

# Serologic Responses and Kinetics of *B. abortus* Biotype 1 Infection in Sprague-Dawley Rats

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## Abstract

**Background:** Brucellosis is an important zoonotic disease in animals and humans caused by *Brucella abortus*. The pathogenesis and immune responses of *B. abortus* is mainly studied in mice and cattle. The serologic responses as well as the kinetics and distribution of bacteria were measured for 8 weeks after infection of Sprague-Dawley (SD) rats with *B. abortus* biotype 1. **Methodology:** Thirty-two SD rats received an intraperitoneal dose of  $1 \times 10^{10}$  colony-forming units (CFU) of *B. abortus* biovar 1 isolated from cattle in Korea. At regular intervals after infection the rats were randomly bled for serologic studies and euthanized for bacteriological examinations. **Results:** Sera were found positive for *B. abortus* specific antibody response by rose bengal plate test (RBPT), tube agglutination test (TAT) and plate agglutination test (PAT) at one week after infection. The peak antibody titers were recorded at 5 weeks after infection by the TAT and PAT then the titer gradually decreased until the end of the study. After experimental infection *B. abortus* was disseminated in blood and lymphoid tissues (spleen, liver and kidney). The persistence of *B. abortus* in blood was noted for short time, up to 5 weeks of infection. Bacterial persistence was recorded in the spleen throughout the experiment. No bacteria were cultured from the liver and kidney at 6 weeks after infection. **Conclusion:** The serologic responses and distribution of *B. abortus* in SD rats following experimental infection suggests that SD rats can clear the infection after a single exposure which will be helpful for the diagnosis of brucellosis.

## Keywords

*B. abortus* Biotype 1, Sprague-Dawley Rat, Acute Infection, Bacterial Persistence, Serologic Response

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## 1. Introduction

Brucellosis is an important zoonotic disease, caused by the several species of the genus *Brucella* that have their natural reservoir in domestic animals [1, 2]. Bovine brucellosis caused by *Brucella abortus* has far reaching animal health and economic impacts at both the local and national levels [3]. *Brucella abortus* is a gram-negative facultative intracellular bacterium whose pathologic manifestations are hepatitis, arthritis and meningitis in humans and spontaneous abortion in cattle, respectively [4].

Brucellosis is an emerging infectious disease in many developing countries and in some developed areas of the

world [2] despite the animal control and eradication projects has become a serious hazard to public health [5]. Infection in humans is either through direct contact with infected animals [6] or from contaminated dairy products [7]. Another most likely source of introduction of brucellosis in livestock and humans is free ranging wildlife [8] since *B. abortus* was isolated from a wide range of wildlife including rats [9, 10, 11]. Generally, rat infections occur in areas with large numbers of infected cattle [9]. The potential role of wild rodents as reservoirs of *Brucella* has been reported by Garcia-Carrillo [12].

The pathogenesis and immune responses of *B. abortus* is mainly studied in mice and cattle. Following invasion of the

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body by *Brucella*, it is taken up by the phagocytic cells, the bacteria proliferate within mononuclear phagocytes and the infected cells play a crucial role in the dissemination of the bacteria to specific locations of the body such as spleen, brain, heart, and bones [13]. Protection against *B. abortus* is mediated both by humoral and cellular immune responses. Humoral immune response is mediated by antibodies directed against lipopolysaccharide (LPS) molecules of *B. abortus* [14]. Most of the current understanding of serologic response, the kinetics and distribution of *B. abortus* has arisen from studies in cattle. However, serologic response, distribution and kinetics of *B. abortus* biotype 1 have not been studied in the rats. Accordingly, the main objective of this work was to characterize the serologic responses, the kinetics and distribution of *B. abortus* biotype 1 in SD rats.

## 2. Materials and Methods

### 2.1. Bacterial Strain and Growth Condition

*B. abortus* biotype 1 lyophilized stock culture was obtained from the laboratory repository. *Brucella* was inoculated onto *Brucella* agar medium (Difco, Kansas City, Missouri, USA) and incubated at 37°C for 7 days under 5% CO<sub>2</sub>. The bacteria were harvested in sterile phosphate buffered saline (PBS).

### 2.2. Rats and Inoculation Procedure

Adult Sprague-Dawley (SD) rats (n=36), weighing 200 to 250 g, 8 weeks old, were purchased from a specific pathogen free (SPF) laboratory animal company (Koatech, Pyungtaek City, Gyeonggido 451-864, Korea). The rats were housed in a stringently hygienic, climate-controlled environment and supplied with commercial feed and water *ad libitum*. All experiments were carried out in compliance with the humane protocols approved by the Chonbuk National University, Jeonju, Republic of Korea. All rats were culture negative and seronegative prior to experimental infection. Thirty-two SD rats were inoculated intraperitoneally with 0.1 ml sterile injectable pyrogen free solution containing  $1 \times 10^{10}$  CFU/ml of *B. abortus* biotype 1. Four rats were used as uninfected controls.

### 2.3. Collection of Specimens

Rats were anesthetized by intraperitoneal administration of 10 mg/kg of Tiletamine and Zolazepam (Zoletil 50, Virbac Laboratories-06515 Carros-France) 5 min before aseptic cardiac puncture. Blood samples were collected in EDTA vacuum tubes (Greiner) with and without anticoagulant. Blood and organ collection was performed from the inoculated rats at 1, 2, 3, 4, 5, 6, 7 and 8 weeks after infection. The rats were then euthanized and spleen, liver and kidney were aseptically removed for bacteriological studies. Blood

samples with anticoagulant were also examined for bacteriological studies. Blood samples without anticoagulant were allowed to clot for 6 hours at 25°C before centrifugation. Serum samples were collected and stored frozen at -20°C until testing. All other samples were stored at 4°C until cultured. Blood and others tissue samples were also collected from the four uninfected control rats.

### 2.4. Serological Studies

Serum samples were tested by RBPT, TAT and PAT using *B. abortus* 1119-3 whole cell antigen according to the procedures described elsewhere [15,16].

### 2.5. Bacteriological Analyses

The EDTA-blood was subjected to osmotic shock with distilled water and centrifugation at 3000 rpm for 60 min at room temperature. The resulting blood pellet was resuspended in *Brucella* broth. Then, 50 µl of suspension was immediately plated in duplicate on *Brucella* agar medium and incubated at 37°C for 7 days in a 5% CO<sub>2</sub> atmosphere. Growth of bacteria was noted 15 days after plating. Spleen, liver and kidney samples were macerated separately in a stomacher (Costa Brava, Spain). For the isolation of *Brucella* and to determine the number of CFU per spleen, macerated samples were cultured in two petri dishes with *Brucella* agar medium containing antibiotics (cycloheximide, bacitracin and polymyxin) which were incubated at 37°C for 5-7 days in a humid atmosphere enriched with 5% CO<sub>2</sub> respectively. The colonies grown were identified following the classical methodology described by Alton *et al.* [15].

### 2.6. Polymerase Chain Reaction

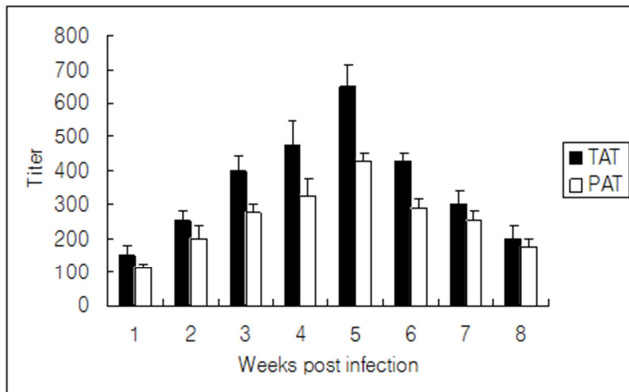
Bacteria harvested from culture positive specimens of rats were confirmed to be *B. abortus* by AMOS (abortus, melitensis, ovis, suis) polymerase chain reaction (PCR) as described by Bricker [17]. For AMOS-PCR, DNA was extracted from *Brucella* suspected colonies of spleen, liver and kidney by a genomic DNA extraction kit (Accuprep DNA Extraction Kit, Bioneer, Daejeon, Korea) using the manufacture's protocol. From whole blood, extraction of DNA was performed by QuickGene DNA whole blood kit S (Life Science, Fujifilm Cooperation, Japan).

## 3. Results

### 3.1. Serological Responses

Sera samples of rats collected at 1, 2, 3, 4, 5, 6, 7, 8 weeks post infection tested positive to *Brucella* by the RBPT, TAT and PAT. Sera collected from control rats were found negative to *Brucella*. *B. abortus* biotype 1 elicited antibody response at 7 days after infection. The mean reciprocal

antibody titers in sera measured by TAT at 1, 2, 3, 4, 5, 6, 7 and 8 weeks after infection were recorded as  $150 \pm 29$ ,  $250 \pm 29$ ,  $400 \pm 41$ ,  $475 \pm 75$ ,  $650 \pm 65$ ,  $425 \pm 25$ ,  $300 \pm 41$  and  $200 \pm 41$ , respectively. On the other hand PAT mean reciprocal sera antibody titers at 1, 2, 3, 4, 5, 6, 7 and 8 weeks after infection were recorded as  $112 \pm 13$ ,  $200 \pm 41$ ,  $275 \pm 25$ ,  $325 \pm 48$ ,  $425 \pm 25$ ,  $287 \pm 31$ ,  $250 \pm 29$  and  $175 \pm 25$ , respectively. The mean reciprocal sera antibody titers measured by TAT and PAT are presented in Fig. 1.



**Fig. 1.** TAT and PAT antibody titers in sera at 1,2,3,4,5,6,7 and 8 weeks post infection with *B. abortus* biotype 1. The titers are presented as mean  $\pm$  SEM.

### 3.2. Kinetics and Distribution of *B. abortus* Biotype 1 Infection

*B. abortus* biotype 1 was identified from culture of spleens from 1 week to throughout the experiment. *B. abortus* was also isolated from liver and kidney at 1 week to 6 weeks. Bacteria were isolated from blood at 1 week to 5 weeks. After 5 weeks there was no bacteria isolation from the blood

cultures. *B. abortus* biotype 1 was negative in culture of blood, spleen, liver and kidney of the control group. Table 1 summarizes the bacterial isolation from blood and lymphoid organs at different time points after infection with *B. abortus* biotype 1.

**Table 1.** Summary of *B. abortus* biotype 1 isolation from blood and lymphoid organs of SD rats experimentally inoculated with *B. abortus* biotype 1.

Weeks after infection	No. of rats	Blood (n=15/36)		Spleen (n=30/36)		Liver (n=20/36)		Kidney (n=16/36)	
		N <sup>a</sup>	% <sup>b</sup>	N <sup>a</sup>	%	N <sup>a</sup>	%	N <sup>a</sup>	%
0	4	0	0	0	0	0	0	0	0
1	4	3	75	4	100	3	75	3	75
2	4	3	75	4	100	3	75	3	75
3	4	4	100	4	100	4	100	4	100
4	4	3	75	4	100	4	100	3	75
5	4	2	50	4	100	4	100	2	50
6	4	0	0	4	100	2	50	1	25
7	4	0	0	3	75	0	0	0	0
8	4	0	0	3	75	0	0	0	0

<sup>a</sup> N, Number of rats from which *B. abortus* was isolated.

<sup>b</sup> Percentage of *B. abortus* isolated from the tissue in culture-positive animals.

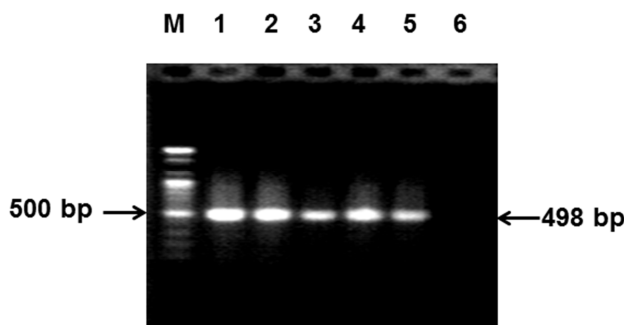
The number of organisms present in the spleen at specific time points after infection was assessed in SD rats infected with  $1 \times 10^{10}$  CFU/ml *B. abortus* biotype 1. One week after infection the number of bacteria was  $3 \times 10^5$  CFU/ml of homogenized spleen. However by week 3, the number was  $1.9 \times 10^6$  CFU/ml and again reached peak at 5 weeks after infection ( $2.2 \times 10^6$  CFU/ml) then the number decreased at 8 weeks after infection ( $2.1 \times 10^4$  CFU/ml).

Table 2 summarizes the bacterial burden in the spleen at different time points after infection.

**Table 2.** Brucella burden in the spleen of SD rats infected intraperitoneally with *B. abortus* biotype 1.

Bacterial strain	CFU of <i>B. abortus</i> biotype 1 cells in spleen			
<i>Brucella abortus</i> biotype 1	1 week (% of animals with negative culture) $3 \times 10^5$	3 week (% of animals with negative culture) $1.9 \times 10^6$	5 week (% of animals with negative culture) $2.2 \times 10^6$	8 week (% of animals with negative culture) $2.1 \times 10^4$

Colony forming units (CFU/spleen) and the proportion of animals in each group with negative cultures (in parentheses) are shown. Data are expressed as mean values of four rats  $\pm$  SD.



**Fig. 2.** AMOS PCR assay for amplification of *B. abortus* biotype 1. M: 100 bp size DNA marker (Bioneer, Daejeon, Korea); Lane 1: bacterial DNA of blood culture; Lane 2: bacterial DNA of spleen; Lane 3: bacterial DNA of kidney; Lane 4: bacterial DNA of liver; Lane 5: positive control with *B. abortus* 1119-3; Lane 6: negative control without DNA.

### 3.3. Polymerase Chain Reaction

All culture positive bacterial colonies obtained from blood and lymphoid organs were confirmed as *B. abortus* biotype 1 by AMOS-PCR with the predicted 498-base pair PCR amplicons (Fig. 2).

## 4. Discussion

Infection in wildlife with *B. abortus* poses a significant threat in the eradication of brucellosis. Eradication of brucellosis in livestock animals as well as humans can not be achieved without controlling brucellosis in wildlife. The knowledge of serologic response, kinetics and distribution of *B. abortus* in

free ranging wildlife is important for controlling brucellosis in wildlife reservoir.

Production of antibodies of *B. abortus* is directed against LPS O side chain that can be measured by conventional serological tests [18]. In this study, we measured antibody response of *B. abortus* biotype 1 infected SD rats during the course of acute infection by RBPT, PAT and TAT. Antibody production was detected in SD rats at 7 days after infection in all serological tests. Similar results were reported by Beh [19] who detected antibody responses in the sera of cattle at one week post infection. Sera antibody titers measured by TAT and PAT were increased from the 7 days post infection and reached at the peak value at 35 days after infection (Fig. 1). Then the titer gradually decreased throughout the course of acute infection. Studies on the antibody response of cattle to *B. abortus* infection have shown an evaluation of the antibody response at one week post infection [19]. In our study screening of sera was performed by RBPT since it is very sensitive for diagnosis of *Brucella* in animals [21]. Serum antibody titers were measured by TAT and PAT. The TAT is the most frequently used confirmatory serological test in many countries. It is recommended for collection of quantitative information on immune responses in brucellosis [22]. The PAT is the routine test for brucellosis that is rapid and would approximate the results of the TAT [23].

It is well known that lymphoid tissue is the predilection site for *B. abortus* [24, 25] that localizes initially in the regional lymph nodes, and then disseminates haematogenously to the cells of the reticuloendothelial system [26]. The data from the bacteriological studies in this experiment suggest that *B. abortus* infection disseminated to all tissues following experimental infection. Persistence of *B. abortus* was recorded in blood, liver and kidney up to 35, 42 and 42 days after infection, respectively. Large numbers of viable bacteria were isolated from the tissues of *B. abortus*-infected mice 30 days after infection [27]. A consistent persistency of bacteria was recorded in the spleen throughout the study period. Our findings are in agreement with High *et al.* [28] who isolated *Brucella abortus* 2308 bacteria from spleen in mice at 5 and 8 weeks after infection. Damir *et al.* [29] isolated *B. abortus* from the spleen but not from the kidney in female camel after experimental infection. Persistence of *B. abortus* was recorded in spleen up to 20 weeks in mice and 6 months in wolves [30, 31]. In case of blood persistence of *B. abortus* was recorded up to 5 weeks of infection in this study. Garcia-Carrillo [12] found a transitory bacteremia in the laboratory infected mice and the bacteria were slowly eliminated some weeks after the onset of the infection. The constant presence of *B. abortus* in the spleen in our study indicated that it is the preferred colonization site in rat for *Brucella* when compared to blood, liver and kidney. The

bacterial kinetics in the spleen indicated that number of bacteria increase from one week after infection and reached a peak at 5 weeks post infection. Bacteria then gradually decreased until the end of the experiments.

## 5. Conclusions

The data of this experiment suggest that SD rats infected with *B. abortus* biotype 1 become bacteremic for a short period, develop disseminated infection and maintain the bacterium in lymphoreticular tissues for at least 8 weeks. The declining serologic titers and the declining numbers and distribution of bacteria observed in SD rats suggest that rats can clear the infection after a single exposure.

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