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Comparative study of immunopathophysiological responses induced by *B. melitensis* and its lipopolysaccharide in mouse model infected via intranasal route of exposure

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ABSTRACT

In this study, we developed a mouse model and characterized the effects of intranasal inoculation of virulent *Brucella melitensis* strain 16M and its lipopolysaccharide (LPS). The effects of the exposure were compared with respective control groups. Both *Brucella melitensis*-infected and LPS-infected groups showed no significant clinical presentation with minor relevance in the mortality associated with the infection. In *Brucella melitensis*-infected group, significant histopathological changes in comparison to the LPS infected group with increase bacterial burden in the lungs, reproductive and reticuloendothelial organs were observed. However, both infected groups showed elevated levels of pro-inflammatory cytokine expression (IL-1β and IL6) and antibody production (IgM an IgG) as early as 3 days post-infection with predominance in LPS infected group. In contrast, low levels of sex related hormonal changes was recorded in both infected groups throughout the experimental period. This is the first detailed investigation comparing the infection progression and host responses in relation to the immunopathophysiological aspects in mouse model after intranasal inoculation with *B. melitensis* and its lipopolysaccharide. The study revealed a significant difference between infected and control groups with overlap in clinical, pathological, and immunological responses as well as sex related hormonal changes resulting from the infections.

1. Introduction

Brucella melitensis is a highly contagious pathogen that infects a wide range of mammalian species, causing severe economic losses due to abortions and infertility in animal industry worldwide [1,3,2]. The disease is manifested as fertility related issues causing abortion followed by retained placenta, weak offspring and metritis. Rams experience orchitis, epididymitis and polyarthritis [4]. In humans, it is manifested as a systemic infection with a very heterogeneous clinical spectrum involving fever and focal forms, with osteoarticular and genitourinary forms being the most common [5]. The disease is transmitted to humans from infected animal reservoirs [6]. The mechanisms of transmission, however, in both humans and animals are similar of nature and include, inhalation, ingestion and can enter the body through breaks in the skin [4].

The bacterium has the propensity to localize inside macrophages of the liver, spleen, bone marrow, uterus, heart and brain with protean clinical manifestations [7]. The disease is of economically important in farm animals and constitutes globally a major public health problem unless strict control measures are undertaken in the livestock industry [8]. Protective immunity seems to be mediated by both humoral and cellular immune responses [9]). Host protection against *Brucella* spp. depends on cell-mediated immunity involving mainly activated macrophages, dendritic cells and T-cells [33]. T-helper 1 (Th1) immune response is essential for the clearance of Brucella spp. infection and LPS is believed to induce typical T-dependent response that includes

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antibody and cytokine production [9]. *Brucella pp*. can survive within the cells of immune system subverting innate immunity and evading adaptive immune mechanisms [10]. Understanding of this peculiar characteristic is essential in order to reveal its unique pathophysiology. Different mechanisms, however, can be postulated as to the basis for the understanding of the biological behavior of *B. melitensis* and its LPS but a comprehensive knowledge is still lacking. Therefore, the present study was designed to investigate the clinical signs, histopathological changes, cytokine and antibody immune response as well as the sex related hormonal changes in mice experimentally infected with *B. melitensis* and its lipopolysaccharide via intranasal route of exposure.

2. Materials and methods

2.1. Ethics statement

All experiments were conducted according to protocols reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) with reference no. UPM/IACUC/AUP-R049/2015.

2.2. Animals

Eighty four clinically healthy mice of approximately 6–8 weeks of age were divided into three groups with equal gender distribution of 36 animals each in Group 1 (*B. melitensis*) and Group 2 (LPS). The animals in Group 3 (Control) consisted of 12 mice with equal gender distribution. Animals were kept in individual cages, fed on pellets and drinking water was given *ad libitum*. Access to veterinary care was available at all times and the animal wellbeing was assessed regularly.

2.3. Experimental design

The mice of Group 1 were exposed intranasally to 0.4 ml of the inoculum containing 10^9 cfu/ml of live *B. melitensis*. Mice of Group 2 were exposed intranasally to 0.4 ml of the inoculum containing 10^9 cfu/ml of lipopolysaccharide extracted from *B. melitensis*. Animals in Group 3 were exposed intranasally to 0.4 ml of sterile PBS.

All mice were observed at 12 h, 48 h, 72 h and then at 7 days interval for clinical signs of *B. melitensis* and its lipopolysaccharide for up to 24 days post-infection. The clinical signs included appetite, eye discharge and ruffled fur. Any animal that developed increased clinical signs with consistent of tremors was killed humanely to minimize the degree and duration of suffering. Animals were observed for 3 weeks post-initial exposure and the experiment was terminated at day-24 post-exposure (Fig. 1).

2.4. Synchronization

Induction and synchronization of oestrus and ovulation in mice were performed with slight modifications as previously described [11]. A total of 42 BALB/c females, divided into three groups were considered. These female mice were treated with two intraperitoneal doses of 0.5 μg of cloprostenol on Days −3 and 0 day.

2.5. Bacterial strain and media

A stock culture of *B. melitensis*, an epidemic strain that was previously isolated from an outbreak in Malaysia, was used to prepare the inoculums. All bacteria were routinely grown on *Brucella* agar which contained growth supplements; namely biotin, thiamin, and nicotinamide. The optimum growth temperature is 36°C-38°C whereby the pure colonies of *B. melitensis* could be visible after 4–5 days of incubation period. Thereafter, the bacteria were suspended and diluted in sterile phosphate buffered saline

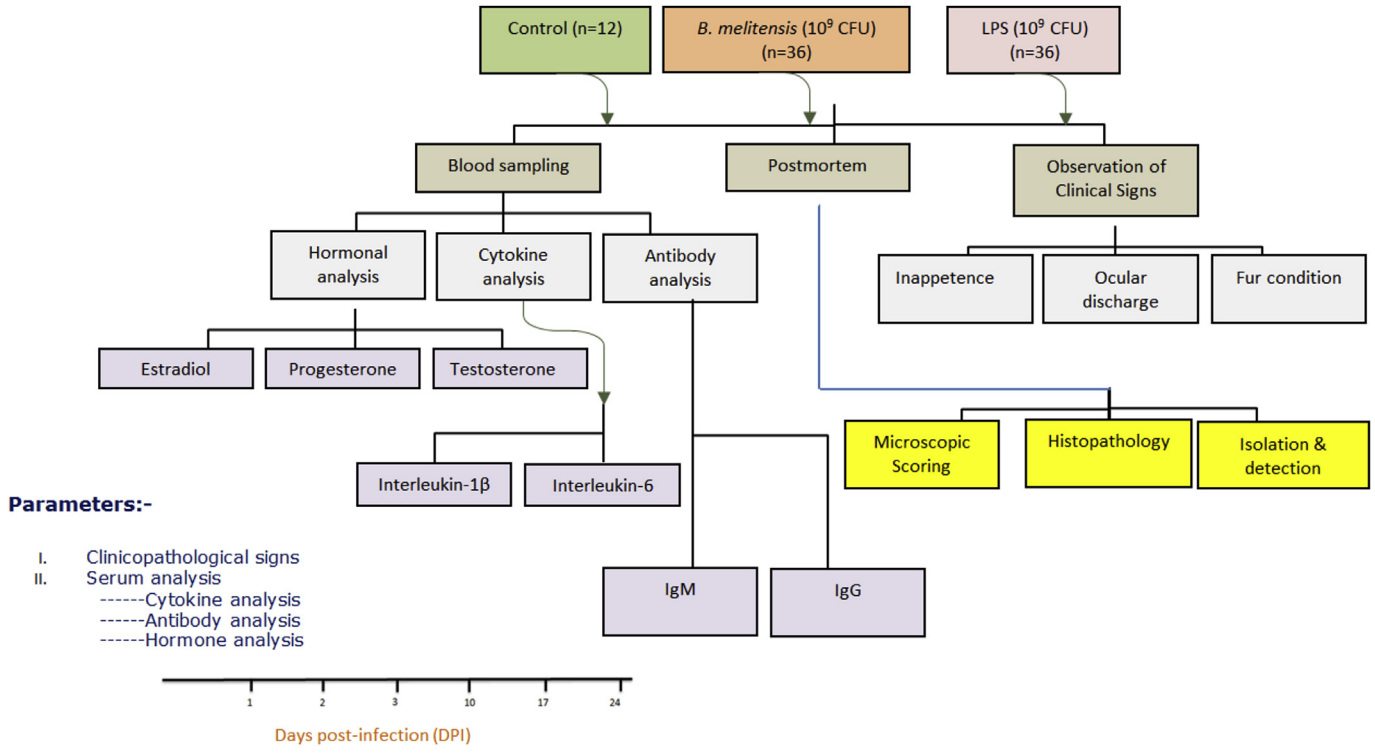

Fig. 1. Diagrammatic representation of the experimental design.