An Alternative Test Procedure for the Protective Efficacy of Brucella Vaccines

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Abstract

Administration of live Brucella vaccine is required to prevent the spread of ruminant brucellosis affected livestock herds. This study optimized the mouse model to test the protective efficacy of live Brucella vaccine. To optimize the protective efficacy test procedure, the absolute infective doses were determined in mice as 10 Colony Forming Units (CFU)/mouse for B. melitensis 16M and 50 CFU/mouse for B. abortus 2308, which were then used as the challenge doses for the respective strains. The optimal vaccination doses of vaccine Brucellaeusis S2 in mice were 10^{2.25} CFU and 10^{3.5} CFU/mouse, which could confer ≥ 80% protection in mice against challenge by B. melitensis 16M or B. abortus 2308, respectively. In addition, challenge with B. melitensis 16M or B. abortus 2308 should occur just at 3.63 weeks and 4.75 weeks post-inoculation, respectively. The protective efficacy test not only was more accurate and took less time but also was consistent with the evaluation index in host animals. Our study indicated that the mouse model could be used to test the protective efficacy of live Brucella vaccines during their production and development.

Keywords: Brucella vaccine; Mouse model; Protective efficacy; Test procedure

Introduction

Brucellosis is an important zoonotic disease with great socio-economic impact in many countries. Infected animals and the resulting contaminated animal products are the main source of human infection [1]. Although animal brucellosis may be controlled by means of adequate testing and slaughtering programs in areas with a high prevalence of the disease and low socioeconomic conditions, vaccination has become the only practical way to control the disease in ruminants [2]. The vaccines used extensively in epidemic areas include B. abortus vaccine S19 for cattle and B. melitensis vaccine Rev.1 and B. suis vaccine S2 for cattle, sheep, and goats [3]. However, low degree of protective immunity and serious adverse effects have been reported for both B. abortus vaccine S19 and B. melitensis vaccine Rev.1 vaccines in some vaccination trials. Such variability might be due, at least in part, to the heterogeneity of the vaccines used mainly because of unsuitable quality control during the manufacturing process [4]. So, it is very important to conduct quality tests of vaccine products during the manufacturing of brucellosis vaccines.

Until recently, testing procedures for brucellosis vaccines were performed according to the procedures in the Manual of Diagnostic Tests and Vaccines for Terrestrial Animals [3], in which mice were used in the protective efficacy test. Given that experiments in ruminants, humans, and primates have economical and ethical concerns, a standardized mouse model has been preferred in brucellosis research areas including pathogenesis, vaccine development, and therapies [5]. For immunogenicity tests, mice have been vaccinated subcutaneously with a dose of 10^5 CFU of tested vaccine and reference vaccine S19 and then were challenged with a dose of 2 \times 10^5 CFU of B. abortus2308 after 30 days [3]. With this procedure, the vaccine strains often survive long beyond the time of the challenge, which interferes with the isolation and bacterial count of the challenge strain. To solve this problem, duplicate plates are required to differentiate the challenge strains from the vaccine strains, but spontaneous mutation and interference between vaccine and challenge strains may lead to an underestimation of the protective efficacy of Brucella vaccines. Therefore, a more convenient and effective test is needed for Brucella vaccine products. The present study aimed to optimize the mouse model for protective efficacy test of Brucella vaccines with S2 as a representative by optimizing the immunizing dose, the challenge dose, and the time interval between inoculation and challenge.

Material and Methods

Ethics statement

All animal research was approved by the Beijing Association for Science and Technology. The approval ID is SYXK (Beijing) 2007-0001, and the animal research complied with the guidelines of Beijing laboratory animal welfare and ethics of Beijing Administration Committee of Laboratory Animals.

Bacterial strains and media

Virulent B. abortus 2308, B. melitensis 16M, and vaccine strain B. suis S2 were all kindly donated by Dr. Qianni He (Institute of Veterinary Research, Xinjiang Academy of Animal Sciences, Urumqi, China). These strains were originally collected and preserved in the Chinese Veterinary Culture Collection Center (Beijing, China). Freeze-dried rehydrated bacteria were routinely grown in Tryptic Soy Broth (TSB) or Tryptic Soy Agar (TSA) at 37. To prepare organisms for animal infection, the Brucella strains were harvested from the
surface of the plates after 3 days of incubation using Phosphate Buffered Solution (PBS), adjusted to the required concentration, and injected into mice as indicated for each experiment. Exact doses expressed in CFU were verified retrospectively by serial dilution, plating, and counting. All work with live Brucella virulent strains was performed in Biosafety Level 3 facilities at China Agricultural University.

Mice

Female BALB/c mice (6 to 8 weeks old) were purchased from Weitong Lihua Laboratory Animal Services Centre (Beijing, China), raised in individually ventilated cage rack systems, and subsequently transferred to the Biosafety Level 3 facilities at the beginning of the infection and challenge experiments. All experiments involving animals followed the regulations enacted by the Beijing Administration Office of Laboratory Animals.

Determining the absolute infective dose (ID100) of B. melitensis16M and B. abortus2308 in mice

To confirm the challenge doses for B. melitensis 16M and B. abortus2308 in mice, the ID100 of each of these two wild-type strains was determined. Six groups of four mice each were inoculated intraperitoneally with B. melitensis 16M at a dose of 1,000, 500, 100, 50, 10, and 5 CFU respectively, and another six groups were inoculated with B. abortus 2308 at the same doses gradient. Two weeks post-infection, all mice were euthanized via carbon dioxide asphyxiation. The protection units of a vaccine strain are typically determined 2 weeks after challenge [3] and bacterial survival curves indicate that the number of wild-type Brucella bacteria peak in mice 2 weeks post-inoculation [6]. Thus, to optimize the challenge doses of wild-type Brucella strains, the ID100 values of B. melitensis16M and B. abortus 2308 were determined 2 weeks post-infection. All of the mice were infected at 10 CFU for B. melitensis 16M (Figure 1A) and at 50 CFU for B. abortus2308 (Figure 1B). Thus, the ID100 values for B. melitensis 16M and B. abortus 2308 in mice were determined as 10 CFU and 50 CFU respectively.

Figure 1: Infection with the different doses of B. melitensis 16M (A) and B. abortus 2308 (B) in mice. Six groups of four mice each were inoculated intraperitoneally with B. melitensis 16M or B. abortus 2308 at a dose of 1,000, 500, 100, 50, 10, or 5 CFU. Two weeks post-infection, all mice were euthanized, and the bacteria recovered from the spleens were counted. Data are expressed as the number of CFU in each spleen.

Persistence time in mice for different doses of vaccine strain S2

To measure the persistence time in mice for different vaccine strain S2 doses, we inoculated BALB/c mice with four doses of vaccine S2 and determined the bacterial loads in the infected mice at different time points post-inoculation. The number of viable bacteria recovered from the spleens of mice in each group decreased continuously. The vaccine strain S2 had disappeared from the mice in the 100 CFU vaccination group by 3 weeks post-inoculation, whereas it persisted until 5 weeks post-inoculation in the 1,000 CFU and 10,000 CFU vaccination groups and until 6 weeks in the 100,000 CFU group.
These results indicated that the persistence time of vaccine strain S2 in mice was positively correlated with the inoculation dose (P<0.05).

Figure 2: Persistence of different vaccination doses of vaccine strain S2 in mice. Twenty-five mice were inoculated with each strain at a dose of 100, 1,000, 10,000, or 100,000 CFU/mouse. Five mice per group were euthanized at 2, 3, 4, 5, and 6 weeks post-inoculation, and the persistence time of vaccine strain S2 was determined based on the number of CFU recovered from each spleen, which is expressed as the mean ± SD.

Protective efficacy of vaccine strain S2 against a challenge by wild-type strains

Because the vaccine strain S2 was not detected in spleens 6 weeks after inoculation, all vaccinated mice were challenged at 6 weeks post-inoculation with B. melitensis 16M or B. abortus 2308 at the respective ID<sub>10</sub>. The challenge strains caused 100% infection in the control groups (Figure 3 and Table 1). The protection ratio was somewhat dose dependent in the vaccination groups. The protection ratio against challenge with either strain was 100% after inoculation with 100,000 CFU of vaccination strain S2. However, at the lowest dose (100 CFU/mouse), the protection ratios were 80% against B. melitensis 16M and 60% against B. abortus 2308.

Table 1: Protective efficacy of vaccine strain S2 with different vaccination doses.

<table>
<thead>
<tr>
<th>Challenge strain</th>
<th>S2 vaccination dose</th>
<th>No. of mice in test</th>
<th>No. of mice infected</th>
<th>Protection ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. melitensis 16M</td>
<td>100</td>
<td>5</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>5</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td>5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100,000</td>
<td>5</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>PBS</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B. abortus 2308</td>
<td>100</td>
<td>5</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>5</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td>5</td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>100,000</td>
<td>5</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

The optimal immunizing dose and optimal time interval between vaccination and challenge to determine the greatest protective efficacy test procedure of brucellosis vaccination that can occur over a short period of time, the immunizing dose and time interval between vaccination and challenge were optimized. As described above, we observed that the vaccine strain S2 survival time in mice was linear correlated with the vaccination dose, and the protective efficacy was also linear related to the vaccination dose in a range of doses. Thus, the optimal vaccination dose and the optimal time interval between vaccination and challenge was determined for vaccine strain S2 by linear curve fitting. When the vaccine strain S2 conferred at least 80% protection for the animals, the optimal vaccination dose against challenge with B. melitensis 16M or B. abortus 2308 was 10<sup>2.25</sup> CFU or 10<sup>3.5</sup> CFU per mouse, respectively (Figure 4A and 4B). The optimal time interval between vaccination and challenge was determined for vaccine strain S2 at the optimal vaccination dose of vaccine strain S2 (Figure 4C).

Discussion

Brucella infection in mice can persist throughout their lives and is accompanied by characteristic pathological signs [5]. Therefore, mice have been used to evaluate the in vivo character of Brucella vaccine and mutation strains [8-11]. For protective efficacy test of Brucella vaccine, different inoculation doses, time intervals between vaccination and challenge, and challenge doses have been used in in mice [12,13-15]. To unify and optimize these factors, we first
determined the ID_{100} values for *B. melitensis* 16M and *B. abortus* 2308, which were 10 CFU and 50 CFU in mice, respectively. While the ID_{100} values for *B. melitensis* 16M in our study was lower than that extrapolated by Teske et al. [16], which may be resulted from the differences in the mouse age, the different source and cultivation conditions of *Brucella* strains, and the bacteria-counting methods. We subsequently used the ID_{100} as the challenge doses. Although these challenge doses were also significantly lower than that (2 × 10^5 CFU/mouse) proposed by Office International des Epizooties and other studies [3,17] they successfully infected all mice.

![Image](45x444 to 283x610)

**Figure 4**: The optimal vaccination doses for vaccine strain S2 against challenge by *B. melitensis* 16M (A) and *B. abortus* 2308 (B); (C) The optimal time interval between vaccine and challenge for the optimal vaccination dose of vaccine strain S2 against *B. melitensis* 16M and *B. abortus* 2308 (*n* = 8 challenges).

The live attenuated smooth *B. suis* strain S2 was developed in China by *in vitro* serial passages of a field strain, which was isolated in 1953 from an aborted sow. The vaccine has been widely utilized against *B. melitensis* and *B. abortus* infection in cattle, goat, and sheep herds in China since 1958 [18,19]. The vaccine is administered through drinking water; a dose of 10^{10} bacteria gives 1–3 years of protection for cattle. In the middle of 1980s, *B. suis* S2 vaccine was introduced to the other countries, e.g. Spain, Turkey, Libya, Britain, France, Germany, and Zambia, and was recorded in “The Manual of Diagnostic Tests and Vaccines for Terrestrial Animals” [18]. In the present study, we selected vaccine S2 to optimize the vaccination dose and the corresponding time interval between vaccination and challenge. At lower vaccination doses, vaccine strain S2 died more quickly in mice. At 6 weeks post-inoculation, we observed that vaccine S2 was absent from all the mice of the different dose groups. In the protective efficacy test, we used the protection ratio as the evaluation index, which was similar to that used in the host animals [7,20], instead of comparing the relative protection units of the given vaccine strains to the reference vaccine or PBS control group. We observed that the protection ratio of vaccine strain S2 was 60% or higher even at the low vaccination dose. To optimize the results, when the protection ratio expected to be 80% the optimal vaccination dose of vaccine S2 was determined by linear curve fitting to be no more than 10^{3.5} CFU/mouse, which is much lower than 10^7 CFU, as proposed by others [3]. Corresponding to the optimal vaccination doses, the optimal time interval between vaccination and challenge was determined to be < 5 weeks, which was not required to consider the interference of the vaccine strains for the number of challenge strain. Therefore, determination of the optimal vaccination dose and survival time would be much practicable for testing the protective efficacy of *Brucella* vaccines in the course of production or development.

This study indicated that vaccination of *B. suis* vaccine S2 with 10^{2.5} CFU/mouse and 10^{3.5} CFU/mouse conferred 80% protection in mice against *B. melitensis* and *B. abortus* 2308, respectively. Furthermore, challenge with 10 CFU of *B. melitensis* 16M and 50 CFU of *B. abortus* 2308 should occur at 3.63 weeks and 4.75 weeks post-inoculation, respectively. These results from mice should be the essential data for the specific *Brucella* vaccine strain S2, which has the intrinsic relations with the data from host animals vaccinated by this vaccine. Therefore, we suggested that the mouse model was used for evaluating the protective immunity of live *Brucella* vaccine, which would provide a reliable and fast method for quality control of the vaccines in production.

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**References**


