In rats, the protective efficacy of the live SRB51 against *B. ovis* has been evaluated using the attenuated *B. melitensis* strain Rev 1 as a reference (Jimenez de Bagues *et al.*, 1995). Cheville *et al.* (1993) demonstrated that subcutaneous inoculation of heifers with SRB51 fully protected them against infection when challenged with virulent *B. abortus* strain 2308. This study demonstrated the protective immunity of SRB51 in

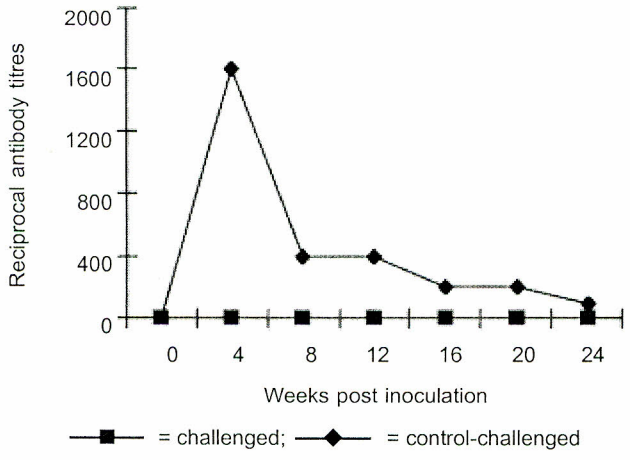

Fig. 1. Reciprocal antibody titres in SD rat sera by dot-blot assay using whole cell antigen of *B. abortus* strain RB51.

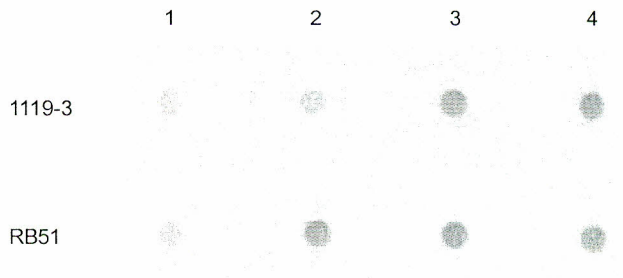

Fig. 2. Dot-blot assay in sera of *B. abortus* strain RB51 inoculated and *B. abortus* strain RB51 inoculated challenged Sprague-Dawley rats using whole cell antigens of *B. abortus* strain 1119-3 and *B. abortus* strain RB51; 1-2 = sera from 0, 4 weeks post from inoculated rats, respectively; 3 = sera from 8 weeks post from challenged rats; 4 = sera from 8 weeks post from control-challenged rats.

SD rats against *B. abortus* biotype 1. *B. abortus* biotype 1 was isolated from the blood of SRB51 challenged rats until 8 weeks, but until the end of experiment, from SRB51 control-challenged rats. It is concluded that the SRB51 produces immunity in SD rats and induces protection against *B. abortus* biotype 1 challenge.

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