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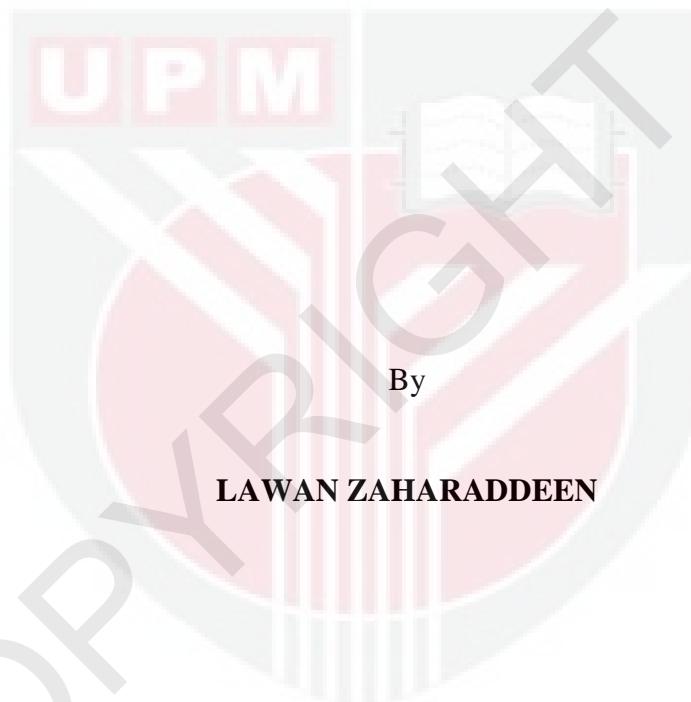
**SEROLOGICAL AND MOLECULAR EPIDEMIOLOGY OF
CONTAGIOUS ECTHYMA AMONG SHEEP AND GOATS IN NEGERI
SEMBILAN, MALAYSIA**

LAWAN ZAHARADDEEN

FPV 2021 8



**SEROLOGICAL AND MOLECULAR EPIDEMIOLOGY OF
CONTAGIOUS ECTHYMA AMONG SHEEP AND GOATS IN NEGERI
SEMBILAN, MALAYSIA**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of
Master of Science**

December 2020

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DEDICATION

The entire this work was dedicated to our beloved prophet Muhammad (S.A.W), His progenies and all those who stand for truth and justice.



Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
of the requirement for the degree of Master of Science

**SEROLOGICAL AND MOLECULAR EPIDEMIOLOGY OF
CONTAGIOUS ECTHYMA AMONG SHEEP AND GOATS IN NEGERI
SEMBILAN, MALAYSIA**

By

LAWAN ZAHARADDEEN

December 2020

Chairman : Professor Mohd Azmi Mohd Lila, PhD
Faculty : Veterinary Medicine

Contagious ecthyma (CE) is a zoonotic and highly contagious epitheliotropic viral disease of goats, sheep and many species of wild ruminants. It is characterized by the development of skin lesions around mouth, lips, ears, nose, and legs. This study is aimed to determine the seroprevalence and possible risk factors associated with CE infection in sheep and goats as well as to determine genetic variation of the virus isolated from scab lesions. A total of 341 serum samples were collected from animals of five (5) different goats and sheep farms. All the farms were selected based on the approval and directive by the Department of Veterinary Services (DVS). The farms were located in Lenggeng (A), Seremban (B), Jelebu (C), Senawang (D), and Mendom (E) in Negeri Sembilan. Only farm C in Jelebu practiced intensive management system while all the remaining farms practiced semi intensive management. The distribution of the animals in relation to 341 samples collected according to the 5 farms are; farm A in Lenggeng with 102 samples (91 goats and 11 sheep), farm B in Seremban with 75 samples all goats, farm C in Jelebu with 60 samples all sheep, farm D in Senawang with 55 samples all goats and farm E in Mendom with 49 samples (36 goats and 13 sheep). In term of gender of the animals, farm A comprises 18 bucks, 73 does, 2 rams and 9 ewes. Farm B has only 31 bucks and 44 does. Farm C has only 3 rams and 57 ewes. Farm D housed 8 bucks and 47 does while farm E comprises of 11 bucks, 25 does, 2 rams and 11 ewes. In term of age of the animals, farm A has 80, 11, 8 and 3 adult goats, kids, adult sheep and lamb respectively. Farm B has only 44 adult goats and 31 kid's contribution to this study. Farm C contributed 53 adult sheep and 7 lamb. Farm D has 48 adult goats and 7 kids while farm E contributed with 30, 6, 11 and 2 adult goats, kids, adult sheep and lambs respectively. Serum samples collected were tested for CE antibody by using of indirect ELISA and serum neutralisation test. Viral culture and molecular confirmation of CE virus was done on six (6) scabs samples obtained from mouth, ear, hooves, and thigh lesions of suspected CE infected animals. Polymerase chain

reaction (PCR) was used to amplify ORFV major envelop glycoprotein (B2L) and immunodominant envelope protein (F1L) genes following extraction of the viral genomic DNA from the processed scab lesions. The virus was first isolated in lamb testicular cells (LT) and Vero cells. The isolate UPM/2019-NS2 obtained from a goat was propagated in Lamb Testicular cells. It was used as a standard viral antigen for the indirect ELISA plates coating. Further information to evaluate possible risk factors was obtained by using a well-designed questionnaire. Based on the total samples tested, the overall seroprevalence for CE was found 71/341 (20.8%). However, only 45 (63.4%) of the seropositive samples were confirmed to have CE viral neutralizing antibodies as determined by using of SNT. The seroprevalence of CE in goat (24.5%) was significantly ($p < 0.05$) higher than in sheep (9.5%). There was a significant variation in the seroprevalence of CE among the five farms studied with farm D having the highest seroprevalence (30.9%) and the least was observed in farm C (8.3%). Sheep and goat kept in semi-intensive system of management showed significantly ($P < 0.05$) higher seroprevalence than those in intensive system. Chi square test showed that variables: species, gender, age, farm, management system, vector control and proximity of farm to another livestock farm played a significant role in the prevalence of CE. Multivariate logistic regression analysis revealed that species and management system of the livestock farms have a significant influence on the presence of antibody against CE. Goats and animals kept under semi intensive management system were found to be 3.085 and 3.377 times more likely to be infected with CE disease as compared to sheep and those animals under intensive management respectively. Only five (5) out of six (6) scab samples were positive to CE virus as tested by PCR for B2L and F1L genes. All the positive samples were processed for Sanger sequencing and results obtained were blasted against NCBI GenBank database. Phylogenetic analysis of these DNA sequences revealed that CE virus isolates were closely related with orf virus UY15/09 from Uruguay, and also clustered together with orf virus TVCC/Shuhama, orf virus OV-1A82 and orf virus SJ1 from India, USA and China respectively. The isolates were also closely related with previous orf virus isolates obtained from different regions in Malaysia. The percentage of similarity for B2L and F1L gene with other global isolates were used for evolutionary assessment within the range of 98-99% and 97-99% respectively. This study revealed the current seroprevalence and possible risk factors associated with CE in sheep and goats in Negeri Sembilan, Malaysia. Seroprevalence of CE was high in goats compared to sheep. More breeds should be investigated because the antibody level could be differ among breeds. Prophylactic measures should be advocated to farmers by the authorities concerned for effective prevention and control measures.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai
memenuhi keperluan untuk ijazah Master Sains

**EPIDEMIOLOGI SEROLOGI DAN MOLEKULAR PENYAKIT
ECTHYMA BERJANGKIT DALAM BEBIRI DAN KAMBING DI NEGERI
SEMBILAN, MALAYSIA**

Oleh

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Contagious Ecthyma (CE) adalah penyakit kulit berjangkit zoonotik yang boleh menjejaskan kesihatan kambing dan kambing biri-biri serta spesies ruminan yang lain. Ianya boleh dikenal pasti dengan perkembangan lesi pada kulit di sekitar mulut, bibir, telinga, hidung, dan kaki. Kajian ini bertujuan untuk mengetahui tahap prevalensi penyakit dan faktor-faktor risiko yang berkaitan dengan jangkitan CE pada kambing biri biri dan kambing di Negeri Sembilan, Malaysia serta pengesahan virus CE daripada luka-luka yang disyaki. Sebanyak 341 sampel sera dari kambing biri biri dan kambing dikumpul dari lima (5) ladang ruminan kecil: Lenggeng (A), Seremban (B), Jelebu (C), Senawang (D), dan Mendom (E), di mana ianya dipilih secara rawak di sekitar Negeri Sembilan. Antibodi CE telah disaring daripada sera tersebut menggunakan teknik ELISA dan teknik ujian peneutralan serum. Kultur virus dan pengesahan molekular virus CE dilakukan daripada enam (6) luka yang diperoleh daripada mulut, telinga, kuku, dan luka paha yang disyaki kes CE positif. Pengesahan molekular dilakukan dengan menggunakan teknik PCR mengamplifikasi gen glikoprotein utama ORFV (B2L) dan gen imunodominant (F1L) selepas pengekstrakan DNA virus dari supernatan yang diperolehi dari sel LT dan vero menggunakan kit ekstraksi DNA (QIAGEN).

Isolat (UPM / 2019-NS2) dari luka kambing yang dibiakkan dalam sel testis kambing biri biri digunakan sebagai antigen virus untuk teknik ELISA. Maklumat bagi faktor risiko yang berkemungkinan menjangkiti penyakit ini diperolehi dengan menggunakan kaedah soal selidik. Sebanyak 71 (20.8%) daripada 341 sampel sera yang diuji adalah seropositif bagi antibodi yang spesifik bagi virus CE dengan menggunakan teknik ELISA. Walau bagaimanapun, hanya 45 (63.4%) sampel seropositif yang disahkan mempunyai antibodi peneutralan virus CE pada SNT. Seroprevalen CE pada kambing menunjukkan (24.5%) jauh lebih tinggi ($p < 0.05$)

daripada kambing biri biri (9.5%). Terdapat variasi dalam seroprevalen di antara lima ladang yang dikaji di mana ladang D mempunyai seroprevalen yang tertinggi (30.9%) dan terendah di ladang C (8.3%). Kambing biri biri dan kambing yang dipelihara dalam sistem pengurusan separa intensif menunjukkan seroprevalen yang signifikan ($p <0.05$) berbanding dengan sistem intensif. Berdasarkan daripada analisis univariat, spesies, ladang, sistem pengurusan, kawalan vektor dan jarak ladang dengan ladang ternakan lain didapati signifikan secara statistik ($P <0.05$). Regresi multivariat menunjukkan bahawa sistem pengurusan ladang ternakan yang disiasat didapati signifikan secara statistik ($P <0.05$). Haiwan yang berada di bawah pengurusan separa intensif adalah 3 kali lebih berkemungkinan untuk dijangkiti oleh virus CE berbanding dengan haiwan di bawah pengurusan intensif. Hanya lima (5) daripada enam (6) sampel luka yang didapati positif bagi hasil ujian PCR. Semua sampel positif dihantar untuk penjujukan Sanger dan keputusan yang diperoleh dikenalpasti dengan proses ‘BLAST’ dan selanjutnya dihantar ke pangkalan NCBI GenBank untuk mendapat nombor edisi khas. Analisis filogenetik urutan genomic menunjukkan bahawa isolat CE dari Negeri Sembilan berkait rapat dengan virus orf UY15 / 09 dari Uruguay, dan juga bercabang bersama-sama dengan virus orf TVCC / Shuhama, virus orf OV-1A82 dan virus orf SJ1 dari India, Amerika Syarikat dan China berdasarkan gen B2L dan F1L. Di Malaysia, isolat tersebut berkait rapat dengan isolat virus orf sebelumnya yang diperoleh daripada negeri yang berlainan di Malaysia. Peratusan persamaan gen B2L dan F1L dengan isolate-isolat lain yang digunakan untuk penilaian evolusi masing-masing berada dalam julat 98-99% dan 97-99%. Sebagai kesimpulan, kajian ini mendedahkan prevalensi semasa dan kemungkinan faktor risiko yang berkaitan dengan penyakit CE pada kambing biri-biri dan kambing di Negeri Sembilan, Malaysia. Lebih banyak baka haiwan harus diselidik kerana tahap antibodi mungkin berbeza antara baka yang berlainan. Pihak bertanggungjawab harus mensarankan langkah-langkah proaktif kepada petani sebagai langkah pencegahan dan kawalan yang berkesan untuk membendung penyakit tersebut.

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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TABLE OF CONTENTS

	Page
ABSTRACT	i
ABSTRAK	iii
ACKNOWLEDGEMENTS	v
APPROVAL	vi
DECLARATION	viii
LIST OF TABLES	xiii
LIST OF FIGURES	xv
LIST OF APPENDICES	xvii
LIST OF ABBREVIATIONS	xviii
 CHAPTER	
1 INTRODUCTION	1
1.1 Background of the Study	1
1.2 Statement of the Problems	2
1.3 Justification of the Study	3
1.4 Hypotheses	3
1.5 Objectives of the Study	3
2 LITERATURE REVIEW	4
2.1 Contagious Ecthyma	4
2.2 Historical Background of Contagious Ecthyma in the World	4
2.2.1 Contagious Ecthyma Infection in Europe, South America, North America and Australia	5
2.2.2 Contagious Ecthyma Infections in Asia	7
2.2.3 Contagious Ecthyma Infections in Africa	13
2.2.4 Historical Background of Contagious Ecthyma in Malaysia	15
2.3 Host Range	15
2.4 Transmission of CE Infection	16
2.5 Replication Cycle of CE Virus	16
2.6 Clinical Signs of Contagious Ecthyma	17
2.7 Epidemiology of Contagious Ecthyma	17
2.8 Immune Responses to Contagious Ecthyma	18
2.9 Contagious Ecthyma Immune Evasion	18
2.10 Diagnosis of Contagious Ecthyma	19
2.10.1 Serological Tests for CE	19
2.10.2 Molecular Detection of Contagious Ecthyma	20
2.11 Treatment of Contagious Ecthyma Infection	20
2.12 Economic Importance of Contagious Ecthyma	21
2.13 Prevention and Control Measures to Reduce the Global Cases of Contagious Ecthyma	23

3	MATERIALS AND METHODS	25
3.1	Ethical and Consent Approval	25
3.2	Study Area	25
3.3	Farms Selection	25
3.4	Sampling Size	28
3.5	Physical Examination and Sample Collection	29
3.5.1	Physical Examination	29
3.5.2	Blood Collection	29
3.5.3	Scab Lesion Sample Collection and Processing	29
3.6	Risk Factors for Contagious Ecthyma Disease	30
3.7	Serological Screening for Contagious Ecthyma Infection	30
3.7.1	Indirect ELISA	30
3.7.2	Serum Neutralisation Test (SNT)	32
3.8	Isolation of Contagious Ecthyma Virus in Embryonated Chicken Egg (ECE)	33
3.8.1	Specific-Pathogen-Free Embryonated Chicken Egg Inoculation	33
3.8.2	CAM Harvesting	34
3.9	Cell Culture	34
3.9.1	Lamb Testicular (LT) Cell Line	34
3.9.2	Vero Cell Line	34
3.9.3	Cell Line Sub-Culturing	34
3.10	Contagious Ecthyma Virus Isolation in Cell Lines	35
3.10.1	CE Virus Harvesting	35
3.10.2	CE Virus Titration in LT and Vero Cell Lines	35
3.11	Molecular Detection of Contagious Ecthyma Virus	36
3.11.1	Viral DNA Extraction	36
3.11.2	Polymerase Chain Reaction	36
3.11.3	Agarose Gel Electrophoresis	36
3.11.4	Identification of B2L and F1L Genes of CE Virus	37
3.11.5	Phylogenetic Analysis	37
3.12	Data Analysis	39
4	RESULTS	40
4.1	Seroprevalence of Contagious Ecthyma in Small Ruminant	40
4.2	Serum Neutralisation Test (SNT)	48
4.3	Risk Factor Assessment	48
4.4	Contagious Ecthyma Viral Isolation from Scab Supernatant using Embryonated Chicken Egg	52
4.5	Contagious Ecthyma Viral Isolation and Titration in Cell Culture	53
4.5.1	Contagious Ecthyma Virus Isolation in Cell Culture	53
4.5.2	Titration of CE Virus Isolates	55
4.6	Molecular Detection of CE	56
4.6.1	Sanger Sequencing for B2L and F1L Genes	57
4.7	The Phylogenetic Analysis of CE Virus Isolates	63

5	DISCUSSION	67
6	SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH	72
6.1	Summary	72
6.2	Conclusion	72
6.3	Recommendations for Future Research	73
REFERENCES		74
APPENDICES		88
BIODATA OF STUDENT		96
LIST OF PUBLICATIONS		97

LIST OF TABLES

Table	Page
2.1 Contagious ecthyma cases in Europe, South America, North America and Australia	6
2.2 Contagious ecthyma morbidity and mortality rate from different countries of the world	7
2.3 Seroprevalence of contagious ecthyma in some countries of the world	8
2.4 Contagious ecthyma cases in Asia	11
2.5 Contagious ecthyma cases in Africa	14
2.6 Diagnostic methods of contagious ecthyma	22
3.1 Body condition scoring of the animals	29
3.2 The serum titers for serum neutralization test	33
3.3 Primer sequences used for the amplification of B2L and F1L gene fragments of contagious ecthyma virus	36
3.4 Details of published B2L gene sequences used for phylogenetic analysis in this study	38
3.5 Details of published F1L gene sequences used for phylogenetic analysis in this study	39
4.1 Seroprevalence of CE among different categories of small ruminant from Farm A in Negeri Sembilan	41
4.2 Seroprevalence of CE among different categories of small ruminant from Farm B in Negeri Sembilan	42
4.3 Seroprevalence of CE among different categories of small ruminant from Farm C in Negeri Sembilan	43
4.4 Seroprevalence of CE among different categories of small ruminant from Farm D in Negeri Sembilan	44
4.5 Seroprevalence of CE among different categories of small ruminant from Farm E in Negeri Sembilan	45
4.6 Overall seroprevalence of CE among different categories of five small ruminant farms from Negeri Sembilan	47

4.7	The comparison of SNT and indirect ELISA on positive small ruminant sera samples in relation to the farms	48
4.8	Association between seropositivity of CE and host factors	49
4.9	Association between seropositivity of CE and farms associated risk factors	51
4.10	Multivariate logistic regression analysis for the risk of CE virus seropositive among sheep and goats in Negeri Sembilan	52
4.11	Titration of CE virus isolates using TCID ₅₀ in LT and vero cells at second passages	56
4.12	List of Accession Numbers obtained from GenBank for all the Nucleotide Sequences	63

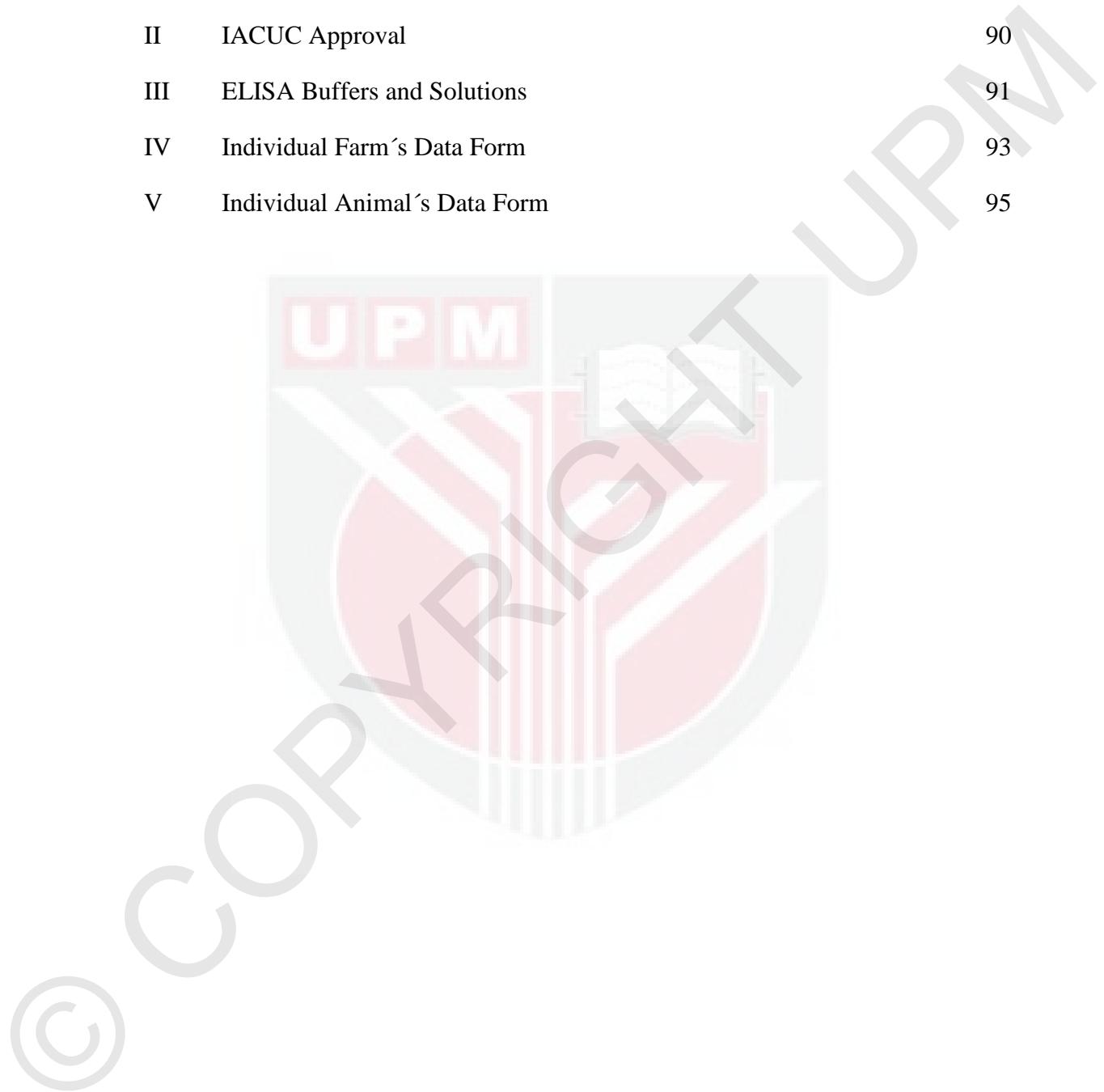
LIST OF FIGURES

Figure	Page
2.1 Transmission cycle of contagious ecthyma	19
2.2 Integrated approach for contagious ecthyma prevention and control	24
3.1 Map of Peninsular Malaysia. Showing area of samples collection as indicated with five-point star	26
3.2 Details location of sampling in Negeri Sembilan, Malaysia	27
3.3 Nature of the livestock houses	27
3.4 Experimental flow chart describing the procedures for seroprevalence and molecular confirmation of CE from selected small ruminant farms in Negeri Sembilan, Malaysia	28
3.5 Suspected cases of contagious ecthyma observed during sample collection	30
4.1 Photomicrograph of embryonated chicken egg (ECE) inoculated with scab lesion supernatant from goats showing CE pathological changes in the CAM associated with CE virus	52
4.2 Photomicrograph of Lamb testicular cells inoculated with supernatant obtained from scab sample of suspected CE infection showing CPE at; (A): Healthy LT cell (B): Infected cells showing rounding of the cells after 48 hours (C): infected cell showing cell attachment and further rounding after 72 hours and (D) Cells detachment from monolayer five days after inoculation at 10x magnification	54
4.3 Photomicrograph of Vero cells inoculated with supernatant obtained from scab sample of suspected CE infection showing CPE at; (A): Healthy vero cell (B): Infected vero cells showing rounding of cells after 48hrs, (C): infected cell vero cells showing cells detachment from monolayer 72 hours after inoculation at 10x magnification	55
4.4 Detection of CE by PCR: gel indicating the product size of 1137 bp after amplification of B2L (major envelop gene) by Polymerase Chain Reaction	56
4.5 Detection of CE by PCR: gel indicating the product size of 1059 bp after amplification of F1L gene by Polymerase Chain Reaction	57
4.6 Comparison of nucleotide sequences of B2L gene of five CE virus isolates (UPM-NS1/19 B2L, UPM-NS2/19 B2L, UPM-NS3/19 B2L, UPM-NS4/19 B2L, UPM-NS5/19 B2L)	60

4.7	Comparison of nucleotide sequences of B2L gene of five CE virus isolates (UPM-NS1/19 F1L, UPM-NS2/19 F1L, UPM-NS3/19 F1L, UPM-NS4/19 F1L, UPM-NS5/19 F1L)	63
4.8	Phylogenetic analysis of the ORFV isolates based on nucleotide sequences of B2L (ORFV011 gene) in comparison to previous ORFV strains globally	64
4.9	Phylogenetic analysis of the ORFV isolates based on nucleotide sequences of F1L (ORFV059 gene) in comparison to previous ORFV strains globally	65
4.10	Phylogenetic analysis of the present isolates of ORFV based on B2L	66

LIST OF APPENDICES

Appendix		Page
I	Cell Culture Media and Buffer	88
II	IACUC Approval	90
III	ELISA Buffers and Solutions	91
IV	Individual Farm´s Data Form	93
V	Individual Animal´s Data Form	95



LIST OF ABBREVIATIONS

AGPT	Agar gel Precipitation Test
ATV	Antibiotic-Trypsin-Versine
BSA	Bovine Serum Albumin
Bp	Base Pair
CE	Contagious Ecthyma
CPDV	Contagious Pustular Dermatitis Virus
CPE	Cytopathic Effect
°C	Degree Celsius
DMSO	Dimethylsulphoxide
DVS	Department of Veterinary Services
DMEM	Dulbecco's Modified Eagle Medium
DNA	Deoxyribonucleic Acid
dsDNA	Double-stranded DNA
EDTA	Ethylenediaminetetraacetic Acid
EEV	Extracellular Enveloped Virion
ELISA	Enzyme-Linked Immunosorbent Assay
FBS	Fetal Bovine Serum
FMDV	Foot-and-Mouth Disease Virus
H ₂ O ₂	Hydrogen Peroxide
HRP	Horse Radish Peroxidase
IACUC	Institutional Animal Care and Use Committee
IBM	International Business Machines Corporation
IgG	Immunoglobulin G
IgM	Immunoglobulin M

Kbp	Kilobase pair
kD	Kilo Dalton
LT	Lamb Testes
Min	Minute
MEGA	Molecular Evolutionary Genetics Analysis
mRNA	Messenger Ribonucleic Acid
MVs	Mature Virions
NaCl	Sodium Chloride
NaHCO ₃	Sodium Hydrogen Carbonate
NCBI	National Center for Biotechnology Information
OD	Optical Density
ORFV	Orf Virus
PBS	Phosphate Buffered Saline
PBST-20	Phosphate Buffered Saline Tween-20
PCR	Polymerase Chain Reaction
pH	Hydrogen Ion Exponent
PI	Post Infection
PPR	<i>Peste des petits Ruminant</i>
%	Percentage
RNA	Ribonucleic Acid
RPM	Revolution Per Minute
SNT	Serum Neutralisation Test
SPSS	Statistical Package for the Social Sciences
TAE	Tris-acetate-EDTA
TCID	Tissue Culture Infective Dose
TEM	Transmission Electron Microscopy

UK	United Kingdom
UPM	Universiti Putra Malaysia
USA	United State of America
UV	Ultraviolet
WV	Wrapped Virion
w/v	Weight per Volume
X ²	Chi Square
µl	Microliter



CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Livestock production is a very vital component in agriculture. It is considered as a large segment that employs many people in the developing countries (Yusuf, 2014). Malaysian livestock farming is among wealth generating activity that supports low income individuals through numerous outputs (meat, milk, wool, skin and hide). It is among the fundamental agricultural activities in the country which provide the most vital source of qualitative animal protein in the diet (Kaur, 2010). Increase in the trend of beef and mutton production has been noticed in Malaysia, but still there is a wide gap to fulfil annually for livestock meat demand (Melissa *et al.*, 2016; FAO, 2007). However, of the numerous challenges affecting the development of the small ruminant sector, diseases constitute an enormous problem to livestock production (Kumar *et al.*, 2015).

Contagious ecthyma (CE) is a highly contagious skin disease of sheep and goats caused by Orf virus, a member of the genus Parapoxvirus. The disease is characterized by proliferative lesions which start from erythematous macule, papule, vesicle, pustule and finally scab formation either on the mouth, lips, nostrils, legs or udder of the affected animal (Zhang *et al.*, 2016; Chi *et al.*, 2013; Adedeji *et al.*, 2018a). The lesions are rarely seen on other organs or tissues but in the case of secondary bacterial or fungal infection it can be highly severe with lesion seen in all part of the body (Nandi *et al.*, 2011; Scagliarini *et al.*, 2012; Midilli *et al.*, 2013). Morbidity associated with CE outbreaks can be up to 100% (Adedeji *et al.*, 2018b) while mortality can reach up 25-50% particularly in small animals due their weak immune system and inability to suckle milk (Hosamani *et al.* 2009; Venkatesan *et al.*, 2011). The prevalence of CE is usually high in the region with a dense population of sheep and goats (Peter *et al.*, 2010; Kumar *et al.*, 2015). The disease has been reported from several regions of the world: Europe (Rebecca, 2012), South America (Abrahao *et al.*, 2009), North America (Tryland *et al.*, 2018), Asia (Oryan *et al.*, 2017), Africa (Adedeji *et al.*, 2018a) and Australia (Maor *et al.*, 2017).

Contagious ecthyma is a disease of great economic importance due to the skin lesions, thus reducing the market value of the animal products (meat, hides and wool) (Nandi *et al.*, 2011). Usually the virus remains viable on the wool and hides for almost one month or more after the lesions have healed, which leads to reinfection and denial of hide for international trade on zoonotic reason (Dalal *et al.*, 2017). As a zoonotic disease, it is an occupational threat to farmers, abattoir workers, veterinarians, and anybody directly working with small ruminant sectors (Hosamani *et al.*, 2009; Nougairede *et al.*, 2013; Zeedan *et al.*, 2015 & Maor *et al.*, 2017). The disease, however, has received less attention in recent time due to the general notion that it is a self-limiting disease (Bala *et al.*, 2018a). Contagious ecthyma is also

known as orf, contagious pustular dermatitis, infectious labial dermatitis, sore mouth and scabby mouth (Winter and Charmley 1999; Kerry *et al.*, 2012).

Apart from the clinical signs, diagnosis of CE is majorly through serological tests such as agar gel immunodiffusion (AGID), indirect immunofluorescence, Enzyme Linked Immunosorbent Assay (ELISA) and neutralization tests (Said *et al.*, 2013, Bora *et al.*, 2016). Other diagnostic tests like polymerase chain reaction (PCR), electron microscopy and cell culture methods have also been used (Bala *et al.*, 2019a), because antibodies can be detected within a week following appearance of the skin lesions (Said *et al.*, 2013). PCR has been considered as a faster and more effective molecular technique for the confirmation of CE infection (Chan *et al.*, 2007; Zeedan *et al.*, 2015; Abdullah *et al.*, 2015; Tedla *et al.*, 2018).

1.2 Statement of the Problems

Livestock industries particularly sheep and goats production were regarded as the vital activities for low income people to sustain their lives either from exchange of live animals or sale of the animals and their products like meat, milk, skin and wool (Yusuf, 2014; Abdullah *et al.*, 2015). While providing a means out of poverty for many, the livestock sector is constantly faced with the threat of endemic animal diseases (FAO, 2010). Contagious ecthyma is one among the diseases that are endemic in sheep and goat herds globally and it posed a serious menace to small ruminant production sector. The disease causes excessive economic losses to farmers as a result of death of infected younger animals and condemnation of other animal's products (meat, milk, skin and wool).

Likewise the disease to some extent lead to the reduction in the qualities of the animal products by rendering the animals less attractive and decrease in their market value. Other problems posed by the disease include stunted growth of infected kids and lambs due to inability to suckle and feed well. Among the consequences of the disease to the farmers there is higher cost of production due to treatment and medications. The disease is highly zoonotic with public health significance in most of the developing countries. Presently, the disease has the potentiality to cross-infect other species of animals and ability to re-infect the same animal (Said *et al.*, 2013).

In Malaysia, cases of CE have also been documented (Bande *et al.*, 2014; Abdullah *et al.*, 2015; Sadiq *et al.*, 2017; Jesse *et al.*, 2018) with prevalence rate of 22.8% reported among small ruminant recently (Bala *et al.*, 2019b). Yet, there is lack of information on CE status from many parts of the country (Abdullah *et al.*, 2015; Jesse *et al.*, 2018; Bala *et al.*, 2019a).

1.3 Justification of the Study

In Malaysia, the livestock industry is one among the fundamental industries in the country's agricultural sector which provides employment and other domestic requirements of meat and milk to Malaysian population (Kaur, 2010). Since CE is a disease of economic importance, the understanding of its epidemiology in different part of the country as well as the possible risk factors associated with the disease will encourage better production management which in turn increase the animal products requirement of the country, thus ensuring adequate food security and reduce dependency on meat and meat product importations. Negeri Sembilan state of Peninsular Malaysia is one among major livestock producing states with many traditional ranchers. Negeri Sembilan has an economy based on agriculture and small ruminants plays a significant role for the development of the state. The state government initiated many programs to improve integrated livestock farming in order to manage the demand for the meat and other animal's products. Livestock farming plays a vital role in eradicating poverty by increasing the individual and government earning (Melissa *et al.*, 2016; Livestock Malaysia, 2020).

1.4 Hypotheses

HO: Seroprevalence of contagious ecthyma is not associated with species, breed, age, gender, and management system, body condition of the animal, vector control and proximity of one farm to another.

H1: Seroprevalence of contagious ecthyma is associated with species, breed, age, gender, and management system, body condition of the animal, vector control and proximity of one farm to another.

1.5 Objectives of the Study

The aim of this study is to investigate the epidemiology of contagious ecthyma among sheep and goats in Negeri Sembilan state of Peninsular Malaysia.

The specific objectives are:

- i. To determine the seroprevalence of contagious ecthyma infection among sheep and goats in selected farms in Negeri Sembilan state of Peninsular Malaysia.
- ii. To identify the possible risk factors associated with contagious ecthyma infection among sheep and goats in Negeri Sembilan state of Peninsular Malaysia.
- iii. To carry out molecular confirmation of contagious ecthyma infection from suspected cases observed during sampling using polymerase chain reaction.
- iv. To assess the evolutionary relationship of the present isolates using phylogenetic analysis.

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