UNIVERSITI PUTRA MALAYSIA

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

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

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It is hereby certified that we have read this project paper entitled "Study on Pathogenicity of OrfVirus Strain Upm 1/14 Malaysia and Upm 2/14 Malaysia in Rat via Different Inoculation Sites with and without Dexamethasone Treatment", by Chook Chian Lin 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- Final Year Project.

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DEDICATION

I would like to dedicate my humble effort to my beloved family for their support and love. A special feeling of gratitude I send to both of parents who inspire me and siblings who provide joy in my life.

I also like to dedicate this dissertation to all my friends who are always beside me whenever I need them. They are always great cheerleaders. Besides, I also dedicate this work and give special thanks to my supervisor and co-supervisor for their guidance and knowledge.

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KAJIAN KEPATOGENAN TERIKAN ORF VIRUS UPM 1/14 MALAYSIA DAN UPM 2/14 MALAYSIA KEPADA TIKUS MELALUI PERBEZAAN LOKASI INOKULASI DENGAN DAN TANPA RAWATAN DEXAMETHASONE

Oleh

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2016

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

Orf virus (ORFV) menyebabkanpenyakitektimamenular yang mengakibatkankerugiandalamsektorekonomi. Kajian ORFV amatpentingtetapikajianmenggunakantikuskurangditerokai.Melaluikajianini, kepatogenan ORFV kepadatikusdinilaiberdasarkankesandaripadaduaterikan virus tempataniaitu ORFV UPM 1/14 Malaysia dan ORFV UPM 2/14 Malaysia, lokasiinokulasi, sertapenindasanimmunisasi.Inokulasisecara intradermal dengan 0.5 ml 1% ORFV UPM 1/14 Malaysia (Kumpulan 1) dan ORFV UPM 2/14 Malaysia (Kumpulan 2) telahdilaksanakandalamkumpulan 1 dankumpulan 2 yang

terdiridaripada 5 tikusmasing-masingpadakulit dorsum (Kumpulan 1A; Kumpulan 2A), dauntelinga (Kumpulan 1B;Kumpulan 2B) sertasudutbibir (Kumpulan 1C; Kumpulan 2C). Selainitu, inokulasisecara intradermal dengan 0.5ml 1% ORFV UPM 1/14 Malaysia telahdilaksanakandalamkumpulan dexamethasone (n=5) dankumpulan non-dexamethasone (n=5). Tanda-tandaklinikaldanperubahanhistopatologitelahdinilaiselama 14 haribagikumpulan 1 dankumpulan 2 manakala 7 haribagikumpulan dexamethasone dankumpulan non-dexamethasone. Hyperemia sederhanatelahdidapatipadakulit dorsum, dauntelingadansudutbibirdaripada 27 tikusdalamkumpulanrawatan. Kumpulan 1 mempunyai rata skorkelukaankulit yang lebihsignifikantinggi($p<0.05$) daripada Kumpulan 2. Kumpulan 1A mempunyai rata skorkelukaankulit yang lebihsignifkantinggi($p<0.05$) daripada Kumpulan 1B dan Kumpulan 1C. Kumpulan dexamethasone jugamempunyai rata skorkelukaankulit yang lebihsignifkantinggi($p<0.05$) daripadakumpulan non-dexamethasone. Keratosis, acanthosisdandegenerasijenisbelontelahdiperolehidaripadatikus yang menunjukkankelukaankulitdalamkumpulanrawatan. Kumpulan dexamethasone mempunyai rata ketebalanlapisanselspinosumdanlapisansel basal sudutbibir yang lebihsignifikantinggi($p<0.05$) berbandingdengankumpulan non-dexamethasone. Rata ketebalanlapisanselspinosumdanlapisansel basal kulit dorsum, dauntelingadansudutbibirtidakmempunyaiperbezaaansignifikanantarakumpulan 1 dankumpulan 2. ORFV telahdikesandaripadakulittikus yang mempunyaikelukaankulitdalamkumpulanrawatandenganteknikreaksirantaipolimerase

(PCR). Kesimpulannya, kepatogenan ORFVmampudihasilkandalamtikusdaniaberbezadisebabkanolehterikan virus, lokasiinokulasidanrawatan dexamethasone. Tikusbolehdigunakansebagai model pengajian ORFV.

Kata kunci: Orf virus,ORFV UPM 1/14 Malaysia, ORFV UPM 2/14 Malaysia, tikus, kepatogenan, lokasiinokulasi, penindasanimmunisasi

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999- Final Year Project.

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

By

Chook Chian Lin

2016

In this study, pathogenicity of ORFV in rat was evaluated by using of two virus strains, ORFV UPM 1/14 Malaysia and ORFV UPM 2/14 Malaysia with variation in inoculation sites and the effects of induced-immunosuppression. Intradermal inoculation of 0.5 ml 1% ORFV UPM 1/14 Malaysia (Group 1) and ORFV UPM 2/14 Malaysia virus suspension (Group 2) were performed in each group of 5 rats 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) respectively. Intradermal inoculation of 0.5 ml 1% ORFV UPM 1/14 Malaysia virus suspension

was performed in dexamethasone-induced immunosuppressed group (n=5) and non-dexamethasone group (n=5). Clinical signs and histopathological changes were evaluated for 14 days post virus inoculation for rats in Group 1 and Group 2 and 7 days for dexamethasone-induced immunosuppressed group and non-dexamethasone group. Mild hyperemia was observed in dorsum, ear pinna and labial commissure of 27 rats in the treatment group. Rats of Group 1 had significantly higher ($p<0.05$) mean skin lesion scores than Group 2. Rats of Group 1A had significantly higher ($p<0.05$) mean skin lesion scores than Group 1B and Group 1C. Dexamethasone-treated group had significantly higher ($p<0.05$) mean skin lesion scores than non-dexamethasone group. Keratosis, acanthosis and ballooning degeneration were observed in rats showed skin lesions in the treatment group. Dexamethasone-treated group had significantly higher ($p<0.05$) mean thickness of stratum spinosum and stratum basale of labial commissure than non-dexamethasone group. There was no significant difference ($p>0.05$) of mean thickness of stratum spinosum and stratum basale of dorsum, ear pinna and labial commissure between Group 1 and Group 2. ORFV was detected by means of PCR on skin tissues of rats with skin lesions in rats. In conclusion, ORFV is pathogenic in rats, and it varies due to strains, inoculation sites and dexamethasone treatment. Disease and lesions produced in rats are similar to that of the normal hosts. Thus, rat is a suitable laboratory animal model to study OFRV infection.

Keywords: Orf virus,ORFV UPM 1/14 Malaysia, ORFV UPM 2/14 Malaysia, rat, pathogenicity, inoculation sites, immunosuppression

1.0 INTRODUCTION

Contagious ecthyma is caused by Orf virus (ORFV) infection. ORFV is species of the genus *Parapoxvirus* which belong to the family Poxviridae and subfamily Chordopoxvirinae. There are several alternative names of contagious ecthyma, which are orf, soremouth, scabby mouth and contagious pustular dermatitis (Smith& Sherman, 2009). The viral infection cause erythematous spots at the beginning, and then formation of papules, vesicles, pustules and scabs which finally become dry and shed. (Spyrou&Valaikos, 2015) Although it is self-limiting, it is an important disease due to its contagious characteristic, zoonotic potential, world-wide distribution and economic importance.

Regarding on its contagious characteristic and zoonotic potential, the virus is transmitted through direct contact via damaged skin, and then replication occurs in epidermal cells. Transmission usually occurs during grazing and through abrasions developed on lips, nostrils and mouth (Spyrou&Valiakos, 2015). Although the disease affects primarily sheep and goat, it has been reported in other animals too, such as camels and camelids, chamois, serows, tahr, steenboks, deer, reindeer, bighorn sheep, dall sheep, musk oxen, mountain goats, dogs, cats and squirrels (Spyrou&Valiakos, 2015). Besides, it is zoonotic. It causes occupational hazard to people working with the animals. According to Spyrou&Valiakos(2015), it is affecting people who are in direct or indirect contact with infected livestock such as farmers, veterinarians, animal caretakers.

As mentioned in the earlier paragraph, ORFV is a pathogen with world-wide distribution which affects livestock economics. According to Essbauer*et. al.*(2010), its world-wide distribution is described with incidence up to 90%. Economic impact of ORFV infection is undoubtedly significant. Haig and Mercer (1998) stated that in severely affected and young animals, the disease reduces food intake, leading to transient growth impairment, and consequently resulting in economic losses. In addition, morbidity is high which is up to 70 % in flocks which the disease is occuring for the first time (Spyrou&Valiakos, 2015). Although mortality is low which is less than 1 % (Spyrou&Valiakos, 2015), but complications such as myiasis (Housawi& Abu Elzein, 2000), co-infections with papilloma virus and sheep pox virus (Spyrou and Valiakos, 2015) and secondary bacterial infections (Zhao et al., 2010) increase the severity, leading to more treatment costs and labour costs.

ORFV infection undoubtedly impairs the development of small ruminant industry in Malaysia with its contagious ability, zoonotic potential and significant economic impact. According to AADGN country report 2013/14, there are only 8195 heads of dairy goats in Peninsular Malaysia, and the ex-farm price of goat milk is RM 20/liter as compared to cow milk of RM 2.20/ liter. Expansion and development of small ruminant industry is impaired by orf disease which is one of the common diseases in small ruminant. Therefore, study of ORFV is extremely important.

Malaysia strains of ORFV should be studied as they are the specific etiological agents involved in outbreak of contagious ecthyma which affects the small ruminant industry in Malaysia. ORFV UPM 1/14 MALAYSIA and ORFV UPM 2/14 MALAYSIA are suggested in this study. These two ORFV strains have been isolated in the study entitled Isolation and phylogenetic analysis of caprineorf virus in Malaysia done by Ashwaq*et al.*(2015) which claimed to be the first study that sequenced partial genome data of ORFV isolated in Malaysia with B2L genes and F1L genes. Their relationships with the existing strains in the database were determined, and the findings were close homology to the Chinese and Indian strains in term of DNA sequence.

ORFV can be studied with laboratory animal model which provide benefits. Studies of biology of ORFV is impaired by the difficulty to find seronegative normal hosts, which are goat and sheep(Cargnelutti, *et al.,* 2010). Experiment in goat or sheep is more expensive. There are also difficulties in obtaining a non-endemic farm. Moreover, studies on suitable animal models would also benefit vaccine and antiviral drug development and testing (DalPozzo*et al*., 2007).

ORFV is underexplored in rat model. This study focuses on the study of pathogenicity of ORFV in rat model to determine more suitable animal models. More variations in suitable animal model undoubtedly will help in more future ORFV study. Virus strain and inoculation sites that are able to provide significant effect in rat are

unexplored. Therefore, by determining the strains and sites of inoculation that produce positive resultin rat, more and more studies can be done.

The objectives of this study are:-

1. toevaluate suitability of rat as animal model for ORFV infection.
2. to determine the effect of inoculation sites on disease development
3. to determine the effect of dexamethasone treatment simulating a stress and non-stress situations on the severity oforf disease.

The hypotheses of this study are:-

1. Different inoculation sites resulted in different disease severity.
2. Dexamethasone treatment resulted in severe Orf disease.
3. Rat is a good experimental animal model to study ORFV.

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