



UNIVERSITI PUTRA MALAYSIA

***ULTRASTRUCTURE EVALUATION OF PASTEURELLA
MULTOCIDA B:2 IN RESPIRATORY ORGANS OF AEROSOLLY-
INFECTED MICE***

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FACULTY OF VETERINARY MEDICINE

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SERDANG SELANGOR

**ULTRASTRUCTURE EVALUATION OF *PASTEURELLA MULTOCIDA* B:2 IN
RESPIRATORY ORGANS OF AEROSOLLY-INFECTED MICE**

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A project paper submitted to the
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CERTIFICATION

It is hereby certified that we have read this project paper entitled “Ultrastructure Evaluation of *Pasteurella Multocida* B:2 in Respiratory Organs of Aerosol-Infected Mice”, by Razila Binti Raazali and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement of the course VPD 4901 – Project.

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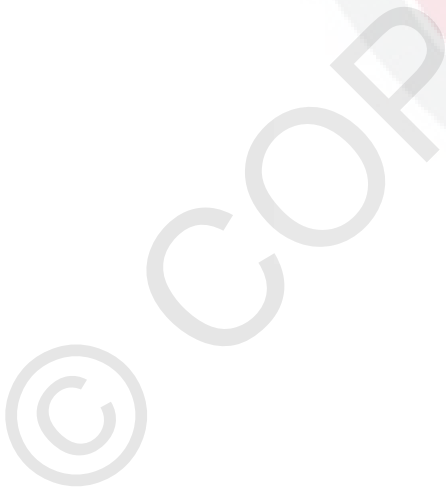
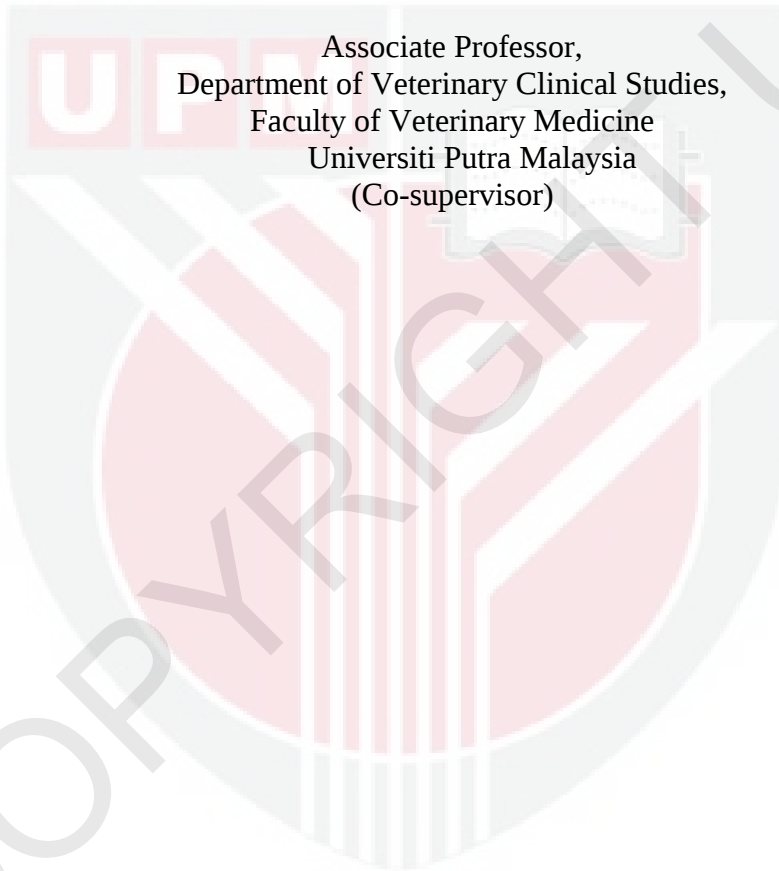
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DEDICATION

Alhamdullilah to Allah SWT.

I dedicate this thesis to my beloved parents and family,

Lecturers,

Loved ones,

Friends,

Thank you for your endless love and support

and everyone who reads and benefits from this thesis.

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Alhamdulillah, greatest thanks to Allah, with all His blessing, I able to complete my final year project successfully. Special thanks to my dedicated supervisor Dr. Annas Salleh for his unwavering guidance, patience and knowledge he poured to me throughout this project. For my co- supervisor Prof. Dato' Dr. Tengku Azmi and Associate Prof. Dr. Firdaus Jesse, million thank you for all your guidance, times and support to complete this project.

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ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine, UPM in partial requirement to fulfil the course of VPD 4999- Final Year Project.

ULTRASTRUCTURE EVALUATION OF *PASTEURELLA MULTOCIDA* B:2 IN RESPIRATORY ORGANS OF AEROSOLLY-INFECTED MICE

By

Razila Raazali

2017

Supervisor: Dr Annas Salleh

Haemorrhagic septicaemia (HS) is an acute, fatal, septicaemic disease of buffaloes and cattle caused by *Pasteurella multocida* B:2. Natural cases of HS occur primarily via the aerogenous route of susceptible animals. This study was conducted to determine and evaluate ultrastructure of respiratory organ with respect to aerosol inoculation of *P. multocida* in mice which mimics natural HS transmission. A group of 30 healthy female mice were divided into two groups, control (n=5) and treatment. The treatment groups were further divided equally into 5 groups (n=5). All treatment group were inoculated aerosol with 10 ml of 1×10^9 cfu/mL of *P. multocida* for 20 minutes. The control group were euthanised at 6 h prior to inoculation, while the treatment groups were euthanised by cervical dislocation at 0, 6, 12, 18, 24 h pi. Clinical signs were observed for 24 h pi. Lungs tissue were collected for ultrastructural examination. Clinical signs of reduced activity were observed starting from 12 to 24 h pi. The ultrastructure evaluation for the treatment groups showed mild to severe degree of pathological changes in the pneumocyte and

endothelial cell. Overall, inoculation of *P. multocida* Type B:2 via aerogenous route revealed significant ($p < 0.05$) cellular changes on the lungs.

Keywords: Haemorrhagic septicaemia, *Pasteurella multocida* serotypes B:2, electron microscope examination, aerosol, mice



ABSTRAK

Abstrak daripada kertas project yang dikemukakan kepada Fakulti Perubatan Veterinar UPM untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek Tahun Akhir.

PENILAIAN ULTRASTUKTUR PASTUERELLA MULTOCIDA JENIS B:2 DALAM ORGAN RESPIRASI BERIKUTAN JANGKITAN MELALUI AEROSOL MENCIT

Oleh

Razila Raazali

2017

Penyelia: Dr Annas Salleh

Penyakit hawar berdarah disebabkan oleh *Pasteurella multocida* merupakan penyakit septisemia akut dalam kerbau dan lembu yang berlaku sepanjang tahun di Malaysia. Kajian ini dilakukan untuk menentukan dan menilai ultrastuktur organ respirasi menggunakan jangkitan melalui aerosol dalam mencit dimana menyerupai natural transmisi penyakit hawar berdarah. Sekumpulan 30 mencit betina yang sihat dibahagikan kepada dua kumpulan, kawalan (n=5) dan rawatan (Kumpulan 1, 2, 3, 4, 5). Kesemua mencit kumpulan rawatan diinokulasi melalui aerosol dengan 10 ml *P. multocida* B:2 dengan konsentrasi 3.7×10^9 jajaan membentuk unit (CFU)/mL selama 20 minit. Kemudian, mencit tersebut dibahagikan kepada lima kumpulan. Mencit dari kumpulan kawalan dan rawatan dieuthanisia pada -6, 0, 6, 12, 18, 24 jam. Tanda-tanda klinikal diperhatikan sepanjang 24 jam selepas jangkitan. Paru-paru dikumpulkan untuk pengasingan-pengesahan bacteria dan pemeriksaan elekton mikroskop. Mencit dari Kumpulan 1 dan 2 tidak menunjukkan sebarang perubahan dalam tingkah laku. Walau bagaimanapun, mencit dalam Kumpulan 3, 4 dan 5 mendedahkan pengurangan dalam tingkah laku. Evaluasi ultrastruktur untuk

kumpulan rawatan menunjukkan sedikit kepada teruk darjah lesi di dalam pneumosit dan sel endothelial. Keseluruhanya, inokulasi *P. multocida* jenis B:2 melalui laluan aerogenus mendedahkan perrubahan ketara sel paru paru.

Katakunci: hawar berdarah, *Pasteurella multocida* jenis B:2, mencit, electron mikroskop



INTRODUCTION

Pasteurella is a genus from Pasteurellaceae family. It is a Gram-negative, small, pleomorphic, non-flagellated coccobacillus. *P. multocida* can be divided into serogroups and serotypes. The serogroups (A, B, D, E and F) are divided based on their antigenicity of the capsule. Meanwhile, the serotypes (1-16) were divided based on the lipopolysaccharide (LPS) antigens which also known to release endotoxin (De Alwis, 1992; Wilkie *et al.*, 2012). *P. multocida* is a causative agent that associated with wide range of veterinary diseases in domestic animals, feral animals and also agricultural species (De Alwis, 1999). This organism can become primary pathogen or can also play a role as secondary pathogen of a disease.

HS is an acute, highly fatal disease that can impose a huge economic loss in livestock industry that is caused by *P. multocida* Type B:2 (Dziva *et al.*, 2008). This organism can be transmitted via inhalation and ingestion of contaminated water or feed with *P. multocida*. In most cases, rapid spread of HS is due to close-contact of infected animals with affected animals within or between the herds and also inhalation of causative agent which originate in nasopharynx of carrier animal (OIE, 2013). Thus, pathological signs mainly in respiratory tract are observed in this disease. According to the Tabatabaei *et al.* (2007), infected animals may show short clinical signs such as depression, high temperature, dyspnoea and mainly respiratory signs and followed by recumbent then lead to death.

In an acute disease, early disease detection is crucial for the treatment to be effective and antibacterial therapy is usually effective at this stage (Benkirine & De Alwis, 2002). However, the most important measures in order to protect animals

against HS is by practicing routine vaccination besides increase hygiene and reduced epizootical factors (Shahet *al.*, 1997). According to the study done by Zamri *et al.* (2007), the animals were succumbed to this organism and produced peracute and acute infection with respect to inoculation of *P. multocida* via subcutaneous (S.Q) and intratracheal (I.T) route. Nevertheless, the study had fail to prove that intranasal (I.N) inoculation can recreate the clinical response of HS in the experimental goat. Therefore, this study was undertaken to evaluate the effect in the ultrastructure of mice lungs exposed with *P. multocida* B:2 via aerogenous route.

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