PLATE AND TUBE AGGLUTINATION TESTS FOR DIAGNOSIS OF BRUCELLA ABORTUS BIOTYPE 1 INFECTION IN SPRAGUE-DAWLEY RATS

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ABSTRACT

The plate and tube agglutination tests were evaluated for the diagnosis of experimentally induced Brucella abortus biotype 1 infection in 45 female, 6 to 10 months old Sprague-Dawley (SD) rats during the period from 2001 to 2002. These 45 rats were divided into two groups A and B. Of which group A consisting of 27 rats used for experimental infection, whereas 18 rats of group B served as uninfected control. Each rat of group A was injected subcutaneously with 0.01 ml colostral forming units (CFU) in 50 ml of bovine pathogenic strain of B. abortus biotype 1 suspension in physiological saline. The SD rats were inoculated at regular intervals by serological and bacteriological methods. The inoculated antibody were 400 through tube agglutination test (TAT) whereas it was 1:800 through plate agglutination test (PAT) at first week of post injection. There was no inoculated antibody in sera of 34 weeks of post-infection both through PAT and TAT despite the presence of bacteria and these tests were evaluated for the first time using sera from 10 rats with brucellosis. PAT using B. abortus strain 1119-3 (S119-3) whole cell antigen was a potential candidate as an improved diagnostic method for field diagnosis of brucellosis in wild animals.

Key words: B. abortus biotype 1, plate and tube agglutination tests, Sprague-Dawley rats

INTRODUCTION

Brucellosis is one of the major zoonoses in South Korea and has been recognized as a cause of reproductive failure in dairy cattle, hereby causing significant economic losses through calving loss and in costs for rearing and eradication programs. Because of the economic importance of brucellosis in domestic animals, diagnosis and prophylaxis of brucellosis in domestic animals have been widely investigated (Ahm et al., 1988; Nicolos, 1990). Although wild animals are crucial in the economy of developing countries, the brucellosis of wild animals have received comparatively little attention. With respect to serological diagnosis there have been studies on wild animals using whole cell antigen and complement fixing test and also on agar immunodiffusion tests using uncharacterized trichomonic acid or sonic extracts (Bell et al., 1976; Palade, 1978, Waghela et al., 1980; Dau-Apap and et al., 1994). However, those studies have not been performed using rat sera from infected or free of brucellosis, and therefore, the actual value of those tests are unknown. B. abortus biotype 1 has been isolated from cattle in different provinces of South Korea following extensive studies (Chung et al., 1988; Park et al., 1998; Park et al., 2001; Rahman, 2005).

The purpose of the present study was to investigate the plate (PAT) and tube (TAT) agglutination tests for diagnosis of B. abortus biotype 1 infection in Sprague-Dawley (SD) rats using whole cell antigen of B. abortus strain 1119-3 (S119-3).

MATERIALS AND METHODS

Culture of B. abortus

B. abortus biotype 1 isolated in South Korea was used in this study for infection. For the preparation of antigen for PAT, TAT, S119-3 was used. B. abortus biotype 1 was cultured in Brucella broth (Difco Co., USA) for 48 hours at 37°C with 5% CO2. The master seed of S119-3 was grown on Brucella agar (Difco Co., USA) for 72 hours at 37°C. Then growth was performed in Brucella broth (Difco Co., USA) in shaking incubator at 37°C with 150 rpm. After 30 hours culture, the organisms were then inoculated at 37°C for 4 h. The bacteria were washed with saline for 3 times and suspended in physiological saline before use.

Experimental design

Healthily disease free 6 to 10 months old female SD rats (n = 45) weighing 200 to 250 gms with no history of exposure to Brucella species were used in this experiment. Rats which were classified into infected group (n = 27) and control group (n = 18), antibody by PAT, TAT were negative for brucellosis (Altun et al., 1975). The infected rats group was equally divided into 9 subgroups for 1, 2, 4, 8, 12, 16, 20, and 24 weeks post-infection consisting of 3 rats of each subgroup. Similar procedure was followed in control group (each subgroup consisting of 2 rats). The rats were maintained under hygienic conditions and provided with commercial feed and water ad libitum.

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A 100 µl containing 1 (bacterial) colony forming unit (CFU) suspension of B. abortus biotype 1 in physiological saline solution was injected intravenously in each of 27 rats of each group. Rats in both control groups were injected subcutaneously only with 100 µl of physiological saline each, housed separately and not exposed to B. abortus biotype 1 organism. All of the rats were examined daily for 1 week to record the clinical signs, rectal temperatures after infection.

Table 1. Sprague-Dawley rats used in plate and tube agglutination test for diagnosis of B. abortus biotype 1 infection

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of rats in weeks post-inoculation</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Infected</td>
<td>3</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
</tr>
</tbody>
</table>

Bacteriologic culture of blood and collection of serum

One ml of blood was collected from the femur of each rat with heparin (100 IU/ml) at every 7 days post-infection after administration with ampicillin hydrochloride (25 mg/kg/mouse) and was cultured at 37°C with 5% CO₂ for 3 days in glass tube containing 3 ml of tryptone soy broth (TSA, Co., USA) with 5% bovine serum. From this tube, cultured bouillon was cultured again at 37°C with 5% CO₂ for 3 days in tryptone soy agar (TSA Co., USA) plate with 5% bovine serum to see bacterial colony (Alton et al., 1998). Another 1 ml of blood was collected following the same procedure without heparin and serum was separated, frozen and stored at -20°C until used.

Tube agglutination test

The preparation of diagnostic antigen and procedure were conducted as described by Hur et al. (2001). The prepared antigen was standardized according to procedure of OIE, 2000. Briefly, inactivated S.119-3 whole cells were washed with 0.5% phenol saline (0.85%) and suspended in 0.5% phenol saline (0.85%) containing preservative, at the concentration of 4.7% (v/v). This concentrated was diluted in phenol saline for use at 1:100 dilution. Thiratides, ratio 0.08, 0.04, 0.02, 0.01, 0.005, 0.00125 ml of serum samples were placed in different tubes and mixed with 2 ml of diluted antigen. The results were read after incubation at 37°C for 24 hours. Positive reaction was one in which the antigen-antibody mixtures was clear and gentle shaking did not disrupt the flocculi. A negative reaction was one in which the antigen-antibody mixture was not clear and gentle shaking revealed no flocculi.

Plate agglutination test

The preparation of diagnostic antigen and procedure were conducted according to the procedure of Ryu et al. (1997). The prepared antigen was standardized according to procedure of OIE (2000). Briefly, inactivated S.119-3 whole cells were washed with 0.18 phenol saline (0.95%) and suspended in 11.0% instead of 4.7% of TAT Crystal violet brilliant green staining solution was prepared by dissolving 2.0 gram brilliant green and 1.0 gram crystal violet in 300 ml of distilled water. Then 6 ml of this staining solution was added into 1,000 ml of cell suspension 0.03 ml of antigen solution was added to 0.06, 0.04, 0.02, 0.01, 0.005, 0.00125 ml of each bovine serum on glass plate and then incubated for 2 hours at room temperature. The time was hand rotated three times, at 4 and 9 hours after mixing and just before reading. Any sign of agglutination was considered positive (Alton et al., 1988).

RESULTS AND DISCUSSION

Clinical signs

All of the rat infected with B. abortus biotype 1 developed lethal, anorectic, anemic and febrile conditions. The highest rectal temperature of infected group was 39°C until 3 days, while in control group the temperature remained 30°C. There were no other adverse reactions or clinical signs after infection.

Bacteriologic findings

Clostridium B. abortus biotype 1 was observed on the lymph node agar plate cultured from blood of all infected rats until 24 weeks post-infection.
Tube agglutination test

The reciprocal antibody titers of TAT in sera of infected SD rats using S119-3 whole cell antigen have been presented in Table 2. The average reciprocal antibody titer was 1:400 at 1st and 2nd weeks post-infection and it increased to 1:1600 at 4th week post-infection. The reciprocal antibody titers gradually decreased to 1:25 at 20th week post-infection, and then there was no reciprocal antibody titer at 24th week post-infection (Table 2). Reciprocal antibody titers were not detected before infection of B. abortus biotype 1 and in control rats. The reaction (agglutination) pattern observed in TAT has presented in Fig. 1.

Table 2. Tube and plate agglutination tests of B. abortus biotype 1 infected SD rats using B. abortus strain 119-3 whole cell antigen.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Reciprocal antibody titers at weeks post-infection</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>TAT</td>
<td>1:400</td>
</tr>
</tbody>
</table>

* TAT = Tube agglutination test, PAT = Plate agglutination test, – indicates no agglutination.

**Fig. 1.** Tube agglutination test in sera of B. abortus biotype 1 infected Sprague-Dawley rats using B. abortus strain 119-3 whole cell antigen; TAT (-ve), negative reaction for brucellosis, positive reaction for brucellosis.

**Fig. 2.** Plate agglutination test in sera of B. abortus biotype 1 infected Sprague-Dawley rats using B. abortus strain 119-3 whole cell antigen; N = Serum before inoculation, P = Serum of day 4th week post-infection of B. abortus biotype 1, A arrow indicates the agglutination sphere.

Plate agglutination test

The reciprocal antibody titers of PAT in sera of infected SD rats using S119-3 whole cell antigen have been presented in Table 2. The average reciprocal antibody titer was 1:400 at 1st and 2nd weeks post-infection and it increased to 1:1600 at 4th week post-infection. The titers gradually decreased to 1:25 at 20th week post-infection, and there was no reciprocal antibody titer at 24th week post-infection. Antibody titers were not detected before infection of B. abortus biotype 1 and in control rats. The reaction (agglutination) pattern observed in TAT presented in Fig. 2.

The diagnosis of brucellosis is confirmed by isolation of Brucella by bacteriological culture or by the detection of an immune response by serological test to its antigens (Biwot, 1989; Oduma et al., 2000). The diagnosis of brucellosis based exclusively on Brucella isolation presents several drawbacks. The slow growth of Brucella may delay diagnosis for more than 7 days (Rodriguez-Torres and Feroone, 1997; Aron, 1996; Yagopolki, 1999). Also, the sensitivity is often low, ranging from 50 to 90% depending on disease stage. Brucella species, culture medium, quantity of bacteria
and culture technique employed (Garcia et al., 1986; Yapisky, 1999). Thus, the serological tests play an important role in the diagnosis of brucellosis.

Among the serological tests, the TAT has become the standard method, as the test recommended for collection of quantitative information on immune responses, and is the most frequently used confirmatory serological test (Lucero and Rolpe, 1998). In many countries, the TAT, which may give false negative results, is the routine test and is sometimes only used if Lucero and Rolpe. (1998) TAT was the first test used for the diagnosis of brucellosis in people and was soon adapted for use in animals (George, 1994).

Brucella species are facultative intracellular pathogens which survive within a variety of cells including macrophages, and the virulence of these species is established by the establishment of long time infection by the host are thought to be essentially due to their ability to avoid the killing mechanisms within macrophages (Badwin and Winter, 1994; Sangot and Anzora, 1996). These macrophages in the presence of Brucella infected rats produce nitric oxide, which can contribute to persist the infection for a long time (Uzun et al., 2000). The involvement of nitric oxide in the anti-brucella activities of macrophages has also been suggested by pharmacological experiments (Jiang et al., 1993).

Bacilli due to brucellosis may persist for varying periods of time depending on the host and Brucella species. In goats infected with B melitensis, bacteria is detectable more than 300 days. In cattle infected with B abortus, the onset of bacteremia may last 5 months or more. In swine infected with B suis, bacilli may persist for more than 3 years (Alton, 1990; George, 1994). Bacilli in B abortus has been shown to be dose dependent and may detectable at 1,120 days (Camichilo, 1990, George, 1994). In the present study, a abscesses biopsies was inoculated into the SD rats subcutaneously at the dose rate of 1.0x10^6 CFU and bacteremia was detected until 24 weeks post-infection.

In the present work, PAT, TAT using S1119-3 whole cell antigen have been evaluated for the first time using Brucella infected sera in rats. The reciprocal antibody titer was 1:400 through TAT whereas it was 1:800 through PAT at first week post-infection. There was no reciprocal antibody titer in sera of 24 weeks post-infection both through PAT and TAT but there were bacteremia until 24 weeks post-infection. The reciprocal antibody titer was 1:25 both by PAT and TAT on 20th week post-infection. Therefore, there was a close relationship between PAT and TAT. PAT requires 48 hours incubation and skilled dilution of the antigen to complete the reaction whereas PAT requires only 5 minutes with no requirements of incubation and dilution of antigen. Therefore, PAT using S1119-3 whole cell antigen can be recommended as a suitable test for field diagnosis of brucellosis in wild animals.

REFERENCES


