

Study of the Placental Pathology in Swiss Mouse Inoculated Intra Peritoneally with Different Dilution of *Brucella abortus*

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ABSTRACT

Sixty female adult mice with fifteen male adult mice were used as breeding stock in this experiment. Day 1 of gestation was assumed to correspond to the day the vaginal plug was observed. Live lyophilized *Brucella abortus* Strain 544 was used and the bacterial suspension was serially diluted with normal saline and 6 fold dilutions were made. All female mice were divided into six experimental groups and one control group. Each experimental group was composed of 8 female mice. The experimental groups were subjected to interaperitoneal inoculation of 0.1 cc of bacterial suspension of *B. abortus* and the control group with normal saline on the 9th day of their gestation. All mice were clinically normal and there was no abortion in all the inoculated animals. Total number of mice were euthanatized on the 18th day of their gestation with carbon dioxide. In *Brucella* infected mice, gross lesions of the placenta varied in size and color, those supporting viable fetuses were dark red and firm, those associated with an edematous or autolyzed fetus were pale and shrunken. Severities of placentitis caused by live lyophilized *B. abortus* strain 544 were very prominent. The pregnant mouse appears to be a convenient model for the study of the pathogenesis of ruminant placental infection with *B. abortus*.

KEY WORDS: Pathology, Placenta, *Brucella abortus*, Female swiss mouse.

INTRODUCTION

Brucella is a coccobacillus gram negative bacteria, they are aerobic and cannot produce spores. This zoonotic bacteria has intracellular life and there are six different discovered species which each of them have preferred host or hosts [1, 2]. Brucellosis or Bang's disease undulant fever is basically a domestic animal's disease which causes abortion in female cattle, ewe, goat and pig, and in the male animals it causes infection of genital organs. Brucellosis does not end abortion in mares, but it appears as serous membrane and joints disorders [1-3]. The disease is diagnosed by acute septicemia, and there is a possibility of long time chronic illness afterward. There is no abortion in human Brucellosis, but it causes pain in the head, muscles and joints, weakness, thinness, perspiration, fever, exhaustion and depression. The common way for infection in human is digestive organ, mostly by contaminated milk and other dairy products specially through throat mucus [2, 3]. But in animals, they get infected through the mucus of mouth, nose, larynx and genital organs [1].

In this investigation, the damages of placental tissue of mouse which were due to different dilution of bacterial infection and the pathogenesis of disease were studied. This study was conducted to evaluate the pathological effect of *Brucella abortus* strain 544 in mouse and finding the mechanism of disease.

MATERIALS AND METHODS

A total of 60 female Swiss mice between the age of 2-4 months old, and also male mice between the age of 4-8 months old were obtained from animal lab of Urmia university. In order to simultaneity in pregnancy, immature 25 days-old mice were separated from their mothers before their sexual maturation, and each males and females were kept in different cages. The phases of sexual cycles were determined after their 50 days of age, in order to this microscopical slides of their vaginal secretions were prepared, and the dispersion rate of integumental cells and the dominancy of cell types were studied. The mice who were in the pro-stress and di-stress of their sexual cycle were put in a cage with a male mouse for 24 h in order to coition. Existence of vaginal plug were determined by microscopical slides in the day after, and that has been considered as the first day of pregnancy (4). These steps were repeated for those who were not in the right sexual cycle.

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Lyophilized *B.abortus* strain 544 were obtained from Razi Institute, and were cultured in blood gelose medium and kept in 37°C, 1 atm and 5 % CO₂ for 48 h. After making serial dilution of bacterial colony, microbial suspension has prepared by Cruick-shank method. Then the bacterial count has been arranged by Miles and Misra method, which counted to 266 × 10⁶ CFU per ml of suspension. In this method, the bacteria is cultured by pouring 0.02ml drops of suspension by pipette from the height of 2.5cm above the medium; this spreads the suspension in an area about 1.5-2cm [1,2].

The female mice were divided in 6 treatment groups including 8 mice in each group, in addition one control group by 12 mice. In the day of 9th of pregnancy, *B.abortus* was inoculated by IP injection via insulin syringe. All mice were euthanized by CO₂ in the 18th day of pregnancy, considering ethical roles. Tissue slides were prepared from placenta and embryos and fixed in 10% Formalin, after opening the uterus. The 5 micrometer thick slides were stained by hematoxylin-eosin method.

RESULTS

All the mice seemed normal and there were no abortion. The placenta of all control group were dark red and firm and all fetus were alive. The placenta of infected mice had lesions and the lesions were vary in color and size. Those with viable fetus were dark red color and firm, and those carrying autolyzed or edematous fetus were pale and shrunk. The trophoblast giant cells were seen in different sides in histopathological study of placentas. These hyper chromatic cells had cytoplasmic granules which indicate presence of bacteria. Necrotic centers were seen in spongiotrophoblast area (Table 1).

Table 1: lesions from infection of *Brucellaabortus* in pragent mouse.

Treatment groups	plethoric	Edema	bleeding	Trophoblast& gall bladder necrosis	Trophoblast necrosis	Thrombosis in placenta vessels	Neutrophils penetration	Plethoric fetus	Edematous fetus	Autolysis fetus
-	-	-	-	-	-	-	2	1	8	First group (10 ⁻⁸)
-	-	-	-	-	-	-	6	4	6	second group (10 ⁻⁷)
-	2	6	-	-	2	1	5	5	5	3th group (10 ⁻⁶)
-	-	-	-	1	4	1	6	6	-	Fourth group (10 ⁻⁵)
-	-	-	3	-	4	2	-	-	-	5 th group (10 ⁻⁴)
2	-	-	5	-	-	5	-	-	5	6 th group (10 ⁻³)

DISCUSSION

In this study, female mouse were observed as a model for evaluation of the pathological effect of *B.abortus* infection in cows placenta. Despite the differences between placenta of these two species (Epitheliochorial and Hemochorial), a lot of similarities have been observed in placental infection pathogenesis of them. The study approved the fact that infection by *B.abortus* in the pregnancy period in mouse can lead to the over growth of the organism in the placenta of the animal and this can cause the necrosis of the placenta tissue, degeneration of vessel's fibrose, Microtjrombosis and penetration of neutrophils in placenta. There will be abortion by a little inflammation in placenta, and infection of *Brucella* in placenta of ruminants leads to the destruction and wide spread of placenta [3,4,5]. Disturbance in placenta functions occurs because of edema in blood vessels and necrosis in the Chorioallantoic which eventually cause the death of fetus in infected ruminants, this phenomenon is usually seen in goat and ewe [5]. There were autolysis in the two mice of which received the high dulation of bacteria (10³). *Brucella's* endotoxin cause the edema in vessels, thrombosis and necrosis so it can be concluded that interruption in the blood transferring process in the placental is the main cause of fetus death. The first attack side of the bacteria is near the placenta of mother and fetus which is reported previously as an infectious site for intracellular bacteria like *Coxillaburnetti*[6], *Listeria monocytogen*[7] and *Brucellaabortus*[8,9]. This place is a very good site for replacement and growth of intracellular bacteria because there are less maternal microphage and T cells for making a proper place for fetus trophoblast growth [7]. Trophoblast cells are present in most of phagocytic stage of pregnancy in mouse and they are the most sensitive cells to brucella infection. Trophoblasts are physiologically active cells and they synthesize many factors like progesterone which stimulates the growth of brucella in vitro system. The endocytosis ability of trophoblasts makes them the first place of bacteria insertion and duplication [4,10]. Trophoblasts become infected in the first stage of brucellosis in the ruminants. The observation showed that the fragility of pregnant animal to brucellosis is not due to general immune system suppression, it is because of topical immune response suppression in placenta. Neutrophils are the only defending cells in the area which is not able to control the infection and become high in the number and lysis of them leads to degeneration and necrosis of the placenta tissue. Neutrophil distribution is intense when the necrosis occurs in trphoblasts. Trophoblasts suppresses

the function of microphages and lymphocyte by releasing some substances like alphaphitoprotein and progesterone. Also, trophoblasts suppresses the function of natural killer cells.4

Erythritol is a alcoholic sugar which is present in the placenta of ungulate and it stimulates the growth and colonizing of brucella. Although erythritol is less in the rodents, *B.abortus* implants in it and duplicates. So it can be concluded that, there are a lot of factors helping this bacteria to growth in the host body [6,11].

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