UNIVERSITI PUTRA MALAYSIA

DETERMINATION SEVERITY OF PNEUMONIA, RESPONSES OF HEAT SHOCK PROTEIN 90 (HSP 90) AND CORTISOL CONCENTRATIONS IN VACCINATED AND NON-VACCINATED PNEUMONIC AND NON-PNEUMOIC GOATS

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FPV 2018 4

DETERMINATION SEVERITY OF PNEUMONIA, RESPONSES OF HEAT SHOCK PROTEIN 90 (HSP 90) AND CORTISOL CONCENTRATIONS IN VACCINATED AND NON-VACCINATED PNEUMONIC AND NON-PNEUMOIC GOATS

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Determination Severity of Pneumonia, Responses of Heat Shock Protein 90 (HSP 90) and Cortisol Concentrations in Vaccinated and Non-Vaccinated Pneumonic and Non-Pneumonic Goats", by Ahmad HafizinBin Ahmad Tarmizi Tan and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project

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DEDICATION

This project paper is dedicated:

To my parents and family,
A small gift for the unending support and affection

To FPV UPM lecturers and staffs,
Especially to Dr Jesse for the support and advice,
and Mr Jefri as well

To my friends,
Supportive DVM 2018 course mates,
and not to forget Vet Buddies

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LIST OF ABBREVIATIONS

%	Percent
^{o}C	Celsius
pg	Picogram
ml	Millilitre
µl	Microlitre
nm	Nanometre
HRP	Horseradish Peroxidase
O.D	Optical density
RPM	Revolutions per minute
ELISA	Enzyme-Linked Immunosorbent Assay
OAV	Oil Adjuvanted Vaccine
APV	Alum Precipitated Vaccine
HSP 90	Heat Shock Protein 90
ANOVA	Analysis OfVariance
et al.	*et alli* (and others)
UPM	Universiti Putra Malaysia
TPU	Taman PertanianUniversiti

ABSTRAK

Abstrak kertas projek dikemukakan kepada Fakulti Perubatan Veterinar, Universiti Putra Malaysia untuk memenuhi keperluan untuk kursus VPD 4999 – Projek Ilmiah Tahun Akhir

PENILAIAN TAHAP KETERUKAN PNEUMONIA, RESPON PROTEIN KEJUTAN HABA 90 (HSP) DAN KORTISOL DALAM KUMPULAN KAMBING YANG DIVAKSIN DAN YANG TIDAK DIVAKSIN YANG DIJANGKITI DAN TIDAK DIJANGKITI PNEUMONIA

Oleh

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Pasteurellosis pneumonik adalah penyakit pernafasan yang biasa dihadapi dalam ruminant kecil, samaada kambing atau bebiri dan agen penyebab umum adalah *Mannheimiahaemolytica* dan *Pasteurella multocida*. Program vaksinasi dilihat sebagai cara yang paling kosefektif untuk mengawal penyakit ini. Protein kejutan haba 90 (HSP 90) adalah protein tekanan yang melibatkan banyak fungsi termasuk perlindungan sel daripada tekanan. Oleh itu peningkatan expresi dijangka apabila haiwan terdedah kepada rangsangan stres. Kortisol adalah glucocorticoid yang juga bertindak balas terhadap rangsangan stress dan peningkatan kepekatan dijangka dapat dilihat dalam keadaan yang tertekan. Terdapat

kekurangan maklumat tentang keterukan radang paru-paru dan tindakbalas protein kejutan haba 90 (HSP90) dan kepekatan cortisol dalam kambing pneumonia yang divaksin dan tidak divaksin. Oleh itu, kajian ini telah dirancang di mana sebanyak 76 ekor kambing dipilih daripada empat lading ruminant kecil yang terpilih.Kambing-kambing tersebut dikelompokkan kepada tiga kumpulan iaitu kumpulan vaksin dan tidak divaksin yang tidak menunjukkan tanda-tanda klinikal, kumpulan pneumonik yang divaksin dan kumpulan pneumonik yang tidak divaksin berdasarkan pemeriksaan klinikal. Keparahan radang paru-paru ditentukan berdasarkan pemarkahan auskultasi dan keterukan dikelaskan sebagai ringan, sederhana dan teruk. Sampel darah diambil dari kambing-kambing dan analisis serum kortisol dan protein kejutan haba 90 dibuat menggunakan teknik ELISA. Hasil kajian menunjukkan bahawa kambing pneumonia yang tidak divaksin mempunyai skorauskultasi yang lebih tinggi dan dikelaskan sebagai tanda-tanda radang paru-paru klinikal yang sederhana dan parah manakala kambing pneumonia yang divaksin menunjukkan skor pneumonia ringan. Purata kepekatan HSP 90 untuk kumpulan vaksin dan tidak divaksin yang tidak menunjukkan tanda-tanda klinikal dan kumpulan pneumonik yang divaksin adalah 32.9pg / ml ± 4.21 dan 33.78pg / ml ±5.71 masing-masing dan kira-kira 1.6 kali peningkatan dalam kepekatan yang dilihat dalam kumpulan pneumonik yang tidak divaksin (54.76pg /mlets±13.6). Purata kepekatan kortisol ialah 17.74pg / ml ± 4.43 dan 22.98pg / ml ± 4.71 dalam kumpulan vaksin dan tidak divaksin yang tidak menunjukkan tanda-tanda klinikal dan kumpulan pneumonik vaksin masing-masing dan hanya 19.67pg / ml ± 3.37 dalam kumpulan pneumonik yang tidak divaksin. Analisis statistik HSP 90 dan kepekatan cortisol menunjukkan tidak terdapat perbezaan yang ketara antara tiga kumpulan. Kesimpulannya, kambing yang dijangkiti penyakit radang paru-paru, kumpulan kambing yang divaksin menunjukkan responselhos yang ringan berbanding kumpulan kamping yang tidak divaksin.

Kata kunci: *Vaksin pneumonia, tahap keterukan, auskultasi, HSP 90, kortisol*

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in requirement for the course VPD 4999 – Final Year Project

DETERMINATION SEVERITY OF PNEUMONIA, RESPONSES OF HEAT SHOCK PROTEIN 90 (HSP 90) AND CORTISOL CONCENTRATIONS IN VACCINATED AND NON-VACCINATED PNEUMONIC AND NON-PNEUMOIC GOATS

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Pneumonic pasteurellosis is a common respiratory disease encountered in small ruminants, either in goats or sheep and the common causative agents are*Mannheimiahaemolytica* and *Pasteurella multocida*. Vaccination program is seen to be the most cost-effective way to control the disease. Heat shock protein 90 is a

stress protein that involves in many functions including protection of cells from stressors, therefore increase expression is expected when the animal is exposed to stress stimuli. Cortisol on the other hand, is a glucocorticoid that also response towards stress stimuli and therefore increase level is expected to see in stressful conditions. There is paucity of information on theseverity of pneumonia and responses of heat shock protein 90 (HSP90) and cortisol concentrations in vaccinated and non-vaccinated pneumonic goats. Therefore, this study was designed where total of 76 goats were selected in this study from selected four small ruminant farms. The animals were grouped into three groups namely normal vaccinated and non-vaccinated group, vaccinated pneumonic group and non-vaccinated pneumonic group based on clinical examination. Severity of pneumonia was determined based on scoring of the auscultation and the severity was classified as mild, moderate and severe. Blood samples were collected from these goats and the samples were subjected for heat shock protein 90 (HSP90) and serum cortisol analyses using ELISA technique. The result of this study showed that the non-vaccinated pneumonic goats had higher auscultation score and were classified as moderate to severe clinical signs of pneumonia. The vaccinated pneumonic goats revealed mild pneumonia score. Mean concentration of HSP 90 for normal vaccinated and non-vaccinated group and vaccinated pneumonic group were 32.9pg/ml±4.21 and 33.78pg/ml±5.71 respectively and approximately 1.6 time increased in the concentration observed in non-vaccinated pneumonic group (54.76pg/ml±13.6). For the mean cortisol concentration were 17.74pg/ml±4.43, 22.98pg/ml±4.71 in normal vaccinated and non-vaccinated group and vaccinated pneumonic group respectively and only 19.67pg/ml±3.37 in non-vaccinated pneumonic group.Statistical analysis of

HSP 90 and cortisol concentration showed there was no significant difference between three groups. In conclusion, the vaccinated group showed mild host cell responses compared to non-vaccinated group in pneumonic goats.

Key words: *Pneumonic Vaccine, severity, auscultation, HSP 90, cortisol*

1.0 INTRODUCTION

Small ruminant farming in Malaysia is a fourth major producer in livestock industrywhich has seen steady increase of output of livestock product of goats and sheep from 2007 until 2011 and linear trend onwards due to increasing trend of per capita consumption of mutton in Malaysia (Department of Veterinary Services, 2017). However, the small ruminant industry is still far behind compared to poultry, swine and cattle industry, as indicated in self-sufficiency level in Malaysia (Department of Veterinary Services, 2017). The reason can be due to majority of goat farmers are smallholder farmers, thus the production output is relatively small and can be contributed by the farm management (Alina Yusoff, Man, and MohdNawi, 2016). Farm husbandry especially in term of management of herd health program, is still lacking among the farmers(Jesse, *et al*, 2015)and therefore development of respiratory diseases is still persist due to presence of multiple stressors.

Pneumonia is one of the notable diseases in small ruminant that has great impact on the animal health thus on the production eventually(Scott, 2017).*Mannheimiahemolytica* is the main causative agent of the respiratory disease in goats, characterized by presence of dyspnea, nasal discharge, pyrexiaand non-specific signs such as depression, lethargy and inappetence (Scott, 2017). The severity of the disease under field condition is variable. Therefore, it its recommended to perform vaccination on the goats to reduce the risk of contracting the disease(Jesse, *et al*., 2014). The vaccines should offer cross protection against

serotypes A2, A7 and A9 for optimum protection as in the use of recombinant vaccine (Sabri, *et. al*, 2012).

Stress is one of the predisposing factors causing the disease in goats. According to Mohamed and Abdelsalam, (2008), stress is the intrinsic condition causing increase susceptibility to infectious diseases due to break down of immunity and can be measured clinically and biochemically. Useful clinical parameters include elevation of heart rate, respiratory rate and body temperature, whereas cortisol, glucose, urea and free fatty acids are useful biochemical parameters to be measured (Mohamed and Abdelsalam, 2008). Stressful factors like transportation stress, high stocking density in pens, environmental changes and concurrent viral infections increase the susceptibility to the disease (Jesse, *et al*., 2014).On the other hand, the most common encountered stressors according to Mohamed and Abdelsalam, (2008) are high humidity environment with extreme cold or hot temperature, poor ventilation, poor management, transportation and overcrowding in small pens.

Cortisol is one of the parameters used in many experimental studies to evaluate the response of the parameter towards the stress factors as seen studies conducted by Ali *et. al*, (2005) andGreenwood and Shutt, (1992). The previous stated studies, mainly investigate the role of acute stress on the cortisol level, whereas studies on chronic stress is more looked into in this study. Another parameter of interest to study the stress response is heat shock protein 90 (HSP 90). Heat shock proteins play important role in physiological processes (Allison, 2012), and also expressed during exposure to stress. Several studies have been done to

study the role of heat shock proteins in response to exposure of stress as studied by Dangi, *et. al*, (2014) and Gaughan, *et. al* 2014.

For the present study, the following hypotheses were proposed:

1. There will be significant clinical pneumonic changes between vaccinated and non-vaccinated goats' farms in relation to pneumonia.
2. There will be significant differences in the levels of cortisol and heat shock protein 90 among pneumonic and non-pneumonic goats from the vaccinated and non-vaccinated goat's farms.

The objectives of the study are:

1. To categorize the severity of pneumonia via auscultation by scoring the sounds obtainable at the lung region.
2. To determine the levels of heat shock protein (HSP) 90 and cortisolamong pneumonic goats from vaccinated and non-vaccinated goat's farms.

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