UNIVERSITI PUTRA MALAYSIA

BOVINE MUCOSAL IMMUNE RESPONSE TO INTRANASAL EXPOSURE WITH LIVE *PASTEURELLA MULTOCIDA* B:2

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By

KHIN MYAT NWE

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BOVINE MUCOSAL IMMUNE RESPONSE TO INTRANASAL EXPOSURE WITH LIVE *PASTEURELLA MULTOCIDA* B:2

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July 2009

Chairman : Professor Mohd Zamri-Saad, PhD

Faculty : Veterinary Medicine

Haemorrhagic septicaemia (HS) is an infectious disease of cattle and buffalo caused by *Pasteurella multocida* B:2. It is endemic in most parts of tropical Asia, Africa and India, causing high mortality in livestock and is considered as one of the most economically important livestock diseases in Southeast Asia. Vaccination has been used to control the disease and the oil adjuvant vaccine is most often used although alum precipitate and broth vaccines are also available. Although the available injection vaccines are effective in providing protection, the low vaccination coverage, particularly among cattle and buffaloes kept extensively, is one of the main reasons that lead to disease outbreaks, particularly among those extensively kept, semi-wild cattle and buffaloes.

Therefore, the disease remains a significant obstacle to sustainable agriculture in the region and attempts should be made to increase the vaccination coverage. The development of live attenuated vaccine that can be administered intranasally may be an answer. This study reports on the use of an attenuated *P. multocida* B:2, that has been created by manipulating the *gdhA* genes, as a component of live haemorrhagic septicaemia vaccine. It is as an alternative way to protect the animals from haemorrhagic septicaemia and in the same time to increase vaccination coverage.

The respiratory tract contains an important component of the mucosal immune system, and the first line of immunological defense since it is exposed continuously to inhaled antigens. Intranasal exposures to live wild-type and *gdhA* derivative of *P. multocida* B:2 not only successfully stimulated the mucosal immunity of the respiratory tract, but also the systemic immunity. This was evidenced by the increased in the size of BALT, the numbers of lymphocytes, the levels of IgA in the lung lavage fluid and the levels of IgG in the serum of exposed calves. However, the nasal associated lymphoid tissues (NALTs) were successfully stimulated only following intranasal exposures to the wild-type *P. multocida* B:2.

Following intranasal exposures of calves to live *gdhA* derivative of *P. multocida* B:2 at 2-week interval and challenged by wild-type *P. multocida* B:2 four weeks later, the exposed calves were not only able to prevent establishment of infection by wild-type *P. multocida* B:2 but also produced similar effects in the susceptible commingled calves. When immuno-suppression was created in calves by subcutaneous injections of

dexamethasone for 3 consecutive days, and immediately followed by the intranasal exposures to the *gdhA* derivative of *P. multocida* B:2, both mucosal and systemic immunities of the immuno-suppressed calves failed to be stimulated. This was obvious when the serum IgA and IgG levels of the exposed, stressed calves were similar to that of unexposed calves. On the other hand, the unstressed calves showed significantly ($p<0.05$) higher serum IgA and IgG levels following intranasal exposures to the *gdhA* derivative of *P. multocida* B:2. Similarly, the IgA and IgG levels in the lung lavage fluid of calves treated with dexamethaosne were significantly ($p<0.05$) lower than those without dexamethasone treatments. It was concluded that dexamethasone reduced the availability of immune cells, thus reducing immune responses.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah Veterinar

TINDAKBALAS KEIMUNAN MUKOSA LEMBU TERHADAP *PASTEURELLA MULTOCIDA* B:2 HIDUP MENERUSI PENDEDAHAN INTRANASAL

Oleh

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Sampar berdarah merupakan penyakit berjangkit yang menyerang lembu dan kerbau disebabkan oleh bakteria *Pasteurella multocida* B:2. Penyakit ini didapati endemik di kebanyakan bahagian tropika Asia, Afrika dan India, di mana menyebabkan kematian yang tinggi terhadap haiwan-haiwan ternakan dan merupakan salah satu penyakit yang menyebabkan kerugian besar daripada segi ekonomi di Asia Tenggara. Pemvaksinan telah digunakan untuk mengawal penyakit ini dan vaksin minyak adjuvan digunakan dengan lebih meluas berbanding vaksin ‘alum precipitate’ dan ‘broth’. Walaupun pemvaksinan melalui suntikan telah terbukti berkesan dalam memberi perlindungan, namun kesulitan dalam memberi vaksinasi terutama pada lembu dan kerbau yang diternak secara separa liar telah menyebabkan berlakunya wabak penyakit.

Oleh yang demikian, penyakit ini telah menjadi halangan besar kepada sektor pertanian di Asia Tenggara dan usaha harus dilakukan untuk mempertingkatkan lagi perlindungan melalui vaksinasi. Penghasilan vaksin hidup yang dilemahkan dan diberi melalui intranasal mungkin menjadi jawapan kepada permasalahan yang dihadapi kini.

Saluran pernafasan adalah komponen penting dalam sistem keimunan mukosa, dan merupakan pertahanan paling hadapan dalam sistem keimunan benda hidup disebabkan oleh pendedahan berterusan kepada antigen yang disedut dari udara. Pendedahan intranasal terhadap *P. multocida* B:2 liar hidup dan *gdhA* diterbitkan dari *P. multocida* B:2 iaitu vaksin yang dilemahkan bukan sahaja telah berjaya merangsang keimunan mukosa di saluran pernafasan, malah turut merangsang penghasilan keimunan sistemik. Ini dibuktikan melalui peningkatan dalam saiz BALT, jumlah limfosit, paras IgA dalam cairan peparu dan paras IgG di dalam serum lembu yang telah divaksinkan. Walaubagaimanapun, NALT hanya berjaya dirangsang melalui pendedahan intranasal terhadap *P. multocida* B:2 liar sahaja.

Berikutan pendedahan lembu-lembu kepada *gdhA* hidup yang diterbitkan dari *P. multocida* B:2 melalui vaksinasi intranasal pada selang dua minggu dan diberi cabaran keupayaan menggunakan *P. multocida* B:2 liar dua minggu kemudiannya, menunjukkan bahawa lembu-lembu tersebut bukan sahaja berupaya melawan jangkitan dari *P. multocida* liar malah turut menghasilkan kesan yang sama terhadap lembu-lembu yang bercampur dengan lembu yang telah diberi vaksin. Seterusnya, lembu-lembu tersebut diberi immunosupressi menerusi suntikan intra-muskular untuk tiga hari berturut-turut,

dan disusuli dengan pendedahan intranasal terhadap *gdhA* yang diterbitkan dari *P. multocida* B:2. Keputusan menunjukkan lembu-lembu yang diberi immunosuppresi gagal merangsang penghasilan keimunan mukosa dan sistemik. Perkara ini jelas sekali dilihat apabila paras antibodi serum IgA dan IgG lembu-lembu yang telah diberi tekanan secara immunosepressi dan didedahkan dengan vaksin yang dilemahkan adalah sama sepertimana lembu-lembu yang tidak diberi rawatan. Dengan kata lain, lembu-lembu yang tidak diberi tekanan dan didedahkan dengan *gdhA* yang diterbitkan dari *P. multocida* B:2 menunjukkan signifikan ($p<0.05$) lebih tinggi dalam paras serum IgA dan IgG. Perkara yang sama dapat dilihat melalui paras serum IgA dan IgG dalam cairan peparu lembu-lembu yang dirawat dengan dexamethasone, dimana secara signifikan ($p<0.05$) lebih rendah berbanding dengan yang tidak diberi rawatan dexamethasone. Kesimpulannya, dexamethasone telah mengurangkan keupayaan sel-sel keimunan dan seterusnya mengurangkan tindakbalas keimunan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The Members of the Supervisory Committee are as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not currently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

KHIN MYAT NWE

Date: 2 October 2009

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BALT	-	bronchus-associated lymphoid tissues
B.H.I	-	brain-heart infusion broth
^{o}C	-	degrees centigrade
cfu	-	colony forming unit
dH_2O	-	distilled water
DEX	-	dexamethasone
EDTA	-	ethylenediamine tetraacetate
g	-	gram
gdhA	-	glutamate dehydrogenase
GALT	-	gut associated lymphoid tissue
HS	-	Haemorrhagic Septicemia
H & E	-	Hematoxylin and Eosin
IgA	-	Immunoglobulin A
IgG	-	Immunoglobulin G
Incontact	-	Incontact group
I/N	-	Intranasal administration group
kg	-	kilogram
L	-	litre
M	-	Molar
mg	-	milligram
mL	-	milliliter
mM	-	milliMolar

NALT	-	nasal associated lymphoid tissue
PBS	-	Phosphate Buffered Saline
PCR	-	polymerase chain reaction
pH	-	isoelectric point
%	-	percent/ pencentage
RT	-	room temperature
rpm	-	revolution per minute
s	-	second
TBE	-	tris-boric-EDTA
Taq	-	*Thermus aquaticus*
µl	-	microlitre
µg	-	microgram
µM	-	microMolar

CHAPTER 1

INTRODUCTION

The Gram-negative bacterium, *Pasteurella multocida* is the etiological agent of hemorrhagic septicemia in cattle, fowl cholera in birds and atrophic rhinitis in pigs (Mannheim, 1984). *Pasteurella multocida* strains have been categorized into 5 serogroups, namely serogroups A, B, D, E, and F based on the antigenicity of their capsule (Carter, 1967; Rimler, 1987). They are further categorized into 16 serotypes; namely serotypes 1 to 16 based on the characteristic of their lipopolysaccharide (LPS) antigens (Heddleston, 1972). The capsular serogroups are generally related to disease predilection, with hemorrhagic septicemia strains belonging to serogroups B or E (Verma and Jaiswal, 1998).

Hemorrhagic septicemia is endemic in most parts of tropical Asia, Africa and India, causing high mortality in livestock (Bain *et al*., 1982). It is considered to be the most economically important disease of cattle and buffaloes in Southeast Asia and causes significant economic losses in India and Africa (Bain *et al.,* 1982; Verma and Jaiswal, 1998). Cattle and buffaloes are the most common hosts, but pigs, sheep, goats, deer and camels are also susceptible to the infection (Dawkins *et al*, 1991; Blackall *et al.,* 2000). Vaccination with killed vaccines is practiced in areas where the disease is endemic and has reduced the incidence of disease, but the duration of immunity is short while the vaccination coverage is low, leading to disease outbreaks (Bain *et al.*, 1982; Verma and Jaiswal, 1998).

The respiratory tract of mammals has a mucosal cell lining, which is constantly exposed to environmental materials including antigens. Therefore, the respiratory tract must either mount an immune response or maintain immunological tolerance to prevent establishment of infection (Holt, 1993; Debertin *et al.*, 2003). Since Bienenstock *et al.* (1973a) called attention to the presence of sub-epithelial lymphoid tissue of the respiratory tract known as the bronchus-associated lymphoid tissue (BALT), the morphology and function of BALT in the respiratory tract of laboratory animals and man has been studied by many investigators (Bienenstock, 1985; Anderson *et al.*, 1986; Mair *et al.*, 1987). Large aggregates of lymphoid follicles, such as those seen in the intestine as Peyer's patches, are also found in the upper airways, especially at bifurcations of the bronchial tree. All of these lymphoid tissues are involved in the immune response in the lung against inhaled antigens.

The specific immune effector lymphocytes are believed to originate either from precursors present in sub-epithelial lymphoid tissue scattered throughout the respiratory tract or from a precursor in the regional and systemic lymphoid tissues. Thus, following intranasal exposure to the antigen, there will be stimulation of the mucosal immunity of the respiratory tract, and the bronchus-associated lymphoid tissues (BALTs) are expected to show morphological changes while a high level of secretory immunoglobulin is expected in the lung lavage fluid and serum. This study describes the morphological changes in BALT and the nasal associated lymphoid tissues (NALT), the antibody levels in the lung lavage fluid and serum following stimulation of the mucosal immunity of respiratory tract by intranasal exposures to live *Pasteurella multocida* B:2.