

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for degree of Doctor of Philosophy

**COMPARATIVE IMMUNOPATHOPHYSIOLOGICAL RESPONSES
INDUCED BY *Brucella melitensis* AND ITS LIPOPOLYSACCHARIDE IN
MOUSE MODEL INFECTED VIA DIFFERENT ROUTE OF EXPOSURE**

By

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July 2016

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Brucella melitensis, which causes a small ruminant brucellosis in sheep and goats and Malta fever in humans, is believed to enter the host via ingestion, inhalation or direct contact of the organism with broken skin or mucous membranes. Among the consequences of the different routes of infection are septicaemia, increased permeability of blood vessels and presence of the organism in several organs. However, the oral and the respiratory tract may not be the only portal of entry and route of spread of *B. melitensis*. Circumstantial evidence had suggested the involvement of gastrointestinal, respiratory and reproductive tract in the pathogenesis of *B. melitensis* and its lipopolysaccharide in ruminants. Nevertheless, the pathogenesis and the immunopathophysiology of the disease following different route of infection have not been well documented since previous reports on the disease were limited to incidental observations.

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The response of gastrointestinal, respiratory, and reproductive tract following oral, intranasal, subcutaneous and intraperitoneal exposure to *B. melitensis* was studied and compared its severity with lipopolysaccharide (LPS) exposure. The cytokine, antibody pattern and sex related hormonal responses following the different route of inoculations to *B. melitensis* and its lipopolysaccharide in mice were also investigated.

The clinical signs observed in these studies include; inappetence, ocular discharge, and ruffled following the different route of exposure to *B. melitensis* and its lipopolysaccharide. Although the severity of the clinical sign varied over time, type of inoculum and route of inoculation, however, mean clinical score were significantly higher in oral and intraperitoneal exposed groups to *B. melitensis* followed by intranasal and subcutaneous groups, respectively. Clinical observations for intranasal and subcutaneous groups were limited mostly to mild and moderate involvement. In contrast to *B. melitensis* infected group, animals challenged with LPS showed mild clinical signs which seemed to be limited in the first 48 h post-infection. Thereafter, normalization was observed in this group as they were not significantly different from those served as a control group. No significant differences were detected among the different sub groups of LPS infected indicating that the clinical presentation did not differ by route of exposure. Animals in control group did not develop any clinical signs throughout the experimental period.

The pathological alterations varied depending on the route of infection, days post-infection and the type of the organs recorded. Spleen, liver, kidney, lung and the reproductive organs that include uterus, ovary, testes, epididymis and seminal vesicle were the most commonly and severely affected organs with predominance in oral and intraperitoneally infected animals of *B. melitensis* group. These organs presented marked infiltration of inflammatory cells, degeneration, necrosis, haemorrhage and oedema. In intranasal and oral group of *B. melitensis*, lungs were the most affected organ than the other route of infection, with an abundance of fibrin admixed with cellular debris. Emphysema, oedema and marked infiltration of inflammatory cells were also recorded in lungs from 24 hours post-infection until the end point of the experiment. In contrast, histopathological changes of the various organs infected with LPS were almost similar presenting mild degrees of lesion involvement in all routes of infection with special reference in lungs and reticuloendothelial organs. Thus, indicating that LPS have preventive properties toward establishment of pathological lesions. Following the different routes of exposure, *B. melitensis* was isolated from the vital and reproductive organs along with intestinal segments of the mice that developed severe lesions scoring. Higher isolation and detection by PCR was noted predominantly in both reproductive tract and reticuloendothelial-rich organs of oral and intraperitoneal expose groups followed by intranasal and subcutaneous groups to *B. melitensis*, respectively.

Concurrently the cytokine and antibody immune response of mice following different routes of inoculation to *B. melitensis* and its lipopolysaccharide was also evaluated. Both *B. melitensis* and LPS elicited sustained and significantly higher serum IL-1 β and IL-6 that has of minor relevance to the route of infection. However, the highest responses were noted in LPS group than *B. melitensis* infected group within the respective route of inoculation. Similarly, the LPS elicited sustained and significantly higher IgM and IgG levels than *B. melitensis* in all different routes of infection. Among the routes of infection, the subcutaneous group yielded highest titers of antibody response followed by intranasal and intraperitoneal groups, respectively. With the presence of severe histopathological evidence along with higher isolation of *B. melitensis* infected group in the reproductive tract, the experiment was conducted to evaluate the serum hormonal changes following different route of exposure to *B. melitensis* and its lipopolysaccharide. Both *B. melitensis* and LPS resulted in significant

decrease in the circulating concentrations of serum progesterone, estradiol, and testosterone levels that has significant ($p < 0.05$) difference when the effect is compared to those served as a control group.

This study showed that *B. melitensis* organisms were present in various segments and tissues of the gastrointestinal, respiratory, and reproductive tract following the different route of exposure. Therefore, it can be concluded that *B. melitensis* infection can be transmitted via the gastrointestinal, respiratory and reproductive tract. Oral, intranasal and subcutaneous routes of administration of LPS elicited high serum cytokine and antibody immune response than *B. melitensis* infected group, although the responses of cytokines were variable. Thus, oral, intranasal and subcutaneous infections with 10^9 of live *B. melitensis* and its lipopolysaccharide were safer than the intraperitoneal route of inoculation. Both of these routes, in particular subcutaneous route, can be considered as potential alternative route for vaccine administration against *B. melitensis* infection in small ruminants. Similarly, it was concluded that the LPS stimulated significantly the innate and acquired immune system without significant systemic dysfunction, suggesting potentiality of the protective properties of this component as alternative vaccine for brucellosis infection.