

STIMULATION OF MUCOSAL AND HUMORAL IMMUNITY BY  
INTRANASAL INOCULATION OF LYOPHILIZED CRUDE OF  
*PASTEURELLA MULTOCIDA* B : 2 IN GOATS

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## ABSTRACT

Haemorrhagic septicaemia (HS) is an acute disease of cattle and buffaloes caused by *Pasteurella multocida* B:2. This disease is considered as one of the most important diseases in cattle and buffaloes causing great economical losses worldwide. Vaccination was used to control of the disease. However, after several years of using Oil Adjuvant Vaccine (OAV) to control of the disease, the result seems to be disappointed. The failure of OAV in controlling the disease is because of the thickness of the vaccine and poor vaccination management. For these reasons, a novel vaccination methods and vaccine was study in the project.

In this study, lyophilized crude of *Pasteurella multocida* B:2 was administrated twice intranasally to animal model with different quantity. Control untreated group was labeled as group C and T1, T2 and T3 were the treated groups. As observed in the study, the crude successfully stimulated the mucosal immunity in lung and intestine of the administrated goats. Bronchus-associated lymphoid tissues (BALT) were observed in the lung of all treated groups after single exposure to the lyophilized crude of *Pasteurella multocida* B:2. However, group T1 and T2 which administrated with 1 mg and 1.5 mg of the crude did not showed further enlargement in BALT after double exposure.

Gut-associated lymphoid tissues (GALT) were also observed in ileum, jejunum and duodenum of intestine. The finding in this study suggested that by using intranasal



inoculation of lyophilized crude of antigen can effectively stimulate mucosal immunity in gut and is important for future study on developing vaccine against gut infection diseases by using intranasal administration route.

The lyophilized crude of *Pasteurella multocida* B:2 not only successfully stimulated the mucosal immunity in lung and gut but also stimulated the humeral immunity in the goats. Ig G and Ig A in lung lavage fluid and serums were examined by using Enzyme-Linked Immunosorbent Assay (ELISA). IgA and IgG level in lung lavage fluid were found significantly high ( $p < 0.05$ ) than control group. In serum, although IgA level in group T1 did not showed significantly high reading above cut-off values, but IgA is only beneficial at the mucosal site but not that important in serum. Antibody that provides protection and prevention to diseases in animals is IgG. Both group T2 and T3 showed highly significant reading ( $p < 0.05$ ) above the cut-off values.

As the conclusion, 1 mg of lyophilized crude of *P. multocida* B:2 is not enough to stimulate the immunity in goats for the protection towards haemorrhagic septicaemia infection. The immunity which include mucosal and humoral immunity, can only be successfully stimulated by intranasal inoculation of at least 1.5mg of the lyophilized crude of *Pasteurella multocida* B:2 in two weeks interval.

## ABSTRAK

Hawar berdarah merupakan sejenis penyakit yang mengakibatkan kematian lembu dan kerbau disebabkan oleh *Pasteurella multocida* B:2. Penyakit ini dikategorikan sebagai salah satu penyakit yang harus diambil perhatian yang serius dalam penternakan industri lembu dan kerbau. Pengawalan secara pencegahan dengan pemvaksinan adalah cara yang terbaik memandangkan penyakit ini boleh memberi impak dari segi ekonomi kepada penternak khususnya dan industri penternakan lembu dan kerbau amnya. Walaupun program pemvaksinan dengan vaksin Oil Adjuvant (OAV) digunakan untuk mengawal penyakit ini, namun vaksin ini tidak memberi keputusan yang memuaskan. Ketidakberkesanan vaksin OAV mengawal penyakit tersebut adalah disebabkan kepekatan vaksin yang tinggi dan sukar disuntik kepada ternakan. Kelemahan dalam pengurusan program pemvaksinan juga merupakan salah satu sebab yang mengakibatkan kegagalan. Justeru, tujuan penyelidikan ini adalah untuk mencari penyelesaian mengatasi masalah tersebut.

Dalam penyelidikan ini, habuk mentah *Pasteurella multocida* B:2 telah disembur sebanyak dua kali pada jarak 14 hari secara semburan intranasal kepada kambing kajian dengan kuantiti yang berbeza. Hasil daripada pemerhatian keberkesanan habuk mentah tersebut dalam merangsangkan immuniti kambing kajian, kehadiran tisu *bronchus-associated lymphoid* (BALT) dapat diperhatikan dalam peparu kambing yang dikaji selepas sekali semburan dengan habuk mentah yang mengandungi *Pasteurella multocida* B:2. Bagi kumpulan T1 dan T2 yang telah diberikan dua kali semburan

sebanyak 1 mg dan 1.5 mg habuk mentah *Pasteurella multocida* B:2 tidak menunjukkan sebarang perkembangan BALT.

Selain tisu BALT, tisu *gut-associated lymphoid* (GALT) juga dapat diperhatikan dalam bahagian ileum, jejunum dan duodenum di usus kecil kambing kajian. Daripada keputusan penyelidikan, penggunaan semburan intranasal dengan habuk mentah *Pasteurella multocida* B:2 dan antigen, sangat berkesan bagi merangsang immuniti mukus dalam usus kecil. Penggunaan semburan intranasal akan dapat membantu dalam penyelidikan dan pembangunan vaksin bagi jangkitan usus kecil untuk tahap seterusnya.

Keberkesanan habuk mentah *Pasteurella multocida* B:2 bukan sahaja berjaya merangsangkan system imunisasi mucosal tetapi juga system imunisasi humoral kambing. Kajian "*Enzyme-Linked Immunosorbent Assay*" (ELISA) digunakan dalam mengesani Ig G dan Ig A dalam serum dan lavage paru paru kambing kajian. Daripada keputusan paras Ig A dan Ig G dalam lavage paru-paru, menunjukkan peningkatan yang tinggi ( $p < 0.05$ ) berbanding kambing yang tidak divaksin. Walaupun dalam serum yang diuji paras Ig A dari kumpulan T1 tidak menunjukkan bacaan yang tinggi tetapi keputusan paras Ig A hanya memberi kelebihan untuk bahagian mukus dan paras IgA tidak memberi kelebihan didalam serum.

Sebagai kesimpulannya, 1 mg habuk mentah *Pasteurella multocida* B:2 tidak mencukupi untuk merangsang sistem immuniti kambing sebagai perlindungan terhadap

jangkitan hawar berdarah. Rangsangan immuniti secara khususnya terhadap mukus dan humoral hanya akan berjaya dengan menggunakan semburan secara intranasal sekurang-kurangnya 1.5 mg habuk mentah yang mengandungi *Pasteurella multocida* B:2 dalam selang masa dua minggu.

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