STUDY OF POSSIBLE IMMUNE PROTECTION WITH GRADED DOSES OF PASTEURELLA MULTOCIDA TYPE B:2 INOCULATED ORALLY IN MICE

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STUDY OF POSSIBLE IMMUNE PROTECTION WITH GRADED DOSES OF *PASTEURELLA MULTOCIDA* TYPE B:2 INOCULATED ORALLY IN MICE .

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It is hereby certified that we have read this project paper entitled "Study of Possible Immune Protection with Graded Dose of *Pasteurellamultocida*Type B:2 Inoculated Orally in Mice", by Tai ShenRong and in our opinion it is satisfactory in term of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999-Project.

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ABSTRACT

Haemorrhagicsepticaemia (HS) is an acute fatal septicaemic disease in cattle and buffaloes caused by Pasteurellamultocida Type B:2 in Malaysia. There is need to develop a vaccine easily administrated compared to those currently used. This study describes the possibility of using graded doses of oral bacterium to induce immunity. A total of 26 mice were divided into treatment groups (Group 1, 2, 3, and 4) each with 5 mice and control (n=6) groups. Treatment groups were inoculated orally with 0.2ml of 103, 105, 107 and 109 colonies forming units (CFU) respectively, while control received 0.2ml of phosphate buffered saline (PBS). Surviving mice were re-challenged with 0.2 ml of 107 CFU orally and observed for another 7 days. Clinical signs, mortality rate and histopathological lesions were examined. All clinical signs were observed to be not significantly ($P > 0.05$) observed in Group 1 and 2 except (level of alertness and ocular discharges) in Group 3 and 4. Presence of inflammatory cells, haemorrhages and congestions were mild to moderately observed in all treatment groups. However, degeneration and necrosis were observed to be moderate to severe in Group 4. All mice in Group 3 and 4 were euthanized at early stage after second challenged, except Group 1 and 2 with 20% of survival. Bacterial culture from survived mice was significantly lower ($P < 0.05$) in heart, lung, liver, spleen and kidney. Pasteurellamultocida was confirmed by Gram and Wright's stains from all positive organ cultures. In conclusion, better survivability was observed in oral bacterium of 103 and 105 CFU with milder clinical signs and histological lesions, while 107 and 109 CFU resulted in detrimental effects on mice. Thus, low but not high dose of oral inoculum was believed to induce immunity and possible to use as oral vaccine.

Keywords: Pasteurellamultocida Type B:2, mice, oral inoculation, clinical signs, histological signs, oral vaccine.

ABSTRAK

Penyakit hawar berdarah adalah penyakit septicaemic mau takut pada lembu dan kerbau yang disebabkan oleh jangkitan *Pasteurellamultocida* Jenis B: 2 di Malaysia. Terdapat keperluan untuk membangunkan satu jenis vaksin mudah diaplikasikan berbanding dengan mereka yang kini digunakan.Kajian ini menerangkan kemungkinan menggunakan dos bergred oral bakteria untuk mendorong imuniti.Seramai 26 mencit dibahagikan kepada kumpulan rawatan (Kumpulan 1, 2, 3, dan 4) masing-masing dengan 5 mencit dan kumpulan kawalan (n = 6). Kumpulan rawatan disuntik secara oral dengan 0.2ml 103, 105, 107 dan 109 jajahan membentuk unit (CFU) masing-masing, manakala kawalan menerima 0.2ml fosfat buffered masin (PBS). Mencit yang hidup pada cabaran pertama telah dicabar semula dengan 0.2 ml 107 CFU secara oral dan diperhatikan untuk 7 hari lagi.Tanda-tanda klinikal, kadar kematian dan lesihistopatologi telah diperiksa. Semua tanda-tanda klinikal yang dapat diperhatikan sebagai tidak bererti (P> 0.05) bagi Kumpulan 1 dan 2 tetapi tidak (tahap kewaspadaan dan cecair okular) dalam Kumpulan 3 dan 4. Kehadiran sel-sel inflamasi, hemoraj dan kongesi yang ringan kesederhana diperhatikan dalam semua kumpulan rawatan. Walaubagaimanapun, degenerasi dan nekrosis adalah didapati sederhana kepada teruk dalam Kumpulan 4.Semua mencit dalam Kumpulan 3 dan 4 telah euthanasia pada peringkat awal selepas cabaran kedua, kecuali Kumpulan 1 dan 2 yang didapati 20% hidup. Bakteria kaltur dari tikus terselamat adalah jauh lebih rendah (P <0.05) di dalam hati, paru-paru, hati, limpa dan buah pinggang.*Pasteurellamultocida* telah disahkan oleh kesan Gram dan Wright dari semua organ positif.Kesimpulannya, kemandirian lebih baik diperhatikan dalam bakteria oral 103 dan 105 CFU dengan tanda-tanda klinikal yang lebih ringan dan lesihistologi, manakala 107dan 109 CFU mengakibatkan kesan memudaratkan mencit. Oleh itu, dos rendah tetapi tidak dos tinggi

inokulum oral dipercayai mendorong imuniti dan mungkin untuk digunakan sebagai vaksin oral.

Kata kunci: Pasteurellamultocida jenis B:2, mencit, oral inokulum, tanda-tanda klinikal, tanda histological, vaksin oral.

1.0 INTRODUCTION

Pasteurellamultocida is a non-motile, gram-negative, coccobacillus that is found in the nasopharynx and gastrointestinal tract of many wild and domesticated animals (Sugan*et al*., 2013). It is the aetiological agent for an acute, fatal septicaemic disease known as haemorrhagicsepticaemia (HS). The disease mainly in found South and Southeast Asia, Africa and India (Hussaini&Jumahat, 2014), which include Malaysia. The death usually occurs quickly and mortality rate without prompt antibiotic treatment in a naïve population is close to 100% (Aktories*et al*., 2012; Jumahat*et al.*,2015). However, treatments of infected animals with *P. multocida* are complex and unsuccessful due to increasing antibiotic resistance strains. Vaccination is the principle method of controlling the disease (Zamri*et al*., 2006)but difficulties in vaccine administration lead to low vaccination coverage and disease outbreaks (Saharee*et al*., 1993). Moreover, the efficacy and safety of available vaccines are limited (Hussaini*et al.*, 2012). Large-scale vaccination of cattle against HS is not practiced in many countries of Africa (FAO, 2005), which is the same scenario in Malaysia. This could be due to the laborious process such as herding and restraining, which involved when vaccinating the cattle by injection. In 2013, Zamri had reported the vaccine coverage of HS for buffaloes in Malaysia is just 17% and it was most probably due to difficulty in vaccine administration. Besides, vaccination via injection might result in adverse reaction such as lumps and abscess at injection sites (Verma and Jaiswal, 1998). Although intranasal HS vaccine has been developed in Myanmar, however, it is still laborious to perform in large scale. Safe and effective vaccines against pasteurellosis are still lacking (Hunt *et al.,* 2000). Therefore there is a need to improve the vaccination approaches. There have been previous studiesdone by (Jesse *et al.,* 2013)on oral inoculation of *P.multocida* using mice model and

shows that it can produce similar clinical signs and pathological lesion in the real host. However, there is still lack of research in immunization against pasteurellosis via oral exposure to the animals. Most of the study is either via subcutaneous route or intraparitoneal route.

Due to the huge economic loses, many research have been carried out to determine which is the protective antigens found in the bacteria. For now, the identified protective antigens are the outer membrane protein-H (OmpH) (Luo*et al.*, 1999), lipoprotein B (Tabatabai and Zehr, 2004), lipopolysaccharide, and one or more iron-responsive OMPs (Ruffolo*et al.,* 1998). However, Boyce and Adler (2006) state that there are "host-respond-proteins" that expressed only during *in vivo* situation and these proteins might be the antigens that able to stimulate full protective immunity against both homogenous and heterogenous infections.

Studying the possible oral immunization of live bacteria in animals can give valuable information regarding the minimal dosage that will protect the host from getting the disease. Besides, this study might result in a new vaccination route where HS vaccine can be given in feed and water. Therefore, this study was conducted to examine the possibility of using oral live bacterium of *P. multocida* to induce immune-protection in mice and the minimal dosage that will protect the host from HS disease.

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