UNIVERSITI PUTRA MALAYSIA

RESPONSE OF GASTROINTESTINAL TRACT TO Pasteurella multocida SEROTYPE B:2 INFECTION IN BUFFALOES(Bubalus bubalis Linnaeus)

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BY

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Doctor of philosophy

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Chairman: **Professor Mohd Zamri-Saad, DVM, PhD**

Faculty: **Veterinary Medicine**

Pasteurella multocida B:2, which causes haemorrhagic septicaemia (HS) of ruminants, is believed to enter the host via the respiratory tract. Among the consequences of the respiratory route of infection are septicaemia, increased permeability of blood vessels and presence of the organism in several organs. However, the respiratory tract may not be the only portal of entry and route of spread of *P. multocida* B:2. Circumstantial evidence had suggested the involvement of gastrointestinal tract in the pathogenesis of HS in ruminants. Nevertheless, the pathogenesis and pathology of the disease following oral infection has not been well documented since previous reports on the disease were limited to incidental observations. The response of gastrointestinal tract following oral exposure to *P. multocida* B:2 was studied and compared its severity with intratracheal exposure. The safety,

antibody pattern and mucosal immune response in the gastrointestinal and respiratory tracts following oral or intranasal exposure to *gdhA* (glutamate dehydrogenase) derivative *P. multocida* B:2 in buffalo calves were also investigated.

The clinical signs observed in this studies includes; dullness, depression, recumbency, pyrexia, dyspnoea, congested mucous membranes, nasal discharge, lacrimation and salivation following single oral exposure to *P. multocida* B:2, however mean clinical score were significantly higher in intratracheally exposed group, inaddition diarrhea was noted in group of calves exposed orally to *P. multocida* B:2 twice, 2 weeks apart. The pathological alterations as the result of oral or intratracheal exposure to *P. multocida* B:2 included generalized lymphadenopathy, acute ulcerative rhinitis, acute fibrinous pneumonia, pleurisy, hydropericardium, hydroperitoneum, haemorrhagic encephalitis, acute enteritis, colitis and necrotizing and haemorrhagic typhilitis and proctitis, however lesions scoring revealed higher scores in gastrointestinal following orally exposure, while respiratory tract showed higher scores in intratracheally exposed group. Following oral or intratracheal exposure, *P. multocida* B:2 was isolated from the intestinal segments of the calves that developed severe clinical HS and they had to be sacrificed at 48 h post-exposure. Similarly, the ultrastructural changes in the infected calves were typical of acute cellular injury, with degeneration of endothelium and vascular walls of the gastrointestinal and respiratory tracts. There were deciliation in the respiratory tract, and microvilli degeneration and disruption in the gastrointestinal tract. Scanning electron microscopy revealed *P. multocida*

B:2 presence on the surfaces of oesophagus, nasal mucosa and trachea of calves in both oral- and intratracheal-exposed group. The lesions were confirmed through the immunoperoxidase technique, to be associated with the inoculated *P. multocida* B:2,. *P. multocida* B:2 antigen was detected not only in the bacterial clusters in the gastric pits, intestinal epithelia and capillaries, Brünner's glands and crypt of Lieberkühn but also seen to interact with infiltrating neutrophils, macrophages and erythrocytes in congested vessels and in haemorrhages.

Concurrently the mucosal-associated lymphoid tissue (MALT) response in the gastrointestinal and respiratory tracts of buffalo calves following oral exposure to live wild-type *P. multocida* B:2 was also evaluated. In calves exposed to both oral and intranasal *P. multocida* B:2, lymphoid nodules with increased sized and lymphocyte number were detected. With the confirmation of bronchus-associated lymphoid tissue (BALT) and gut-associated lymphoid tissue (GALT) involvement in *P. multocida* B:2 infection, an experiment was conducted to evaluate the safety, antibody pattern, and responses of BALT and GALT to exposure to *gdhA* derivative *P. multocida* B:2. Large dose oral and intranasal *gdhA* derivative *P. multocida* B:2 exposures elicited sustained and significantly higher serum IgG and IgA responses. Similarly, the IgG and IgA levels in bronchioaveolar fluid and intestinal washings were higher and the BALT and GALT responses were significant.

This study showed that *P. multocida* B:2 were present in various segments and tissues of the gastrointestinal tract following oral or intratracheal

exposure. Therefore, it can be concluded that *P. multocida* B:2 infection can be transmitted via the gastrointestinal tract. Both oral and intranasal routes of administration of *gdhA* derivative *Pasteurella multocida* B:2 elicited high serum antibody response although mucosal IgA and IgG responses were variable. Thus, oral and intranasal infections with large doses of live attenuated *P. multocida* B:2 were safer than with wild-type *P. multocida* B:2. Both of these routes can be considered as potential alternative route for vaccine administration against HS in buffalo calves.

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

GERAK BALAS SALUR GASTROUSUS TERHADAP JANGKITAN *Pasteruella multocida* SEROTYPE B:2 PADA KERBAU (*Bubalus bubalis* Linnaeus)

Oleh

ABUBAKAR SALISU MUHAMMAD

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Pasteurella multocida B:2, penyebab sampar berdarah (HS) pada ruminan, dipercayai memasuki perumah melalui salur pernafasan. Antara akibat jangkitan melalui jalan pernafasan ini ialah septisemia, peningkatan kebolehtelapan salur darah dan penumpuan organisma dalam beberapa organ. Bagaimanapun salur pernafasan mungkin bukan satu-satunya haluan masuk dan cara sebaran *P. multocida* B:2. Bukti tidak langsung menyarankan ada penglibatan salur gastrousus dalam patogenesis HS dalam ruminan. Sungguhpun begitu, patogenesis dan patologi penyakit berikutan jangkitan oral belum pernah didokumenkan sepenuhnya kerana laporan sebelum ini hanya terhad kepada pemerhatian secara kebetulan. Kami mengkaji gerak balas salur gastrousus berikutan pendedahan oral kepada *P. multocida* B:2 dan membandingkan keterukannya dengan

pendedahan intratrakea. Keselamatan, pola antibodi dan gerak balas dalam salur gastrousus dan pernafasan berikutan pendedahan oral dan intranasum kepada *gdhA* (glutamate dehydrogenase) terbitan *P. multocida* B:2 dalam anak kerbau juga diselidik.

Peranan jangkitan melalui saluran pernafasan sudah terbukti secara ujikaji. Kajian kini menghuraikan persembahan klinikal, termasuk kesuraman, kemuraman, terbaring, pireksia, dispnea, kesesakan membran mucus, lelehan hidung, pelakrimaan dan pengliuran berikutan pendedahan oral dan cirit-birit dalam kumpulan anak kerbau yang terdedah kepada *P. multocida* B:2 sebanyak dua kali dalam jarak 2 minggu antara satu. Perubahan patologi akibat daripada jangkitan ini termasuk limfadenopati menyeluruh, rinitis ulseratif akut, pneumonia berfibrin akut, pleurisi, hidroperikardium, hidroperitoneum, ensefalitis hemoraj, enteritis akut, kolitis dan tifilitis nekrosis dan hemoraj dan proktitis. Berikutan pendedahan oral atau intratrakea, *P. multocida* B:2 telah dipencil daripada segmen usus anak lembu yang menunjukkan HS klinikal teruk dan terpaksa disembelih pada jam 48 pasca-pendedahan. Begitu juga dengan perubahan ultrastrukur pada anak kerbau terjangkit, yang tipikal untuk kecederaan sel akut, dengan degenerasi endotelium dan dinding salur darah pada salur gastrousus dan pernafasan. Pada salur pernafasan berlaku penyahsiliuman dan pada salur usus pula berlaku degenerasi dan kerosakan mikrovilus. Mikroskopi electron imbasan menunjukkan kewujudan *P. multocida* B:2 pada permukaan esofagus, mukosa nasum dan trakea pada anak kerbau dalam kedua-dua kumpulan terdedah oral dan intratrakea. Lesi ini disahkan melalui teknik imunoperoksidas adalah berkaitan dengan *P. multocida* B:2

yang diinokulat. Antigen *P. multocida* B:2 dikesan bukan sahaja pada gugusan bakteria dalam pit gaster, epitelium dan rerambut usus, kelenjar Brünner dan kripta Lieberkühn tetapi juga dapat dilihat bertindak balas dengan neutrofil, makrofaj dan eritrosit yang menyusup ke dalam salur tersesak.

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I certify that a Thesis Examination Committee has met on 21st November, 2012 to conduct the final examination of Abubakar Salisu Muhammad on his thesis entitle "**Response of Gastrointestinal tract to *Pasteurella multocida* Serotype B:2 infection in Buffaloes *(Bubalus bubalis* Linnaeus)**" 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 Doctor of Philosophy.

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This thesis was submitted to the Senate of the 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 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 other degree at Universiti Putra Malaysia or other institution.

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ABUBAKAR SALISU MUHAMMAD

Date: 21 November 2012

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