UNIVERSITI TEKNOLOGI MARA

IDENTIFICATION AND IMMUNOGENICITY STUDY OF SOLUBLE PROTEIN DERIVED FROM *PASTEURELLA MULTOCIDA* SEROTYPE B

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CONFIRMATION BY PANEL EXAMINERS

I certify that a Panel of Examiners has met on 15th June 2015 to conduct the final examination of Noor Masyitah Binti Jumahat on her Master of Science thesis entitled "Identification and Immunogenicity Study of Soluble Protein derived from *Pasteurella multocida* serotype B" in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiners recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

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ABSTRACT

Pasteurella multocida (*P. multocida*) serotype B is associated with hemorrhagic septicaemia (HS) disease endemic in Africa, India and Asian countries. It is causative agent of thriftily significant disease in livestock. Hence, this study purposed and aimed to identify and express an immunogenic soluble protein of *P. multocida* in efforts toward development of HS vaccine. Immunogenic soluble protein was identified as lipoprotein B (plpB) using electrospray mass spectrometry. The size of expressed purified recombinant protein was approximately 39kDa. Immunogenicity study of the recombinant protein plpB was carried out using 6 groups of BALB/c mice. The groups were immunized with recombinant protein (Group 1), soluble recombinant protein (Group 2), insoluble recombinant protein (Group 3), vector (Group 4), soluble protein of *P. multocida* (Group 5) and PBS (Group 6) respectively. Mice in group 4 and 6 showed signs and symptom of HS after challenge with the parental strain (p-value < 0.05). However, immunised mice with purified recombinant protein did not show signs and symptoms of HS. Based on immunoblotting analysis, purified recombinant protein was significantly immunogenic (p-value < 0.05). Additionally, no inflammation was seen in the tissues of organs from mice immunized with purified recombinant protein, which indicates that recombinant protein was 100% protective towards *P. multocida* infection and eventually towards HS disease. Thus, this study shows that the recombinant protein lipoprotein B (plpB) is significantly immunogenic and could be a potential candidate in developing vaccine against HS.

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CHAPTER ONE
INTRODUCTION

1.1 RESEARCH BACKGROUND

In 1887, Trevisan pristinely proposed and described genus *Pasteurella*. It is a group of nonmotile, minute (0.7μm by 0.5μm) gram negative coccobacilli. It often exhibits a characteristic type of bipolar staining (Albrecht et al., 1996). The first pathogen to be analyzed which is called *Pasteurella septic* was shown to cause hemorrhagic septicaemia (Bain et al., 1982) in cattle and sheep and fowl cholera in chickens (Curtis, 1979). Pasteur utilized it for his vaccination studies in 1880 (Trevisan, 1887) and it is now called *Pasteurella multocida* (*P. multocida). P. multocida* is a heterogeneous species which can cause respiratory diseases in domestic and wild animals (Jubb et al, 1985).

P. multocida is named in honor of Louis Pasteur who in a classic experiment in the early 1880s attenuated the agent and so produced the first deliberately developed a vaccine (Rimler, 2001). *P. multocida* has a pathogenic potential in vertebrate animals (Dziva et al., 2008). It is a commensal of the upper respiratory tract of many animal species too. Then again, under inclining conditions the bacterium is the etiological operator of extensive variety of monetarily weighty infections, incorporating fowl cholera in poultry, haemorrhagic septicaemia in dairy calves and bison, atrophic rhinitis in swine and sniffles in rabbits. The living being is moreover apperceived to be the contributory executor of Pasteurellosis in American cow, yak, deer, elephants, camels, stallions, moose and other savage creatures. Besides, *P. multocida* can cause a zoonotic infection in man. It is conventionally because of bites or scratches from pets such as cats and dogs (Hawkins, 1969).

P. multocida comprises of five capsular type A, B, D, E, and F and there is association between the capsular type and illness (Carter, 1952; Boyce et al., 2000). Serotype A causes fowl cholera in avian species and enzootic bronchopneumonia or pneumonic pasteurellosis in bovine. In pigs, atrophic rhinitis plus pneumonia are related essentially by toxigenic strains of serotype D and serotype A, respectively. *P. multocida* serotype B: 2, B: 2, 5 and B:5 cause hemorrhagic septicemia in dairy