UNIVERSITI PUTRA MALAYSIA

ESTABLISHMENT OF INFECTION BY PASTEURELLA MULTOCIDA B:2 IN CALVES

CHAU THI HUYEN TRANG

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MASTER OF VETERINARY SCIENCE
UNIVERSITI PUTRA MALAYSIA

2009
ESTABLISHMENT OF INFECTION BY \textit{PASTEURELLA MULTOCIDA} B:2 IN CALVES

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

ESTABLISHMENT OF INFECTION BY *PASTEURELLA MULTOCIDA* B:2 IN CALVES

By

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 *gdhA* 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 $3.2 \times 10^7$ CFU/mL of live wild-type *P. multocida* B:2 and $7.0 \times 10^7$ CFU/mL of live *gdhA* 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 *gdhA* 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 ($3.3 \times 10^{10}$ CFU/mL) and *gdhA* derivative of *P. multocida* B:2 ($5.4 \times 10^9$ 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 gdhA 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 gdhA derivative (57.3 ± 3.4%) of *P. multocida* B:2.

In conclusion, the intranasal exposures to either wild-type or the gdhA 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 gdhA 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 gdhA 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.
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 gdhA 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°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 gdhA 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 gdhA 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 gdhA. 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 gdhA (57.3 ± 3.4%).

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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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(Member)

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**HASANAH MOHD GHAZALI, PhD**  
Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date: .............
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.

______________________
CHAU THI HUYEN TRANG

Date: ................................
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<table>
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<tr>
<td>ANOVA</td>
<td>One-way analysis of variance</td>
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<tr>
<td>BALT</td>
<td>Bronchus-associated lymphoid tissue</td>
</tr>
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<td>BHI</td>
<td>Brain heart infusion broth</td>
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<tr>
<td>bp</td>
<td>Base pair</td>
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<tr>
<td>cm²</td>
<td>Centimeter square</td>
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<tr>
<td>CFU/mL</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>EMEM</td>
<td>Eagle’s minimum essential medium</td>
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<td>EDTA</td>
<td>ethylene diaminetetra acetic</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>dNTP</td>
<td>Deoxyribonucleotide triphosphate</td>
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<td>gdhA</td>
<td>Glutamate dehydrogenase</td>
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<td>Immunoglobulin G</td>
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<td>IgA</td>
<td>Immunoglobulin A</td>
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<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HS</td>
<td>Haemorrhagic septicaemia</td>
</tr>
<tr>
<td>H &amp; E</td>
<td>Hematoxylin and eosin</td>
</tr>
<tr>
<td>Kbp</td>
<td>Kilobase pair</td>
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<tr>
<td>LPS</td>
<td>Lipopolysaccharide</td>
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<td>LSD</td>
<td>Least significant difference</td>
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<td>PBS</td>
<td>Phosphate buffered saline</td>
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<td>PCR</td>
<td>Polymerase chain reaction</td>
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<td>Symbol</td>
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<td>--------</td>
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</tr>
<tr>
<td>pH</td>
<td>Puissance hydrogen (Hydrogen-ion concentration)</td>
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<td>p.i</td>
<td>Post inoculation</td>
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<td>SEM</td>
<td>Scanning electron microscopy</td>
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<td>sd</td>
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<td>mL</td>
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