Vertical Transmission of *Brucella abortus* Causes Sterility in Pregnant Mice

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ABSTRACT. The mechanisms of abortion and sterility induced by bacterial infection are largely unknown. In the present study, we found that *Brucella abortus*, a causative agent of brucellosis and facultative intracellular pathogen, caused sterility in pregnant mice. We have recently established a mouse model for abortion induced by *B. abortus* infection and high rates of abortion are observed for bacterial infection on day 4.5 of gestation, but not for other days. Infected newborn (first generation) mice showed poor growth compared with uninfected newborn mice and bacterial replication in the spleen of the former was observed over a long period. When infected first generation female mice were mated to infected first generation male mice, the number of fetuses was significantly less than that in uninfected first generation mice. These infected second generation mice also showed poor growth. These results suggest that vertical transmission of *B. abortus* causes sterility in pregnant mice and our mouse model would be useful for the investigating of brucellosis.

KEY WORDS: abortion, *Brucella*, infertility, mouse.

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Brucellosis is a major bacterial zoonosis in the world and an important cause of a serious debilitating disease in humans and abortion and sterility in domestic animals. *Brucella abortus*, a facultative, gram-negative, intracellular pathogen, is the etiologic agent of brucellosis [8]. The establishment of chronic infection depends on the ability of *Brucella* species to survive within phagocytes [3]. In contrast to other intracellular pathogens, *Brucella* species do not produce exotoxins, antiphagocytic capsules or thick cell walls, resistance forms or fimbriae and do not show antigenic variation [2]. Intracellular survival and replication are key virulence features of *Brucella* species, because mutants defective for these attributes are also avirulent [6]. A key aspect of the virulence of *Brucella* species successfully bypasses the bactericidal effects of phagocytes, and their virulence and chronic infections are thought to be due to their ability to avoid the killing mechanisms within host cells [1, 9]. While *B. abortus* induces abortion, sterility, vertical transmission, and poor growth of offspring in cows, rats, and mice [4, 7, 10], little is known regarding the effect of vertical transmission to the offspring of infected animals. We have recently established a mouse model for abortion induced by *B. abortus* infection and high rates of abortion were observed on day 4.5 of gestation, but not for other days [5]. In the present study, we investigated growth and reproductive ability of offspring born from *B. abortus* infected mouse using our mouse model.

To determine if newborn mice derived from *B. abortus*-infected mothers grow into normal adults, ten pregnant mice were divided into two groups, with 5 mice in each group. One group of mice was infected intraperitoneally with approximately 10^4 CFU of the *B. abortus* wild type in 0.1 ml saline on day 6.5 of gestation, since approximately 50% of fetuses were aborted in this condition [5]. After birth, mice were raised in breeding facilities under specified pathogen-free conditions, and their body weight was measured at 1, 3, 6 or 8 weeks after birth. The animal experiments were permitted by Animal Research Committee of Gyeongsang National University. Two of three pregnant mice were infected with *B. abortus*, and 5 female offspring (first generation) were selected in each group. The two groups of 5 female offspring had a lower body weight than the uninfected control until 56 days after birth (Fig. 1A), and similar results were obtained with a group of male offspring (data not shown). To clarify the cause of poor growth in the first generation derived from *B. abortus* infected female mice, newborn mice were reared by foster mothers. Briefly, pregnant mice were divided into infected and uninfected groups, with 5 mice in each group. The former was infected intraperitoneally with approximately 10^4 CFU of *B. abortus* wild-type in 0.1 ml saline on day 6.5 of gestation. After birth, mice born from infected mice were put together with uninfected mother mice. At the same time, mice born from uninfected mice were put together with infected mother mice. Mice were reared, and body weight was measured. Newborn mice derived from infected mothers showed poor growth even though they were reared by uninfected foster mothers, while those from uninfected mothers and reared by infected foster mothers showed normal growth (Fig. 1B). These results indicate that the poor growth of first generation mice born from infected mothers was due to infection via the placenta, but not attributed to other factors such as

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Fig. 1. Poor growth in offspring of infected mice. (A) Two of three pregnant mice were infected with 10^4 CFU of wild-type *B. abortus* on day 6.5 of gestation. Then, body weights of first generation mice were measured at the indicated time of birth. (B) Pregnant mice were divided into two groups. One group of mice was infected with 10^4 CFU of wild-type *B. abortus* on day 6.5 of gestation. The other group of mice was uninfected. Then, first generation mice were raised by uninfected or infected foster mothers. (C) Body weights of second generation mice. The first generation of mice, which were descendant of infected or uninfected group of mice, were mated each other in the same group respectively. (D) The number of fetuses in second generation. Statistically significant difference was indicated by asterisks (*, $P < 0.01$).

poor lactating ability of infected mothers or oral infection by the milk. The vertical transmission of the bacteria from mother to first generation offspring was directly confirmed that the numerous numbers of bacteria were recovered from the livers and spleens of these offspring (Fig. 2 and data of the livers were not shown).

To examine the influence of *B. abortus* infection on second generation, first generation of female and male mice born from *B. abortus* infected mice was mated with each other. Pregnant mice were infected intraperitoneally. At 3 weeks

after birth, mice were isolated from their mother and separated by sex. At 9 weeks after birth, mice born from infected or uninfected mice were individually mated with each other. The number of fetuses was counted on day 18.5 of gestation. Body weight of the second-generation mice was measured at 1, 3, 6 or 8 weeks after birth. The number of fetuses was significantly less than that in uninfected first generation mice (Fig. 1D), suggesting that transmitted *B. abortus* to first generation can cause sterility. Two groups of 5 infected second generation female mice had lower body weights than the uninfected

Fig. 2. The number of bacteria recovered from the spleens of descendant. Pregnant mice were infected with 10^4 CFU of wild-type *B. abortus* on day 6.5 of gestation. And both first and second generation mice were raised as described in the text. Each point shows the mean number of viable bacteria recovered from five mice. Error bars indicate standard deviations.

control until 8 weeks after birth (Fig. 1C), and similar results were obtained for a group of infected second generation male mice (data not shown). However, no replicating bacteria were detected in the spleen or liver of the infected second generation mice after birth (Fig. 2). These results indicate that even though *B. abortus* is not transmitted to second generation, the number of fetus and growth of second generation were significantly influenced.

Although *B. abortus* causes abortion and sterility in domestic animals, the mechanisms are largely unknown. It was reported that intraperitoneal injection of *B. abortus* to female rat induced sterility and poor growth of offspring [4]. In addition, we demonstrated in the present study that vertical transmission of *B. abortus* also causes sterility in mice born from *B. abortus* infected mother. However, the molecular mechanisms of sterility and poor growth of offspring were still unknown. In a bovine model, infection of *B. abortus* between 6–7 months pregnancy resulted in approximately 40% of abortion and 40% of healthy birth [10]. Interestingly, healthy calves born from infected cows showed more intense inflammation in the spleen than aborted calves, suggesting effective immune responses in healthy calves born from infected cows. Our recent study showed that abortion induced by *B. abortus* infection is regulated by IFN- γ during

the period of placental development [5]. Therefore, sterility and poor growth caused by *B. abortus* infection may be involved in immunological responses. To clarify the effect of vertical transmission of *B. abortus* in detail, further studies related to reproductive organ development, localization of the transmitted bacteria, and reproductive ability in second generation mice were needed. Our mouse model will enable to solve those issues and clarify the molecular mechanisms of sterility and poor growth caused by vertical transmission.

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