UNIVERSITI PUTRA MALAYSIA

ESTABLISHMENT OF HAEMORRHAGIC SEPTICAEMIA IN MICE INOCULATED WITH WATER CONTAMINATED WITH PASTEURELLA MULTOCIDA TYPE B:2

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ESTABLISHMENT OF HAEMORRHAGIC SEPTICAEMIA IN MICE USING WATER CONTAMINATED WITH *PASTEURELLA MULTOCIDA TYPE B: 2*

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March, 2014

Chairman: Faez Firdaus Jesse Abdullah, Ph.D.
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Haemorrhagic septicaemia (HS) is an infectious disease of cattle and buffalo inflicted by serotypes B: 2 and E: 2 of *Pasteurella multocida* in Asian and African countries respectively and characterised by an acute, highly fatal septicaemia with high morbidity and mortality. Therefore, the present study aims at evaluating the presence of *P. multocida* type B: 2 in various organs using Polymerase chain reaction (PCR), and host cell response in mice infected with *Pasteurella multocida* type B: 2 in contaminated river water with mice carcasses kept for 24, 48 and 72 hours. This study postulated that the outbreak of HS among buffaloes and cattle could be due to the consumption of river water contaminated with infected HS carcasses and the aerosol routes could perhaps be a readily available route for effective vaccine administration and heightened immunity in animals considering the progressive responses of APPs through this route. Sixty five healthy BALC male mice of eight to ten weeks old were used in this study. The wild-type *P. multocida* B: 2 used in this study were obtained from stock culture. The river water was obtained from Hulu Langat and was cultured to confirm that it was free from *P. multocida* type B: 2, Fifteen mice were initially inoculated with 1.0 mL of *P. multocida* type B: 2 intraperitoneally. Five infected mice carcasses were placed in each tank for 24, 48 and 72 hours and1ml of the contaminated river water containing *Pasteurella multocida* type B: 2 were inoculated via the intraperitoneally and aerosol routes while, 0.4 ml of *Pasteurella multocida* type B: 2 was inoculated orally into five mice in each group and after 48 hours the mice were euthanized by cervical dislocation. Blood samples were collected directly from the heart into plain tubes from the moribund animals to obtain serum for the analysis of serum amyloid A (SAA) and haptoglobin. The fourth group is the control group comprised of five mice and was inoculated with 1.0 mL of sterile Phosphate Buffered Saline (PBS) pH7. Post mortem was conducted and the brain, kidney, heart, spleen, lungs and liver were sampled for histopathology. All the organs were cultured on the blood agar and incubated at 37°C for 24 hours. PCR was performed on the organs from the mice. The concentration of SAA increased significantly ($p<0.05$) in the mice that were infected with the contaminated river water for 72 hours followed intraperitoneal group compared to the control, oral and aerosol group. There were also significant increase ($p<0.05$) in the concentrations of Hp in the group of mice that were infected with contaminated river water for 24 hours intraperitoneally relative to the control, oral and the aerosol groups. The PCR results revealed the presence of *P.*

multocida from the brain, kidney, heart, spleen, lung and liver in the group of mice from the intraperitoneal, oral and aerosol groups. The river water kept for 24 hours was positive for *P. multocida* in the intraperitoneal, oral and the aerosol groups. The river water kept for 48 and 72 hours were positive for *P. multocida* in the intraperitoneal and oral groups. The cellular changes in the vital organs include thrombosis, inflammatory cells, hemorrhage, degeneration and necrosis. In the brain, heart, kidney, liver, lung and spleen, the degeneration and necrosis was significantly high ($p < 0.05$) compared to the other cellular changes in the mice carcasses infected with *P. multocida* in contaminated river water kept for 72 hours. In conclusion, mice model could be used to enhance the understanding of the progression of the disease and control of the natural disease through the various routes of the disease transmission and contaminated river water infected with HS carcasses could be a potential source of infection if the carcasses are not removed from the river water immediately.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains Veterinar

PENGHASILAN HAWAR BERDARAH DI DALAM MENCIT DENGAN MENGGUNAKAN AIR TERCEMAR DENGAN *Pasteurella multocida* JENIS B: 2

Oleh

MOHAMMED MUQDAD KHALEEL

Mac, 2014

Pengerusi: Faez Firdaus Jesse Abdullah, Ph.D.
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Hawar berdarah merupakan penyakit berjangkit bagi lembu dan kerbau disebabkan oleh bakteria *Pasteurella multocida* jenis serotaip B:2 dan E:2 dinegara Asia dan Afrika masing-masing dan penyakit ini mempunyai ciri-ciri akut, septisemia berbahaya, dengan morbiditi dan kematian yang tinggi. Oleh itu, kajian ini bertujuan untuk melihat kehadiran baketria *P. multocida* B: 2 dalam pelbagai organ dengan menggunakan aplikasi reaksi rantai polymerase (PCR) , dan tindak balas sel tuan rumah dalam mencit yang dijangkiti dengan bakteria *Pasteurella multocida* B: 2 dalam air sungai yang tercemar dengan bangkai mencit disimpan selama 24 , 48 dan 72 jam. Kajian ini merumuskan bahawa wabak hawar berdarah dikalangan kerbau dan lembu mungkin disebabkan oleh penggunaan air sungai tercemar dengan jangkitan bangkai hawar berdarah dan laluan aerosol boleh menjadi salah satu laluan sedia ada untuk pentadbiran vaksin yang berkesan dan imuniti yang memuncak pada haiwan. Enam puluh lima ekor mencit jantan BALC yang sihat berumur antara lapan hingga sepuluh minggu digunakan dalam kajian ini. Bakteria jenis liar *P. multocida* B: 2 yang digunakan dalam kajian ini diperolehi daripada stok kultur. Air sungai yang digunakan dalam kajian ini diperolehi dari Hulu Langat dan dikultur untuk mengesahkan bahawa ia adalah bebas daripada bakteria *P. multocida* jenis B: 2, Lima belas ekor mencit pada mulanya disuntik dengan 1.0 mL *P. multocida* Jenis B: 2 melalui intraperitoneum. Lima bangkai mencit yang dijangkiti dengan telah hawar berdarah diletakkan di dalam setiap tangki air sungai selama 24, 48 dan 72 jam and1ml air sungai yang dicemari dengan *Pasteurella multocida* jenis B: 2 telah disuntik melalui intraperitoneum dan laluan aerosol manakala , 0.4 ml air sungai tercemar telah disuntik secara lisan kepada lima ekor mencit dalam setiap kumpulan, dan selepas 48 jam kesemua mencit telah ditakai dengan cara terkehel leher. Sampel darah yang diambil secara langsung daripada jantang ke dalam tiub kosong daripada mencit yang menunjukkan tanda hampir menemui ajal bagi mendapatkan serum untuk analisis serum amiloid A (SAA) dan haptoglobin. Kumpulan keempat adalah kumpulan kawalan terdiri daripada lima ekor mencit yang telah disuntik dengan 1.0 mL steril larutan penampan fosfat (PBS) pH7. Bedah siasat telah dijalankan dan organ seperti otak , buah pinggang, jantung , limpa , paru-paru dan hati telah disampel untuk tujuan kajian histopatologi . Semua organ-organ telah dikultur pada agar darah dan dieram pada 37°C selama 24 jam. PCR telah dijalankan ke atas organ-organ tersebut . Kepekatan SAA meningkat dengan ketara ($p < 0.05$) pada mencit yang dijangkiti dengan air sungai yang tercemar selama 72 jam diikuti kumpulan ‘intraperitoneal’ berbanding dengan kawalan, kumpulan lisan dan ‘aerosol’. Terdapat juga peningkatan yang signifikan ($p < 0.05$) dalam kepekatan Hp

dalam kumpulan mencit yang dijangkiti dengan air sungai yang tercemar selama 24 jam ‘intraperitoneally’ berbanding dengan kawalan, lisan dan kumpulan aerosol. Keputusan PCR mendedahkan kehadiran *P. multocida* pada organ seperti , buah pinggang, jantung , limpa , paru-paru dan hati pada mencit dari kumpulan intraperitoneal, lisan dan aerosol. Air sungai yang disimpan selama 24 jam adalah positif bagi *P. multocida* bagi kumpulan ‘intraperitoneal’, lisan dan aerosol. Air sungai disimpan selama 48 dan 72 jam adalah positif bagi *P. multocida* bagi kumpulan ‘intraperitoneal’ dan lisan. Perubahan struktur sel dalam organ-organ adalah seperti trombosis, sel-sel radang, pendarahan , degenerasi dan nekrosis . Bagi organ otak, jantung , buah pinggang , hati, paru-paru dan limpa, kemerosotan dan nekrosis adalah ketara tinggi ($p < 0.05$) berbanding dengan perubahan sel lain dalam kumpulan yang bangkai mencit dijangkiti *P. multocida* dalam air sungai yang tercemar disimpan selama 72 jam. Kesimpulannya, model mencit boleh digunakan untuk meningkatkan pemahaman perkembangan penyakit dan kawalan penyakit semula jadi melalui pelbagai laluan jangkitan penyakit dan air sungai yang tercemar dijangkiti bangkai hawar berdarah boleh menjadi salah satu sumber yang berpotensi jangkitan wabak penyakit hawar berdarah jika bangkai tersebut tidak dikeluarkan dari air sungai serta-merta.

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I certify that a Thesis Examination Committee has met on 15th March, 2014 to conduct the final examination of Mohammed Muqdad Khaleel on his thesis entitled "establishment of haemorrhagic septicaemia in mice inoculated with water contaminated with *Pasteurella multocida* type B:2" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Veterinary Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Veterinary Science.

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LIST OF ABBREVIATIONS

APP	Acute phase proteins
APR	Acute phase reaction
AGID	Agar gel immunodiffusion
BHI	Brain Heart Infusion
DNA	Deoxyribonucleic acid
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization
Hp	Haptoglobin
HS	Haemorrhagic septicaemia
ICR	Institute of Cancer Research
IU	International unit
IHA	Indirect haemagglutination
$MgCl_2$	Magnesium chloride
NC	necrotic cardiocytes
OIE	Office of International Epizootic
PBS	Phosphate Buffered Saline
PCR	Polymerase chain reaction
SAA	serum amyloid A
TSI	Triple Sugar Iron
VRI	Veterinary Research Institute
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

The production of ruminants in Malaysia is steadily changing from subsistence to intensive operations (Jesse et al., 2013a). The ruminants' population is predominantly made up of cattle, buffaloes, goats and sheep. The ruminant industry is the foremost in the production of food such as milk, meat and the by- products such as hide for the leather industry in Malaysia. The ruminant sector is vulnerable to the deadly and infectious disease outbreaks, which causes enormous losses to farmers and the country. The acute deadly disease is known as haemorrhagic septicaemia (HS) caused by a Gram negative bacterium called *Pasteurella multocida* type B: 2.

The pathogen is significant in animals for a protracted period and emerging as human pathogen (Jesse et al., 2013a) leading to a disease process termed Pasteurellosis. Notwithstanding, Pasteurellae have been indicated as the most dominant microflora of the upper respiratory tract in healthy animals (Abubakar et al., 2012; Jesse et al., 2013b). The organisms usually act as secondary invaders in animals with concomitant diseases or suffering from incapacitating stressful conditions (Abubakar and Zamri, 2011; Jesse et al., 2013c) and the infection frequently occurs via inhalation or ingestion of infected material (Shafarin et al., 2009).

In one study, research was conducted to investigate on the survival rate of *Pasteurella multocida* in water using different temperature where 18°C provides better survival rate then 2°C (Bredy and Botzler, 1989). Depending on the mode of storage and temperature where *Pasteurella multocida* was not isolated in river water and artificial sea water from the first to the 14th day of inoculation (Thomson et al., 1992).

Animals experienced exogenous or endogenous challenge mount a strong response by activating the innate immune systems at the initial stage of acute infection before the onset of the acquired immune system (Eckersall, 2000). The acquired immune system ultimately leads to the development of specific cellular and humoral immune responses. During the preliminary challenge the continued existence of the host depends on the capability of the innate reactions to tussle the causes of disease (Eckersall, 2000).

The acute phase proteins (APPs) are proteins found in the blood and the circulating concentrations of APPs are correlated to the severity of the infection and hence the concentrations of APPs offers a ready means of assessing the presence and extent of the disease progression (Kent, 1992; Eckersall, 2000; Murata et al., 2004; Petersen et al., 2004; Ceron et al., 2005; Baumann and Gauldie, 1994; Tothova, et al., 2013).

However, there are substantial species dissimilarities in the serum concentrations of acute phase proteins (Eckersall and Bell, 2010). Six fold increases in Hp concentrations was observed in infected dairy cows and those with metabolic disease compared to animals with minor lesions (Hirvonen et al., 1999). Seven and forty fold increases in SAA and Hp were also observed in culled dairy cattle with acute lesions relative to healthy beef animals (Tourlomoussis et al., 2004). Isolation of *Pasteurella multocida* from calves with respiratory disease was associated with significant increased concentrations of acute phase proteins (Nikunen et al., 2007; Hodgson et al., 2005).

Jesse et al. (2013c) successfully *isolated Pasteurella multocida* type B: 2 in mice following oral inoculation using polymerase chain reaction. The infection of mice with contaminated river water with *P. multocida* type B: 2 and subsequent alterations of SAA and Hp in a mice model has not been elaborated in previous studies. There is still inadequate information about the pathogenicity and epidemiology of HS through the intraperitoneal, oral and aerosol routes with contaminated river water with *P. multocida* type B:2 mice carcasses kept for 24, 48 and 72 hours. This study is a leap in the knowledge of HS transmission in a mice model. This study also postulated that the outbreak of HS among buffaloes and cattle could be due to the consumption of river water contaminated with infected HS carcasses and the aerosol routes could perhaps be a readily available route for effective vaccine administration and heightened immunity in animals considering the progressive responses of APPs through this route. Therefore, the present study aims at evaluating the presence of P. multocida type B: 2 in various organs using Polymerase chain reaction (PCR), and host cell response in mice infected with Pasteurella multocida type B: 2 in contaminated river water with mice carcasses kept for 24, 48 and 72 hours.

Therefore, the objectives of present study are as follows:

1. To detect the presence of *P. multocida* type B: 2 in various organs using Polymerase chain reaction (PCR) in mice through different routes of inoculation with river water contaminated with the bacteria.

2. To determine acute phase protein response associated with *Pasteurella multocida* type B: 2 infections in mice through different routes with river water contaminated with *Pasteurella multocida* type B: 2.

3. To determine the severity of cellular changes in various organs associated with *Pasteurella multocida* type B: 2 infections in mice through different routes with contaminated river water with *Pasteurella multocida* type B: 2.

Therefore the hypotheses of study were as outlined below:

1. Infection of mice with contaminated river water via different routes of inoculation will lead to the establishment of HS in mice and will be associated with clinical responses.

2. Contaminated river water with *P. multocida* type B: 2 inoculations through different routes can be detected in the various organs using Polymerase chain reaction (PCR).

3. Inoculation of mice through different routes with contaminated river water containing *Pasteurella multocida* type B: 2 will lead to changes in the concentrations of acute phase proteins.

4. There will be severe cellular changes in the various organs associated with the infection of *Pasteurella multocida* type B: 2 in mice inoculated through different routes with contaminated river water.

Therefore, the present study will provide a better comprehension of the characteristics of *Pasteurella multocida* type B; 2 infection and pathogenesis in the host post inoculation with contaminated river water.

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