UNIVERSITI PUTRA MALAYSIA

INVASIVE PROPERTY OF AN ATTENUATED GDHA- DERIVATIVE OF *Pasteurella multocida* B:2

FARAHANI MUHAMMAD AZAM

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By

FARAHANI BINTI MUHAMMAD AZAM

Thesis Submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Bachelor of Science (Hons.)

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Haemorrhagic septicaemia (HS) is a disease of buffaloes and cattle that can cause acute and fatal mortality if contracted. The animals are infected by the bacteria through the intranasal pathway which translocated intracelullarly into the vascular system. Live-attenuated vaccine, containing attenuated live bacteria is the alternative vaccine that has been shown to confer longer period of immunity towards the field-animals. The attenuated GdhA$^-$ derivative of *Pasteurella multocida* B:2 has been proven to be a suitable candidate as a live-attenuated vaccine towards HS among other live-attenuated strains tested. A further insight into the intracellular viability and pathogenicity of this disease can be discovered by understanding the mechanism of interaction between the host cell and the vaccine candidate. In this study, the GdhA$^-$ derivative strain was shown to adhere and to invade bovine aortic endothelial cells (BAECs) *in vitro* alongside another derivative strain, the AroA$^-$ derivative and the wild-type of *P. multocida* B:2 strains as a control. Infection of GdhA$^-$ derivative towards BAECs had shown no cytotoxicity effect with >80% viability towards the cell lines through trypan blue viability assessment. Adhesion and invasion of the GdhA$^-$ derivative was shown to be more efficient with highest adhesion rate (45.75 ± 5.74 number of bacteria/BAEC cell) and invasion rate (1.41 ± 0.27 number of bacteria/BAEC cell) when compared to the AroA$^-$ derivative and the wild-type strains.

Abstrak tesis yang dikemukakan kepada Jabatan Biologi Sel dan Molekul sebagai memenuhi keperluan untuk ijazah Sarjana Muda Sains (Kepujian)

PENCIRIAN INVASIF OLEH DERIVATIF GDHA ATENUAT DARIPADA *Pasteurella multocida* B:2

Oleh

FARAHANI BINTI MUHAMMAD AZAM

Sampar berdarah (HS) adalah penyakit berjangkit yang menyerang populasi kerbau dan lembu, menyebabkan maut dengan kadar kematian yang tinggi. Secara amnya, haiwan-haiwan tersebut telah dijangkiti bakteria melalui laluan intranasal yang ditranslokasikan secara intraselular ke dalam sistem vaskular. Vaksin hidup-atenuat, mengandungi bakteria hidup yang teratenuat adalah vaksin alternatif yang telah terbukti memberikan imuniti dalam tempoh masa yang panjang terhadap haiwan tersebut. Di antara strain-strain hidup lain yang dilemahkan dan telah diuji, derivatif GdhA$^-$ yang teratenuat daripada *P. multocida* B:2 telah terbukti menjadi calon yang sesuai sebagai strain hidup yang dilemahkan terhadap HS. Penyingkapan terhadap kebolehhidupan intraselular dan patogenisiti penyakit ini boleh difahami dengan menyelidiki mekanisme interaksi antara sel tuan rumah dan calon vaksin. Melalui kajian ini, derivatif GdhA$^-$ yang dilemahkan telah menunjukkan dapat melekat kepada dan menakluki sel-sel endothelial aorta lembu (BAECs) secara *in vitro* bersama strain hidup yang lain; strain derivatif AroA$^-$ dan strain jenis liar daripada *P. multocida* B:2 sebagai kawalan. Jangkitan daripada derivatif GdhA$^-$ terhadap BAECs telah tidak menunjukkan sebarang kesan sitotoksisiti dengan viability >80% terhadap barisan sel melalui penaksiran kebolehhidupan “trypan blue”. Pelekatan dan penaklukkan derivatif GdhA adalah lebih berkesan dengan nilai tinggi kadar pelekatan (45.75 ± 5.74 nombor bakteria/sel BAEC) dan kadar penaklukan (1.41 ± 0.27 nombor bakteria/sel BAEC) apabila dibandingkan dengan strain derivatif AroA$^-$ dan jenis liar.

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FARAHANI MUHAMMAD AZAM

APPROVAL

This thesis was submitted to the Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences and has been accepted as fulfillment of the requirement for the degree of Bachelor of Science (Hons.). The member of the Supervisory Committee was as follows:

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DECLARATION

Declaration by undergraduate student:

I hereby confirm that:

- This thesis is my original work;
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Declaration by Supervisor

This is to confirm that:

- The research conducted and the writing of this thesis was under supervision.

Signature:_____

Name of Chairman of Supervisory Committee:_____

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BAECs	-	bovine aortic endothelial cells
BHI	-	brain-heart infusion broth
bp	-	basepairs
CO_2	-	carbon dioxide
CFU	-	colony forming unit
EDTA	-	ethylenediamine tetraacetate
^0C	-	degree Celcius
gdhA	-	glutamate dehydrogenase
HS	-	haemorrhagic septicaemia
h	-	hour
IgG	-	immunoglobulin G
kb	-	kilobasepairs
µl	-	microliter
ml	-	mililitre
min	-	minute
MOI	-	multiplicity of infection
rpm	-	rotation per minute
SEM	-	standard error of mean
TEM	-	transmission electron microscopy
UV	-	ultraviolet
V	-	voltage

CHAPTER 1: INTRODUCTION

Hemorrhagic septicemia (HS) is a major disease in cattle and buffaloes which is caused by the infection of *Pasteurella multocida* B:2 (Abubakar and Saad, 2011). In Asia, the serotype of the bacteria responsible for the disease is *P. multocida* B:2 (Tabatabaei et al., 2001). HS when contracted will exhibit symptoms like depression, fever (pyrexia), throat swelling (submandibular oedema), breathing difficulties (dyspnea) and death (Abubakar and Saad, 2011). Transmission occurred from diseased animals or carriers through intranasal and oral routes (Abubakar and Saad, 2011). Invasion of the bacteria through endothelial cells resulted in rapid infiltration of the animals' bloodstream (Galdiero et al., 2001). Vaccine for HS usually was registered prior to rainy season using oil-adjuvant vaccine or alum-precipitated vaccine. Despite both vaccines contained bacterin, only short-termed protection was detected (Othman, 2007). Live attenuated vaccine consisted of live organisms such as the attenuated bacteria with reduced virulence compared to the wild-type (Saad, 2013). GdhA$^-$ derivative, known as GDH7, is the attenuated derivative generated through the disruption of the *gdhA* gene by the insertion of kanamycin cassette (Othman, 2007). This resulted in the metabolism interference and hence arrested its pathogenicity. Since currently available vaccines such as alum-precipitated vaccine and oil-adjuvant vaccine were discovered to be less effective, a new alternative is paramount. The aforementioned *gdhA* gene disruption has been found to be a promising manipulation for non-pathogenic *P. multocida* B:2 vaccine development (Othman et al., 2012). However, the invasion mechanism of the derivative bacteria was still unknown. Therefore, in this study, the invasion mechanism of the GDH7 strain of *P. multocida* B:2 will be assessed in order to understand the effectiveness of the vaccine to further develop the vaccine strain as a DNA delivery vehicle.

There were a few problem statements recognized prior to this study. A mutant derived from *P. multocida* B:2, the AroA$^-$ attenuated derivative, also known as JRMT12, was non-pathogenic. Tabatabaei et al., (2007) and Dagleish et al., (2007) proved that the JRMT12 vaccine strain when intramuscularly (i.m.) injected had provided strong immunity towards a cattle

population when challenged with the wild-type. This vaccine strain had shown a higher rate of adhesion and invasion towards embryonic bovine lung cells as compared to the parent strain (Othman et al., 2012). In another study, another mutant of *P. multocida* B:2, GDH7 was developed from local isolated strain to be manipulated as a live-attenuated vaccine for HS and this strain showed positive protection efficacy in cattle even during stressful conditions (Rafidah et al., 2013; Othman, 2007). In her study, Rafidah et al., (2013) also proved that through intranasal (i.n.) vaccination of GDH7 strain and allowing the vaccinated cattle to mingle freely with the non-vaccinated cattle in a grazing area. This managed to confer sufficient protection and lasting immunity towards HS in a larger group. This vaccination route (i.n.), was considered as an advantage towards the poor farmers especially in the countries with endemic outbreaks like Malaysia instead of the intramuscular (i.m.) route requiring individual injection of cattle that would be laborious and expensive.

In this study, the adherence and invasive properties of the GDH7 strain are of interest and worthy to be investigated to provide a better insight on the pathogenicity of HS. The investigation of the mechanism in the infection process between the host cell lines and the GDH7 vaccine strain was evaluated based on the methods by Othman et al., (2012). Therefore the objective of this study was to assess the adherence and the invasive properties of the attenuated derivatives of *P. multocida* B:2 and its wild-type strain towards BAEC cell lines.

This study is expected to contribute significant knowledge in the adherence and invasive property of the GDH7 derivative strain compared to other strains of *P. multocida* B:2, to be developed as a vaccine. Hence, through the analysis of this study, this strain can be used as a delivery vehicle of DNA vaccine for HS in the future.

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