Original Article

Rats born to *Brucella abortus* infected mothers become latent carriers of *Brucella*

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Abstract

Introduction: Rats are known to be infected with *Brucella*. Vertical transmission of brucellosis was recorded in rats. The study was performed to judge whether rats born from *Brucella abortus* infected mothers can act as latent carriers of *Brucella* infection.
Methodology: Female Sprague Dawley (SD) rats were experimentally infected with *B. abortus* biotype 1 and subsequently bred 10 days post infection (PI). Serum samples of rats (n = 48) born from infected dams were tested using the Rose Bengal plate test (RBPT), tube agglutination test (TAT), and enzyme-linked immunosorbent assay (ELISA) at one, two and three months of age. Tissue samples were plated onto *Brucella* agar and blood agar media and incubated at 37°C with 5% CO_2 for five to seven days for isolation of bacteria..
Results: *B. abortus* was isolated from 18 out of 48 rats born to infected dams, and the isolates were confirmed as *B. abortus* by AMOS (*B. abortus*, *melitensis*, *ovis* and *suis*) PCR assay with the production of a 498 bp PCR amplicon. Serum samples of rats (n = 48) born from infected dams were tested negative using the RBPT, TAT and ELISA at all time points.
Conclusion: We conclude from the study that rats born to infected dams may become latent carriers of *Brucella* infection potentially providing a reservoir for future transmission.

Key words: rats; *Brucella abortus* biotype 1;latent carrier

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Introduction

Brucellosis, a worldwide zoonotic disease caused by members of the genus *Brucella*, affects a large range of domesticated livestock, wildlife, marine mammals, and humans [1,2]. In cattle, the major causative agent of brucellosis is *Brucella abortus*[3]. *Brucella* spp. is a pathogen that affects the reproductive tract of animals [4] and causes abortion in domestic [5] and wild mammals [6,7]. Economic losses due to brucellosis result from abortion, retention of placenta, infertility, loss of calves, decreased milk production, increased calving interval and birth of weak calves [8].

Brucellosis is emerging as a serious animal and public health issue in many parts of the world [9,10] despite animal control and eradication programs. Control of *Brucella* is mainly based on the testing and slaughtering of sero-positive animals. Calves born from infected mothers often become sero-negative, leading to difficulties in controlling brucellosis [11]. The other most likely source of infection to humans and livestock is from free-ranging wildlife [12] including rats. *B. abortus* was isolated from rats with active brucellosis trapped from a cattle farm [13]. Infected rats could play a role in the maintenance and transmission of brucellosis among domestic animals and humans since wild rats harbor *Brucella* [14].

Vertical transmission of *B. abortus* was recorded in experimentally infected Sprague-Dawley rats [15]. In cattle, vertically infected calves can become latent carriers of brucellosis [11,16]. To test the hypothesis that vertical *Brucella* transmission in rats can lead to latent carriers, we determined the *Brucella* infection status of offspring born to infected rats using serological, bacteriological and molecular methods.

Methodology

Experimental rats

Eight- to twelve-week-old female (n = 8) and male (n = 4) Sprague Dawley (SD) rats weighing 300 to 400 grams were used. The parent stock was obtained from a commercial rat breeder (Koatec,

Pyeongteak, South Korea). Rats were housed in a stringently hygienic, climate-controlled environment and supplied with commercial feed and water *ad libitum.* The rats were fed and handled according to standard humane protocols under the supervision of licensed veterinarians.

Bacterial strain

A bovine pathogenic strain of *B. abortus* biotype 1 obtained from the laboratory repository was used for experimental infection. Bacterial cells were maintained as frozen glycerol stocks and cultured on Brucella agar medium (Difco, Kansas City, MO, USA) for 5 to 7 days at 37ºC with 5% CO_2. Cultured bacteria were harvested in normal saline.

Experimental inoculation

Female rats (n = 8) were intraperitoneally injected with 0.1ml saline solution containing 1×10^{11} CFU/ml *B. abortus*. Prior to experimental inoculation rats were found to be healthy and free from brucellosis as determined by culture and antibody testing.

Breeding protocol

At day 10 post-infection, eight female rats were bred with four healthy males (one male for two females). Males were housed with female rats for one month; after this time, the number of pregnant rats and offspring were recorded. Offspring remained caged with their mothers until one month of age.

Specimen collection

Blood samples from rats (n = 48) born to infected dams were collected at one (n = 16), two (n = 16) and three months (n = 16) of age by aseptic cardiac puncture after general anesthesia induced by intraperitoneal administration of 10 mg/kg of tiletamine and zolazepam (Zoletil 50, Virbac Laboratories, Carros, France) for bacteriological and serological examinations. The rats were killed humanely and specimens of spleen and liver were collected aseptically. Sera were stored in small aliquots at -80 °C until tested. Specimens of blood, liver, and spleen were also collected from infected dams.

Serological study

Serum samples were tested by the Rose Bengal plate (RBPT) and tube agglutination tests (TAT) as described by Alton *et al.* [17]. An indirect ELISA was standardized and performed to test serum samples as described elsewhere [18,19].

Bacteriological study

Tissues were macerated in a stomacher (IUL Instruments, Costa Brava, Spain). All macerated samples were plated onto *Brucella* agar media (Difco) supplemented with antibiotics (cycloheximide, polymixin B and bacitracin that inhibit growth of bacteria other than *Brucella*) as well as blood agar media and incubated at 37 °C with 5% CO_2 for 5 to 7 days. Identification of the isolates in the culture-positive specimens was conducted by routine methods [17].

Polymerase chain reaction

DNA extracted from spleen as well as bacteria harvested from culture positive specimens of rats were tested for *B. abortus* biotype 1 by AMOS (*B. abortus, melitensis, ovis, suis*) polymerase chain reaction (PCR) described previously [20]. For AMOS-PCR assay, DNA was extracted from the spleen of progeny rats as well as bacteria from culture positive specimens by a genomic DNA extraction kit (AccuPrep DNA Extraction Kit, Bioneer, Daejeon, Korea) using the manufacturer's protocol.

Results

Clinical signs and reproductive profile of infected rats

Female rats inoculated with *B. abortus* biotype 1 were monitored for clinical signs over a period of seven days. Elevation of rectal temperature (38.8 ºC) was recorded 24 hours following infection. Other clinical signs manifested were lethargy, reduced appetite, and increased thirst. Following breeding, six of eight infected rats became pregnant. A total of 48 viable and 9 dead offspring were birthed by the infected mothers.

Serological study

A total of 48 serum samples from rats born to infected mothers, collected at one month (n = 16), two months (n = 16) and three months (n=16) of age tested negative by RBPT, TAT and ELISA. In contrast, serum samples of infected parturient (n = 6) and non-pregnant rats (n = 2) tested positive. The positive TAT titer was up to 1:100, a titer of 1:50 was suspicious, and 1:25 was considered negative for brucellosis. In ELISA, the absorbance value of serum

Table 1. Results of three serological tests used for screening serum samples

Serum source (n)	RBPT		TAT				ELISA	
	Negative	Positive	Negative	Positive at end point titer			Negative	positive
				1:25	1:50	1:100	OD≥0.0605 ± 0.041	OD≤0.8355 ± 0.041
Rats born from infected mothers (48)	48	0	48	48	0	0	48	0
Infected parturient rats (6)	0	6	0	0	0	6	0	6
Infected non-pregnant rats (2)	0	2	0	0	0	2	0	2
Male rats (4)	4	0	4	0	0	0	4	0

samples of infected and uninfected female rats as well as rats born to them were compared with the absorbance value of the known positive and negative control serum samples. The positive absorbance value of ELISA was established as 0.84 ± 0.10 and negative absorbance value was 0.06 ± 0.04 at an optical density (OD) of 492 nm. The OD values for the serum samples of infected female rats ranged from 1.23 to 1.42 (mean = 1.30; SD = 0.10) and for offspring born to infected female rats ranged from 0.09 to 0.03 (mean = 0.04; SD = 0.02). The results of the serological tests are shown in Table 1.

Bacteriological findings and speciation of Brucella

Colonies characteristic of *B. abortus* (3-5 mm in diameter and opaque in color) were cultured from all infected female rats (n = 8). A total of 18 out of 48 offspring born from infected mothers were found to be culture positive (Table 2). Among the 18 culture-positive isolates, 7 were isolated from one-month-old, 5 from two-month-old, and 6 from three-month-old offspring. All culture-positive bacterial isolates were confirmed as *B. abortus* using the AMOS-PCR; amplification of a 498-bp region of the *B. abortus* genome is shown in Figure 1.

Discussion

Rats are known to be carriers of *Brucella* spp. in many parts of the world [21]. As a reservoir of *B. abortus*, rats pose a significant threat to eradication programs for bovine brucellosis because cattle can get the disease through close contact with the infected animals. Latent carrier stages are known to occur with cattle, and introduction of these animals to previously unaffected farms may result in outbreaks of brucellosis [22]. Vertically infected cattle usually become latent carriers of brucellosis [11]. Although vertical transmission of brucellosis is recorded in wildlife as well as Sprague-Dawley rats [23, 15], there is no study concerning the latent carrier status of rats. The lack of knowledge in this area of *Brucella* pathogenesis prompted us to evaluate the latent carrier stage in rats born from *B. abortus* infected mothers.

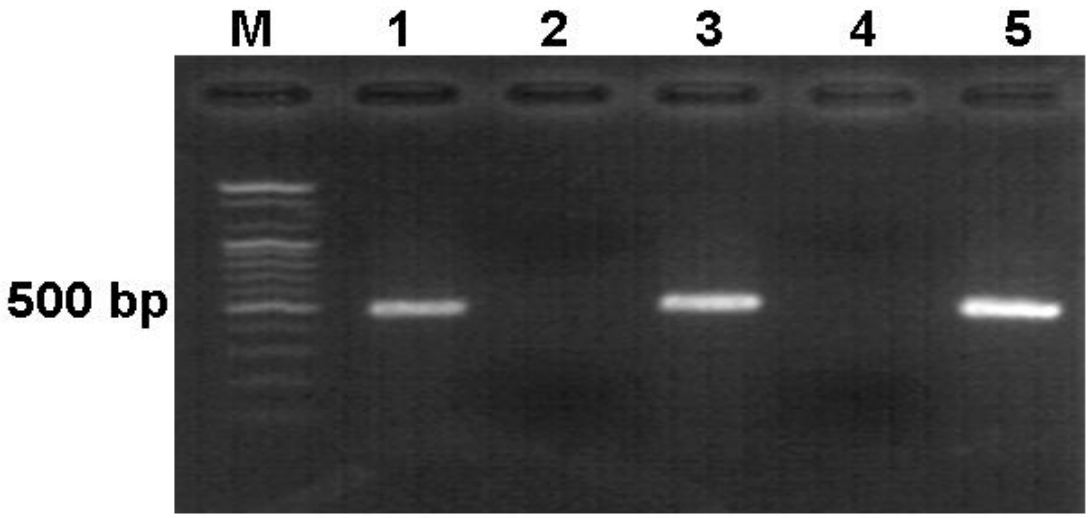
Serological testing is often used for the confirmation of brucellosis [24]. In our experiments, *Brucella* specific serum IgG and IgM were measured by three serological tests: RBPT, TAT and ELISA. In acute *Brucella* infection IgM, and in chronic infection IgG, antibodies are produced. The RBPT is a simple screening test used for confirmation of brucellosis [25]. Specific agglutinating antibodies (IgG and IgM) are detected by RBPT [26]. This test can give a false positive reaction with other organisms having antigenic similarities to *Brucella*. The TAT is the most frequently used confirmatory serological test that can detect the early stage of the disease, when IgM antibodies are elicited. [27]. Since this test detects infection early, there is little risk of missing infected animals. In our study ELISA was used to detect specific IgG antibodies against *Brucella* spp. since it is the most sensitive serological test for chronic infections [27]. False negative reactions are occasionally reported using the IgG ELISA, especially in the early stages of acute infection**.**

B. abortus was confirmed in 38% rats born from infected dams. Our findings are in agreement with those of Robertson [30], who isolated *B. abortus* from tissues of sero-negative calves born to infected

Table 2. Results of isolation and identification of *B. abortus* from different groups of rats

Rat groups (n)	No of culture positive samples (%)	No of culture negative samples (%)	No. of isolates confirmed as *B. abortus* by AMOS-PCR (%)
Rats born from infected mothers (48)	18 (37.50)	30 (62.50)	18 (37.50)
Infected parturient mother rats (6)	6 (100)	0 (0)	6 (100)
Male breeding rats (4)	0	4 (100%)	0(0)

Figure 1. AMOS-PCR profile for identification of *B. abortus* in rats born to infected mothers



Lane M: 100 bp size DNA marker (Bioneer, Daejon, South Korea); lane 1: Amplification of DNA from a culture positive spleen of rat born from infected mother; lane 2: No amplification of DNA extracted from a cultured negative spleen of rat born from infected mother; lane 3: Amplification of DNA extracted from a culture positive bacterial colony of rat born to infected mother; lane 4: Negative control with water; lane 5: Positive control with DNA extracted from *B. abortus* strain 1119-3.

dams. Catlin and Sheehan [28] also isolated *B. abortus* biotype 1 from a calf born to an infected dam. In our experiment, there was no sero-conversion in any of the culture-positive rats up to three months of age indicating they were latently infected. Similarly, in cattle, calves born from infected dams generally show no serological reaction [11].

Nicoletti [3] reported that calves may be infected by *B. abortus in utero* or infected via ingesting colostrum. In sheep, latent infection of *B. melitensis* is acquired through the ingestion of infected colostrum or milk [29]. In our experiment, the transmission of *B. abortus* from infected mother rats to offspring may have occurred during pregnancy or after birth due to ingestion of infected colostrum [15].

Prepubescent animals are innately resistant to *Brucella* infection [31]. Our study examined sero-conversion status of the rats born to infected mothers up to sexual maturity. *B. abortus* was isolated from all age group sero-negative rats born to infected dams. Robertson [30] detected *B. abortus* in sero-negative calves when they became pregnant.

The present study documents latent infection in rats, which might pose a significant threat for global eradication of brucellosis from humans and domesticated animals. This first report of latent infection of *Brucella* in rats will be helpful for understanding the epizootiology of *Brucellla* infections.

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