UNIVERSITI PUTRA MALAYSIA

STUDY ON PATHOGENICITY OF ORF VIRUS STRAIN UPM 1/14 AND 2/14 IN MICE AT DIFFERENT SITES OF INOCULATION WITH AND WITHOUT DEXAMETHASONE TREATMENT

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STUDY ON PATHOGENICITY OF ORF VIRUS STRAIN UPM 1/14 AND 2/14 IN MICE AT DIFFERENT SITES OF INOCULATION WITH AND WITHOUT DEXAMETHASONE TREATMENT

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CERTIFICATION

It is hereby certified that we have read this project paper entitled "Study on Pathogenicity of Orf Virus Strain UPM 1/14 and 2/14 in Mice at Different Sites of Inoculation With and Without Dexamethasone Treatment", by Tay Kimmy and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999 – Project

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DEDICATION

This project paper is dedicated

To my family,

Father

Mother

Brother

& Sister

And to all my teachers who have committed themselves towards the noble cause of education.

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It is with deepest appreciation and gratitude that I thank all those who have made this project paper a reality.

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LIST OF ABBREVIATIONS

%	Percent
℃	Degree Celsius
kg	Kilogram
ug	Microgram
mg	Milligram
ml	Milliliter
nm	Nanometer
x g	Times gravity
bp	Base pair
CE	Contagious ecthyma
DNA	Deoxyribonucleic acid
G+C	Guanine+Cytosine
kbp	Kilo-base pair
MDBK	Mardin-Darby bovine kidney
MDOK	Mardin-Darby ovine kidney
ORFV	Orf virus
p.i.	Post-inoculation
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
TAE	Tris-acetate-EDTA
UPM	Universiti Putra Malaysia

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 –Projek Tahun Akhir

PENYELIDIKAN KEPATOGENAN ORFV UPM 1/14 DAN 2/14 PADA MENCIT DI TEMPAT INOKULASI YANG BERLAINAN DENGAN DAN TANPA RAWATAN DEXAMETHASONE

Oleh

Tay Kimmy

2016

Penyelia: Prof. Dato' Dr. Mohd Azmi Mohd Lila

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Dr. Faez Firdaus Jesse Abdullah

Sejak kebelakangan ini, dua jenis ORFV (UPM 1/14 Malaysia; UPM 2/14 Malaysia) telah diasingkan tetapi tiada penyelidikan telah dijalankan pada mencit dengan menggunakan dua jenis ORFV ini. Penyelidikan ini bertujuan untuk memerihalkan kesan dua jenis ORFV ini, tempat inokulasi serta kesan dexamethasone pada kepatogenan jangkitan ORFV pada mencit. Suntikan intradermis 0.2ml 1% UPM 1/14 Malaysia (Kumpulan 1) dan UPM 2/14 Malaysia (Group 2) telah dilakukan dalam

kumpulan berlainan yang terdiri daripada 5 mencit dalam Kumpulan 1 dan Kumpulan 2 di dorsum (Group 1A; Kumpulan 2A), cuping telinga (Group 1B; Kumpulan 2B) dan sudut bibir (Group 1C; Kumpulan 2C). Suntikan intradermis 0.2ml 1% UPM 1/14 Malaysia telah dilakukan dalam kumpulan dexamethasone (n=5) dan kumpulan bukan dexamethasone (n=5). Mencit dalam kumpulan dexamethasone dirawat dengan dexamethasone, 5mg/kg/hari, intraperitoneum tiga hari sebelum cabaran ORFV dan diteruskan sehingga hari kelima selepas cabaran ORFV. Secara umum, hiperemia diperhatikan dalam semua kumpulan rawatan. Hasil statistik menunjukkan tiada perbezaan yang signifikan dalam min lesi skor antara kumpulan tempat inokulasi ($p>0.05$) dan antara kumpulan dexamethasone dan kumpulan bukan dexamethasone ($p>0.05$). Kumpulan 1 dan Kumpulan 2 juga menunjukkan tiada perbezaan yang signifikan dalam min lesi skor ($p>0.05$). Sungguhpun begitu, Kumpulan 2B dan Kumpulan 2C mempunyai min stratum ketebalan yang lebih tinggi ($p<0.05$). Secara keseluruhan, pemeriksaan histopatologi menunjukkan keratosis, akantosis dan penggelembungan degenerasi. ORFV telah dikesan dalam tisu kulit mencit yang menunjukkan lesi kulit melalui tindak balas reaksi rantai polimerase (PCR). Kesimpulannya, inokulasi intradermis dengan menggunakan kedua-dua ORFV tempatan ini mampu menghasilkan lesi kulit dan perubahan histopatologi pada mencit. Selain itu, terdapat tiada kesan yang berbeza pada kepatogenan jangkitan ORFV dengan menggunakan tempat inokulasi yang berlainan pada mencit. Dalam penyelidikan ini, dexamethasone tidak mempunyai kesan yang signifikan pada lesi kulit ORFV. Oleh itu,

drug alternatif seperti cyclosporin boleh dicadangkan untuk mengganti dexamethasone dalam kajian dari segi aspek ini.

Kata kunci: Virus Orf, mencit, tempat inoculasi, dexamethasone, reaksi rantai polimerase (PCR)

ABSTRACT

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

STUDY ON PATHOGENICITY OF ORF VIRUS STRAIN UPM 1/14 AND 2/14 IN MICE AT DIFFERENT SITES OF INOCULATION WITH AND WITHOUT DEXAMETHASONE TREATMENT

by

Tay Kimmy

2016

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Dr. Faez Firdaus Jesse Abdullah

Recently, two local ORFV strains (UPM 1/14 Malaysia; UPM 2/14 Malaysia) have been isolated. However, there is no study done in mice using these strains. This study aims to describe the effect of different ORFV strains and inoculation sites as well as dexamethasone effect on pathogenicity of ORFV in mice. Intradermal injection of 0.2ml 1% UPM 1/14 Malaysia (Group 1) and UPM 2/14 Malaysia (Group 2) were done in each group of 5 mice in Group 1 and Group 2 at dorsum (Group 1A; Group 2A), ear

pinna (Group 1B; Group 2B) and labial commissure (Group 1C; Group 2C). Intradermal injection of 0.2ml 1% UPM 1/14 Malaysia was performed in dexamethasone group (n=5) and non-dexamethasone group (n=5). Mice in dexamethasone group were treated with dexamethasone, 5mg/kg/day, intraperitoneally three days prior to challenge and continued until day five post-challenge. In general, mild hyperaemia was observed in all treatment groups. There were no significant difference in mean lesion score among the groups of inoculation site ($p>0.05$) and between dexamethasone-treated group and non-dexamethasone group ($p>0.05$). Mice in Group 1 and Group 2 showed no significant difference in mean lesion score as well ($p>0.05$). However, mice in Group 2B and Group 2C had significantly higher mean stratum thickness ($p<0.05$). Overall histopathological examination revealed keratosis, acanthosis and ballooning degeneration. ORFV was detected by means of PCR on skin tissues of mice with skin lesions. In conclusion, intradermal inoculation of both local strains is able to produce mild skin lesion and histopathological changes in mice. Besides, there is no significant effect of variation in inoculation sites on pathogenicity of ORFV in mice model. In this study, dexamethasone has no statistical effect on pathogenicity of ORFV. Therefore, alternative drug such as cyclosporine can be used for further studies on this aspect.

Key words: Orf virus strains, mice, inoculation site, dexamethasone, Polymerase Chain Reaction (PCR)

1.0 INTRODUCTION

Orf virus (ORFV) is the prototype species of the genus *Parapoxvirus* (PPV) of the *Poxviridae* family that includes *Pseudocowpox* (PCPV), *Bovine papular stomatitis virus* (BPSV) and the *Parapoxviruses of red deer in New Zealand* (PVNZ) (Fleming *et al*., 2007). It is the etiological agent of contagious ecthyma (CE), a severe exanthematic dermatitis that affects domestic and wild small ruminants (Peralta *et al*., 2015). It is commonly known as contagious pustular dermatitis, scabby mouth, sore mouth or orf (Fleming *et al*., 2007). It has also been reported in camels and camelids, members of the Cervidae family and various other ruminants (chamois, serows, tahr, steenboks); dogs, cats and squirrels can also been affected (Spyrou *et al*., 2015). The disease also has a zoonotic potential particularly to people working with animals such as veterinarians, farmers and animal attendants (Kumar *et al*., 2013).

Orf virus usually gains access to host's tissue through breaks and abrasions of skin and replicating in regenerating epidermal keratinocytes (Markey *et al*., 2013). This viral replication will result in oedematous and granulomatous inflammation of dermal cells (Spyrou *et al*., 2015). It is clinically recognized by the appearance of papules, vesicles, pustules and rapidly growing scabs confined to the lips and muzzle of the affected animals (Cargnelutti *et al*., 2011). CE is not usually lethal, and lesions typically disappear within 2 to 4 weeks, but death may result if secondary complications, such as bacterial infections or myiasis, develop (Wilson *et al*., 2012). The most frequent

invaders includes Staphylocci, alpha haemolytic Streptococci and Corynebacteria. *Dermatophilus congolensis* and *Fusobacterium necrophorum* can also be found (Nandi *et al*., 2010). Morbidity rates can be up to 70% in flocks where the disease occurs for the first time (Zhao *et al*., 2010). Besides disruption of the national and international trade of animal and animal products, the lesions produced can also affect the optimum productivity and reduce the market value of the meat, leather and wool (Nandi *et al*., 2010). In immunocompromised animals, extensive and recurrent lesions can occur (Guo *et al*., 2003). This will undoubtedly produce certain economic loss to small stock farming. Although gross clinical signs can be used as a good reference to diagnose this disease, the gold standard is to carry out virus isolation (Chan *et al*., 2003).

Many researches had been done to study the unique genes of ORFV and to develop functional vaccines. These advances would not be possible without the use of laboratory animals such as mice. According to Cargnelutti *et al*. (2010), clinical lesions were successfully reproduced accompanied by virus isolation in mice inoculated with ORFV despite consistent failure by other researchers. This gives rise to the questions where choice of viral strains and sites of inoculation can be the determining factors for successfulness of ORFV research in mice model. Isolation of caprine ORFV was carried out recently to give more insight into the Orf viral strains in Malaysia and ORFV strain UPM 1/14 Malaysia, ORFV strain UPM 2/14 Malaysia and ORFV strain UPM 3/14 Malaysia had been isolated (Abdullah *et al*., 2015). To date, there is still lack of work in determining the effect of these viral strains differences on pathogenicity in mice

model. Besides, Dexamethasone has the ability to suppress immune function thereby increases susceptibility to infections and their severity. Thus, this study is to:

1. Determine the severity of ORFV (UPM 1/14 and UPM 2/14) in mice.

2. Determine differences in lesion produced following different inoculation sites in mice.

3. Study the effects of Dexamethasone (simulating stress/non-stress situations) on the severity of Orf.

For this research, the following hypotheses were proposed:

1. ORFV infection in mice causes relevant skin lesions similar to that of the natural host.

2. Different inoculation sites resulted in different disease severity.

3. Use of Dexamethasone resulted in more severe ORFV lesion in mice.

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