UNIVERSITI PUTRA MALAYSIA

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MASTER OF VETERINARY SCIENCE
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2009

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By

CHAU THI HUYEN TRANG

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Veterinary Science

May 2009

DEDICATED TO …….

My Father and Mother,

CHAU VAN DONG
DUONG THI NO

My Brothers and Sisters,

My beloved husband,

TRAN CHI KY

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Veterinary Science

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CHAU THI HUYEN TRANG

May 2009

Chairman : Professor Mohd Zamri Saad, DVM, PhD

Faculty : Veterinary Medicine

Infection by *Pasteurella multocida* B:2 leading to haemorrhagic septicaemia in cattle and buffaloes has been reported in many countries and is considered to be one of the most economically important livestock diseases in Southeast Asia, including Malaysia.

This study was conducted to investigate the role of intranasal vaccination on colonization of wild-type *P. multocida* B:2 and *gdh*A derivative of *P. multocida* B:2 onto the nasal mucosa and lungs of calves. Three healthy local calves, about 6 months of age, were used. The first and second calves were exposed intranasal twice at two-week interval with 3.2×10^7 CFU/mL of live wild-type *P. multocida* B:2 and 7.0×10^7 CFU/mL of live *gdh*A derivative of *P. multocida* B:2, respectively. The third calf was untreated. Two weeks after the second

exposure, all calves were killed and *in vitro* explants of the lung and nasal mucosa were immediately prepared before they were challenged with 0.5 mL of the inoculum containing 10^9 CFU/mL of live wild-type *P. multocida* B:2 and incubated at 37°C. At 2, 6, and 12 hours post-challenge, three explants from each calf were removed and processed for scanning electron microscopic (SEM) examination to determine the rate of colonization.

In vitro colonization of wild-type *P. multocida* B:2 onto the lung explants of calves exposed to either the wild-type or *gdh*A derivative of *P. multocida* B:2 was significantly ($p<0.01$) less severe than the untreated calf when mild to moderate colonization were observed. However, colonization onto the nasal mucosa showed no significant difference ($p>0.05$) between the three calves throughout the entire 12-hour study period.

Following intratracheal introduction to high dose of wild-type (3.3×10^{10} CFU/mL) and *gdh*A derivative of *P. multocida* B:2 (5.4×10^9 CFU/mL) into calves, the phagocytic efficiency of alveolar macrophages were determined at 48 h post-inoculation. Wild-type *P. multocida* B:2 resulted in clinical signs typical of haemorrhagic septicaemia, which include dullness, fever, mucous nasal discharge and salivation. Subcutaneous oedema was obvious at the lower jaw, neck and brisket areas. Post-mortem examination was concentrated primarily on the respiratory tract. The lungs, trachea and epiglottis were congested and oedematous while the associated lymph nodes were congested with petechial

haemorrhages. These changes were not observed in calves inoculated with *gdh*A derivative of *P. multocida* B:2. There was significant (p<0.05) difference in the phagocytic efficiency of alveolar macrophages and neutrophils between calves inoculated with wild-type (45.1 ± 4.1%) and those inoculated with *gdh*A derivative (57.3 ± 3.4%) of *P. multocida* B:2.

In conclusion, the intranasal exposures to either wild-type or the *gdh*A derivative of *P. multocida* B:2 was significantly reduced colonization of the respiratory tract by wild-type *P. multocida* B:2. Similarly, intra-tracheal exposures of calves to the *gdh*A derivative of *P. multocida* B:2 failed to establish the disease due to the more efficient phagocytosis by the neutrophils and macrophages compared to the wild-type *P. multocida* B:2. Therefore, the *gdh*A derivative of *P. multocida* B:2 was found to be easily eliminated by phagocytosis and was unable to survive for long period of time in the host.

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

PENGHASILAN JANGKITAN OLEH *PASTEURELLA MULTOCIDA* B:2 PADA ANAK LEMBU

Oleh

CHAU THI HUYEN TRANG

Mei 2009

Pengerusi : Professor Mohd Zamri Saad, DVM, PhD

Fakulti : Perubatan Veterinar

Jangkitan oleh *Pasteurella multocida* B:2, yang boleh menyebabkan penyakit hawar berdarah pada lembu dan kerbau, telah dilaporkan berlaku dalam banyak negara. Penyakit ini dianggap sebagai salah satu penyakit ternakan yang berkepentingan ekonomi di Asia Tenggara, termasuk Malaysia.

Kajian ini dibuat untuk menyiasat peranan memberi vaksin secara intra-nasum ke atas pengkolonian *P. multocida* B:2 liar pada mukosa hidung dan paru-paru anak lembu. Tiga ekor anak lembu sihat, berumur kira-kira 6 bulan telah digunakan. Anak lembu pertama didedahkan kepada 3.2×10^7 CFU/mL *P. multocida* B:2 liar secara intra-nasum sebanyak dua kali, sementara anak lembu kedua didedahkan kepada 7.0×10^7 CFU/mL *P. multocida* B:2 terbitan *gdh*A hidup. Anak lembu ketiga tidak didedahkan. Dua minggu selepas dedahan

kedua, semua anak lembu dibunuh dan eksplan *in vitro* daripada tisu paru-paru dan mukosa nasum dibuat serta-merta sebelum eksplan dicabar menggunakan inokulum 0.5 mL yang mengandungi 10^9 CFU/mL *P. multocida* B:2 liar dan dieramkan pada suhu 37^{o}C. Pada jam ke 2, 6 dan 12 selepas dicabar, sejumlah tiga eksplan dari setiap anak lembu dialih dan diproses untuk kajian mikroskop electron imbasan (SEM) bagi menentukan kadar pengkolonian.

Pengkolonian *in vitro* oleh *P. multocida* B:2 liar ke atas eksplan paru-paru yang didedah sama ada kepada *P. multocida* B:2 liar atau *P. multocida* B:2 terbitan *gdh*A menunjukkan pengurangan bermakna (p<0.01) berbanding anak lembu tidak didedah. Akan tetapi, kadar pengkolonian ke atas mukosa nasum tidak menunjukkan perbezaan bermakna (p>0.05) di kalangan ketiga-tiga anak lembu terbabit.

Selaras dengan pemberian intra-trakea dos tinggi *P. multocida* B:2 liar dan terbitan *gdh*A kepada anak lembu, kecekapan fagositosis oleh makrofaj alveolus dikaji selepas 48 jam. *P. multocida* B:2 liar menghasilkan petanda klinikal mirip penyakit hawar berdarah. Ini termasuklah kemurungan, demam, lelehan hidung dan pengliuran. Edema subkutis jelas kelihatan pada bahagian rahang bawah, leher dan brisket. Pemeriksaan bedah-siasat yang ditumpukan kepada trakus pernafasan menunjukkan kesesakan dan edema pada paru-paru, trakea dan epiglotis sementara nodus limfa berkaitan turut sesak dan pendarahan petekia. Perubahan ini tidak pula dilihat berlaku ke atas anak lembu yang diberikan *P. multocida* B:2 terbitan *gdh*A. Terdapat perbezaan bermakna (p<0.05) pada

kadar kecekapan fagositosis oleh makrofaj alveolus dan neutrofil di antara anak lembu yang diberi organisma liar (45.1 ± 4.1%) dengan yang diberi organisma terbitan *gdh*A (57.3 ± 3.4%).

Sebagai kesimpulan, pendedahan intra-nasum sama ada kepada *P. multocida* B:2 liar atau terbitan *gdh*A menghalang secara bermakna pengkolonian ke atas trakus pernafasan oleh *P. multocida* B:2 liar. Begitu juga, pendedahan intra-trakea kepada *P. multocida* B:2 terbitan *gdh*A gagal untuk menghasilkan penyakit kerana kecekapan fagositosis oleh neutrofil dan makrofaj berbanding dedahan kepada *P. multocida* B:2 liar. Oleh itu, *P. multocida* B:2 terbitan *gdh*A didapati mudah untuk dihapuskan melalui fagositosis dan tidak dapat kekal dalam hos.

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Thank you.

I certify that an Examination Committee has met on 20th May 2009 to conduct the final examination of Chau Thi Huyen Trang on her Master of Veterinary Science thesis entitled " Establishment of infection by *Pasteurella multocida* B:2 in calves" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of Examination Committee are as follows:

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This thesis 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. The members of the Supervisory Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at UPM or other institutions.

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CHAU THI HUYEN TRANG

Date: .....

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LIST OF ABBREVIATIONS

ANOVA	One-way analysis of variance
BALT	Bronchus-associated lymphoid tissue
BHI	Brain heart infusion broth
bp	Base pair
cm^2	Centimeter square
CFU/mL	Colony forming unit
EMEM	Eagle's minimum essential medium
EDTA	ethylene diaminetetra acetic
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
*gdh*A	Glutamate dehydrogenase
IgG	Immunoglobulin G
IgA	Immunoglobulin A
h	hour
HS	Haemorrhagic septicaemia
H & E	Hematoxylin and eosin
Kbp	Kilobase pair
LPS	Lipopolysaccharide
LSD	Least significant difference
M	Molar
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction

pH	Puissance hydrogen (Hydrogen-ion concentration)
p.i	Post inoculation
SEM	Scanning electron microscopy
sd	Standard deviation
TAE	Tris-acetate-EDTA buffer
UPM	Universiti Putra Malaysia
UV	utraviolet
%	Percent/percentage
µl	Microlitre
µg	Microgram
mL	Millilitre
^{o}C	Degree centigrade