UNIVERSITI PUTRA MALAYSIA

PATHOLOGICAL, BACTERIOLOGICAL AND PREVALENCE STUDIES OF OVINE FOOTROT

KARIM ALWAN MOHAMED AI-JASHAMY

FPV 2003 1
PATHOLOGICAL, BACTERIOLOGICAL AND PREVALENCE STUDIES OF OVINE FOOTROT

By

KARIM ALWAN MOHAMED AI-JASHAMY

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of Requirements for the Degree of Doctor of Philosophy

April 2003
PATHOLOGICAL, BACTERIOLOGICAL AND PREVALENCE STUDIES OF OVINE FOOTROT

By
KARIM ALWAN MOHAMED AL-JASHAMY

April 2003

Chairman: Associate Professor Dr. Jasni Sabri, DAHIP, DVM, PhD
Faculty: Veterinary Medicine

Ovine footrot, is a disease associated with infection by the bacterium *Dichelobacter nodosus*. It is a disease that limits the productivity of sheep-farming enterprises throughout the world. Both wool production and body weight are adversely affected during the clinical phase of the infection.

Ovine footrot has become an important contagious disease in Malaysia. The first confirmed case of footrot was reported in a government sheep farm in mid-1980s. The disease is now present in other farms throughout the country, and local vaccine is being used to reduce the disease.

Previous studies have identified *D. nodosus* in three sheep farms in Malaysia and only serogroup B was identified. The possible presence of other *D. nodosus* serogroups and serotypes is unknown. This study attempts to isolate...
and identify the unknown serogroups and serotypes so as develop a better vaccine candidate using local isolates of \textit{D. nodosus}.

Eight sheep farms were investigated in this study. Four sheep farms were found to be infected with \textit{D. nodosus}. Two hundred and ninety-three \textit{D. nodosus} isolates were obtained from 741 foot samples. Five serogroups were identified in Malaysia. This is the first study where serogroups A, C, F and I with their serotypes A1, A2, C1, F1 and F2 were identified in the infected sheep farms. Serogroup B was the predominant serogroup isolated (78.2\%) while the isolation percentages for serogroups F, A, I and C were 7.9\%, 7.5\%, 3.8\% and 2.7\% respectively.

The information on the pathogenesis of the disease is still lacking despite previous studies on ovine footrot. Interdigital cutaneous changes associate with footrot in sheep is not well documented. The disease was induced experimentally in sheep by topical application of bacterial isolates on the interdigital skin of the hoof, and light and electron microscopy studies of the lesions were conducted.

Virulent footrot was observed by a gross progressive separation of the horny tissues from the soft tissues. On day 21 post inoculation (p.i.), a complete separation of the hoof from the underrunning structures and lameness were evident. The benign footrot was observed with mild interdigital dermatitis and all infected feet completely recovered on day 21 p.i..
Histopathological changes in virulent footrot were observed in the interdigital skin layers and hoof matrix. These ranged from acute dermatitis to hyperkeratosis, parakeratosis and acanthosis of the epidermis. Oedema and leukocytic infiltration with neutrophils, macrophages and scanty lymphocytes were also evident in the dermis. Furthermore, vasculitis and perivascular cuffing, lymphangitis and inflammation of the sweat glands were observed in the dermis. The histopathological changes of benign footrot were less severe than virulent form in the epidermis and there were no pathological changes in the dermis.

In scanning electron microscopy, a severe zone of lysis appearing as a surface depression around bacteria in the horny layer of the interdigital skin of the hoof was detected in virulent footrot, while this lesion was less severe in the benign form. Transmission electron microscopy revealed degeneration in the epidermis and dermis. Degeneration in the basal cell layer of the epidermis and the basement membrane in virulent form of footrot, which have not been reported previously was observed in this study.

*Dichelobacter nodosus* was observed in the lesions of the epidermis and dermis of virulent footrot. Its isolation from characteristic foot lesions indicated that it was associated with footrot. Immunohistochemistry observations validate the relationship between the lesions seen in footrot and virulent *D. nodosus*. Immunogold staining technique facilitates to detection and localisation of *D. nodosus* for electron microscopy. Specific reactions were labelled in
components and the matrix of epidermis and dermis of the interdigital skin. *Dichelobacter nodosus* antigen labelled with 5 nm gold particles was observed in the intracellular and intercellular spaces of the epidermis. This is the first report where immunogold labelling technique have been used in the study of footrot lesions in sheep for electron microscopical observations.

The total monthly rainfall and mean daily temperature have a relation to the prevalence rate of the disease. These conditions provide suitable environment propagation of *D. nodosus*. The overall prevalence of footrot in the eight farms investigated was 3.3%. The highest prevalence was recorded in April (0.8 %), while the lowest in August (0.3%) in IHK farm by survey study. Observations described in this study were made to define the prevalence are related to seasonal conditions, but the effect of rainfall overrides all other factors for footrot to occur.

Adults were more susceptible than weaners. No cases were detected in preweaners. The prevalence by sex which was 4.4% in the male and 7.7% in the female was significant (p=0.009). No significant difference in prevalence rates between breeds was detected.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

KAJIAN PATOLOGI, BAKTERIOLOGI DAN PREVELANS BURUK KAKI OVIN

Oleh

KARIM ALWAN MOHAMED AL-JASHAMY

April 2003

Pengerusi: Profesor Madya Dr. Jasni Sabri, DAHP, DVM, PhD
Fakulti: Perubatan Veterinar

Buruk kaki adalah penyakit yang disebabkan oleh bakteria *Dichelobacter nodosus*. Penyakit ini mengurangkan produksi bebiri di seluruh dunia. Berat badan dan pengeluaran bulu bebiri terjejas akibat jangkitan bakteria ini.


Kajian yang lepas telah mengesan *D. nodosus* di tiga ladang bebiri di Malaysia dan hanya serogroup B sahaja yang dapat dikesan. Samada terdapat serogroup dan serotip lain di sini tidak ditemui lagi. Kajian ini dijalankan untuk mengesan serogroup dan serotip yang tidak di ketahui sebelum ini untuk membolehkan penghasilan vaksin yang lebih baik dengan menggunakan isolat tempatan. Kajian
telah dilakukan di lapang ladang ternakan bebiri. Dua ratus dan sembilan puluh
lima isolat *D. nodosus* telah berjaya dipenculkan dari 741 sampel kaki. Lima
serogroup telah dapat dikesan. Buat pertama kalinya serogroup A, C, F, dan I
dengan serotip A1, A2, C1, F1 dan F2 telah dikesan di ladang bebiri yang telah
dijangkiti. Serogroup B adalah serogroup yang paling banyak di isolat (78.2%)
dan peratusan isolat untuk masing-masing serogroup F, A, I dan C adalah 7.9%,
7.5%, 3.8% dan 2.7%.

Pengetahuan tentang patogenesis penyakit ini masih lagi kabur walaupun
banyak kajian telah dilakukan sebelum ini. Tidak banyak laporan tentang
perubahan interdigital kutaneous yang berlaku semasa buruk kaki. Penyakit
buruk kaki virulen telah dihasilkan dengan menyapu isolat bakteria pada kulit
interdigital pada kuku keras dan lesi dikaji dengan menggunakan mikroskop
cahaya dan elektron. Buruk kaki virulen dilihat sebagai berlakunya pemasaran
yang progresif tisu keras daripada tisu lembut kaki. Pada hari ke 21 selepas
disuntik, pemisahan lengkap kuku keras daripada struktur bawahan
menyebabkan ketempangan berlaku. Dalam buruk kaki benigna, dermatitis
interdigital yang tidak teruk berlaku dan semua kaki sembuh dengan sempurna
pada hari ke 21 selepas suntikan bakteria. Perubahan histopatologi dalam buruk
kaki virulen dapat dilihat pada lapisan kulit interdigital dan matrik kuku keras.
Lesi yang berlaku adalah dari dermatitis akut ke hiperkeratosis, parakeratosis
dan akantosis di epidermis. Edema dan penyunuspan neutrofil, makrofaj and
sedikit limfosit juga kelihatan di dermis. Selain daripada itu, terjadi vaskulitis
dan "cuffing" perivaskular, limfangitis dan inflamasi kelenjar peluh di dermis.
Perubahan histopatologi buruk kaki benigna adalah kurang teruk daripada buruk kaki virulen di epidermis dan tiada perubahan patologi berlaku di dermis. Melalui mikroskopi elektron imbasan, satu zon lisis yang teruk yang mempunyai satu lekukan di sekeliling bakteria pada lapisan kuku keras interdigital kulit kelihatan dalam buruk kuku virulen. Lesi ini kurang teruk dalam buruk kuku benigna. Melalui mikroskopi elektron transmisi, degenerasi dilihat di epidermis dan dermis. Degenerasi sel basal epidermis dan selaput basemen buruk kaki virulen yang tidak pernah dilaporkan sebelum ini juga di temui dalam kajian ini.

_Dichelobacter nodosus_ dapat dilihat di epidermis dan dermis buruk kaki virulen. Pemencilan bakteria ini daripada lesi buruk kaki menunjukkan bahawa lesi ini berkaitan dengan kehadiran bakteria ini.

Pemeriksaan secara imunohistokimia menyokong yang kejadian lesi buruk kaki berkaitan dengan kehadiran dan virulen _D. nodosus_. Pewarnaan “immunogold” telah digunakan untuk mengesan dan mencari lokasi _D. nodosus_ menggunakan mikroskop cahaya dan elektron. Satu reaksi yang spesific dilabel di komponen intrasel dan matriks epidermis dan dermis kulit interdigital.

Antigen _D. nodosus_ yang dilabel dengan 5 nm zarah emas dilihat dalam ruang intersel dan intrasel epidermis. Reaksi pewarnaan imuno lesi buruk kaki benigna adalah kurang berbanding buruk kaki virulen di lapisan kulit interdigital. Teknik
perlabelan “immunogold” ini adalah pertama kali digunakan untuk mengkaji buruk kaki pada bebiri dengan menggunakan mikroscop cahaya dan elektron.

Jumlah taburan hujan bulanan dan min suhu harian ada kaitan dengan prevalen penyakit ini. Keadaan ini menyediakan persekutuan yang sesuai untuk pembiakan *D. nodosus*. Prevalen penyakit buruk kaki di lapan ladang yang dikaji keseluruhannya adalah 3.3%.

Prevalen yang paling tinggi telah direkod pada bulan April (0.8%) dan yang terendah pada bulan Ogos (0.3%) di ladang IHK secara “survey”. Kajian ini menunjukkan bahawa prevalen berkaitan dengan musim, tetapi hujan yang berlaku melebihi faktor yang lain dalam menyebabkan kejadian buruk kaki. Umur adalah sangat bererti pada bebiri dewasa berbanding bebiri yang sudah di cerai susu. Prevalen yang mengikut jantina adalah 4.4% pada bebiri jantan dan 7.7% pada bebiri betina adalah bererti (p=0.009). Prevalen mengikut baka didapati tidak bererti.
ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious, Most Merciful. All praise to Almighty Allah. Had it not been due to his will and favour, the completion of this study would not have been possible.

I wish to express my profound appreciation and gratitude to all who have provided guidance and assistance towards the completion of this thesis.

I extend my sincere gratitude and appreciation to my supervisor, Associate Professor Dr. Jasni Sabri, who devoted much of his time in providing me with invaluable guidance, advice, supervision and support throughout the course of this study.

I am especially grateful to my co-supervisor, Professor Dato’ Dr. Sheikh Omar Abdul Rahman for his invaluable advice, support and guidance. His encouragement and unbounded optimism have kept me in high spirits well in the most difficult times. Sincere thanks are also due to my co-supervisors, Assoc. Prof. Dr. Abdul Rahim Mutilib, Assoc. Prof. Dr. Mohd Azmi Lila and Dr Siti. Zubaidah Ramanoon who provided advice and helpful suggestions that have enlightened and improved this study.
Special thanks to Dr. Jothi Panandan from the Department of Animal Science, Agriculture Faculty, Universiti Putra Malaysia and my colleague, Mr. Aqeel Noori who helped me in statistical analysis.

I wish to thank the Department of Veterinary Services Malaysia for granting me permission to collect samples in the sheep farms. I would also like to thank the Ministry of Science, Technology and Environment Malaysia for financial assistance through IRPA grants no. 51011 and 51488.

Grateful thanks are extended to my supportive wife, Dr. Karima Akool Al-Salihi, my son, Mohamed and my daughter, Hajir, who have had to put up with me working late nights and weekends to complete my Ph.D study.

I have been very fortunate in receiving assistance from a number of colleagues and friends. I would like to acknowledge Dr. Karim Al-Aajeli, Dr. Muthafar Al-Haddawi, Dr. Zunita Zakaria and Dr. Mohammed Firoz Main.

Finally, I wish to express my deepest gratitude to Miss Azilah Abdul Jalil and Mr. Ho Oi Kuan of the Electron Microscopy Unit, Institute of Bioscience, Universiti Putra Malaysia and also to all the academic and support staff of the Faculty of Veterinary Medicine, Universiti Putra Malaysia for their assistance and support during the course of the study.
I certify that an Examination Committee met on 26th April 2003 to conduct the final examination of Karim Alwan Mohamed Al-Jashamy on his Philosophy Degree of Science thesis entitled “Pathological, Bacteriological and Prevalence Studies of Ovine Footrot” in agreement with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) regulation 1981. The Committee recommended that the candidate be awarded the relevant degree. Members of the examination committee are as follows:

Zamri Saad, Ph.D.,
Professor,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Jasni Sabri Ph.D.,
Associate Professor,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Sheikh Omar Abdul Rahman M.R.C.V.S.,
Professor,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Abdul Rahim Mutalib Ph.D.,
Associate Professor,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Siti Zubaidah Ramanoon MS.,
Lecturer,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Takashi Umemura Ph.D.,
Professor,
Graduate School of Veterinary Medicine
Hokkaido University
(Independent Examiner)

GULAM RUSUL RAHMAT ALI, Ph.D.
Professor, Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

22 Aug 2003
This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee are as follow:

**Jasni Sabri Ph.D.,**  
Associate Professor,  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
*Chairman*

**Sheikh Omar Abdul Rahman M.R.C.V.S.,**  
Professor,  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Abdul Rahim Mutalib Ph.D.,**  
Associate Professor,  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**Siti Zubaidah Ramanoon MS.,**  
Lecturer,  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

---

_AINI IDERIS, Ph.D._  
Professor/ Dean,  
School of Graduate Studies,  
Universiti Putra Malaysia

_Date: 12 SEP 2003_
I hereby declare that the thesis is based on my original work except for quotations and citation, which have been duly acknowledged. I also declare that it has been not been previously or concurrently submitted for any other degree at Universiti Putra Malaysia or other institutions.

Karim Alwan Mohamed Al-Jashamy
Date: 10 July, 2003
# TABLE OF CONTENTS

ABSTRACT ........................................................................................................ ii
ABSTRAK .......................................................................................................... v
ACKNOWLEDGEMENTS ................................................................................... viii
APPROVAL ........................................................................................................ x
DECLARATION .................................................................................................. xii
LIST OF TABLES ............................................................................................... xvii
LIST OF FIGURES ............................................................................................ xviii

CHAPTERS

I GENERAL INTRODUCTION ................................................................. 1

II REVIEW OF LITERATURE ................................................................. 5

2.1 General Introduction ............................................................................ 5
2.2 Historical Background ......................................................................... 6
2.3 Etiology of Ovine Footrot ................................................................. 7
2.4 Morphological and Bacteriological Characteristics of *Dichelobacter nodosus* ................................................................. 8
2.5 Ultrastructural Morphology of *D. nodosus* ..................................... 10
2.6 Serogrouping and Serotyping of *D. nodosus* ..................................... 11
2.7 Virulence of *D. nodosus* ................................................................... 14
2.8 Antigen of *D. nodosus* ...................................................................... 15
2.9 Virulence Determination ...................................................................... 16
2.10 Clinical manifestation of Footrot ....................................................... 19
    2.10.1 Clinical Signs and Differential Diagnosis ............................... 19
    2.10.2 Clinical Pathology of Footrot ................................................... 21
2.11 Pathophysiology .................................................................................. 22
2.12 Pathology .............................................................................................. 24
    2.12.1 Clinical Scores ........................................................................... 24
    2.12.2 Histopathology .......................................................................... 25
    2.12.3 Ultrastructural Changes of the Skin .......................................... 26
2.13 Epidemiology and Predisposing Factors .......................................... 27
    2.13.1 Host Factors .............................................................................. 28
    2.13.2 Environmental Factors .............................................................. 29
        2.13.2.1 Biotic Factors ................................................................. 29
        2.13.2.2 Abiotic Factors ............................................................... 29
    2.13.3 Immunity to *Dichelobacter nodosus* ...................................... 30
    2.13.4 Vaccination ............................................................................... 31
    2.13.5 Transmission of infection ......................................................... 32
    2.13.6 Incidence and Prevalence ......................................................... 32
III  ISOLATION AND IDENTIFICATION OF D. NODOSUS ....... 34

3.1  Introduction.................................................. 34
3.2  Materials and Methods........................................ 36
   3.2.1  Area of Study........................................... 36
   3.2.2  Sampling Procedures.................................... 36
   3.2.3  Direct Smear Examination............................... 37
   3.2.4  Preparation of Culture and Media....................... 37
      3.2.4.1  Hoof Agar (HA)..................................... 37
      3.2.4.2  Ovine Hoof Powder................................ 38
      3.2.4.3  Trypticase Arginine Serine (TAS) Media........ 38
      3.2.4.4  Isolation and Cultivation of D. nodosus..... 39
   3.2.5  Serology.................................................. 39
      3.2.5.1  Preparation of Antigens......................... 39
      3.2.5.2  Slide Agglutination Tests for Serogrouping.... 40
      3.2.5.3  Microtitre Plate Agglutination Tests for
               serotyping.......................................... 41
   3.2.6  Virulence of D. nodosus................................. 42
      3.2.6.1  Elastase Test..................................... 42
      3.2.6.2  Gelatin Gel test................................... 43
         3.2.6.2.1  Preparation of Substrate Gel.............. 43
         3.2.6.2.2  Broth Antigen Preparation.................. 43
         3.2.6.2.3  Gelatin Gel .................................. 44
   3.2.7  DNA Isolation and Polymerase Chain Reaction ...... 45
      3.2.7.1  Rapid DNA Extraction of D. nodosus
               from Pure Culture ...................................... 45
      3.2.7.2  Preservation of Foot Lesions Material........ 46
         3.2.7.2.1  Rapid DNA Extraction of D.
                        nodosus  from Lesion Materials .............. 47
      3.2.7.2.2  Boiling DNA Extraction of D.
                        nodosus  from Lesion Material............... 47
      3.2.7.3  Boiling DNA Extraction of D. nodosus
               from Pure Cultures................................. 48
   3.2.8  Polymerase Chain Reaction............................. 48
   3.2.9  Ultrastructural Study of D. nodosus.................. 49
      3.2.9.1  Negative Staining of Bacteria................... 49
      3.2.9.2  Transmission Electron Microscopy (TEM)
               of D. nodosus........................................ 50

3.3  Results.......................................................... 51
   3.3.1  Direct Smear Examination.............................. 51
   3.3.2  Bacterial Isolation................................... 52
   3.3.3  Polymerase Chain Reaction............................ 55
   3.3.4  Serogrouping and Serotyping.......................... 57
   3.3.5  Virulence Assessment.................................. 60
   3.3.6  Ultrastructural Morphology of D. nodosus ........... 64

3.4  Discussion...................................................... 67
   3.4.1  Direct Gram-Stain and Culture........................ 67
3.4.2 Morphology of the Bacterial Colonies.............. 69
3.4.3 Serogrouping and Serotyping of *D. nodosus*...... 69
3.4.4 Virulence studies.................................. 71
3.4.5 Polymerase Chan Reaction.......................... 72
3.4.6 Ultrastructural Morphology of *D. nodosus*..... 74
3.4.7 Conclusion.......................................... 75

IV PATHOLOGY OF EXPERIMENTALLY INDUCED OVINE
FOOTROT.................................................... 77

4.1 Introduction........................................... 77
4.2 Materials and Methods................................ 80
  4.2.1 Sheep.................................................. 80
  4.2.2 Bacterial Strains.................................. 80
  4.2.3 Experimental Design................................. 81
  4.2.4 Preliminary Experimental Reproduction of Ovine
       Footrot.................................................. 81
  4.2.5 Infection of Sheep.................................. 82
  4.2.6 Bacteriology........................................ 83
  4.2.7 Pathology........................................... 83
       4.2.7.1 Lameness and Lesion Scoring.................. 83
       4.2.7.2 Light Microscopy.................................. 84
       4.2.7.3 Electron Microscopy.............................. 84
       4.2.7.4 Transmission Electron Microscopy............. 85
4.3 Results................................................. 86
  4.3.1 Bacteriology......................................... 86
  4.3.2 Pathology........................................... 87
       4.3.2.1 Clinical Scores................................. 87
       4.3.2.2 Histopathology.................................. 95
  4.3.3 Ultrastructural Pathology.......................... 100
       4.3.3.1 Scanning Electron Microscopy of
               Footrot Lesions................................. 100
       4.3.3.2 Transmission Electron Microscopy
               of Footrot Lesions................................. 100
       4.3.3.2.1 Epidermis...................................... 100
       4.3.3.2.2 Dermis......................................... 102
4.4 Discussion............................................. 117
  4.4.1 Clinical Scores...................................... 117
  4.4.2 Histopathology...................................... 120
  4.4.3 Ultrastructural Pathology............................ 121
  4.4.4 Conclusion.......................................... 124
6.3.1.2 Endemic Farm with Footrot ........... 157
  6.3.1.2.1 Prevalence of Ovine Footrot by Age.......................... 161
  6.3.1.2.2 Prevalence of Ovine Footrot by Sex.......................... 161
  6.3.1.2.3 Prevalence Ovine Footrot by Breed.......................... 162
  6.3.2 Cross-Sectional Study of Footrot ....................... 162
  6.3.3 The Distribution of Footrot by Scoring System... 164

6.4 Discussion......................................................... 165
  6.4.1 Prevalence of Ovine Footrot....................... 165
  6.4.2 The Prevalence of Footrot Related to Environmental Factors............. 166
  6.4.3 The Prevalence by Age, Sex and Breed ............. 167
  6.4.4 Source of Infection................................. 169
  6.4.5 Conclusion.................................................. 171

VII GENERAL DISCUSSION.................................................... 172

REFERENCES........................................................................ 179
APPENDICES........................................................................ 191

Appendix

2.1 Biochemical and Phenotypic Properties of Dichelobacter nodosus....................... 191
2.2 The Scoring System.................................................. 191
2.3 Serogrouping of D. nodosus determined used Cross-tube agglutination reaction........ 193
3.1 Gram Stain.......................................................... 194
3.2 Formal Phosphate Buffer Saline (FPBS)................................ 194
3.3 Tris EDTA Na2 (TE).................................................. 195
3.4 3 M Sodium Acetate.................................................. 195
4.1 Modified–Haematoxylin-Eosin Stain.............................. 195
4.2 Buffered Glutaraldehyde (pH 7.0)................................ 195
4.3 0.1 Sodium Cacodylate Buffer (pH 7.0).......................... 196
4.4 1% Buffered Osmium Tetroxide..................................... 196
4.5 Resin Mixture....................................................... 196
4.6 Uranyl Acetate Stain.................................................. 196

BIODATA................................................................. 198
## LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Number of foot samples, positive direct smear, positive growth culture on 4% HA, PCR results and number of isolates</td>
<td>53</td>
</tr>
<tr>
<td>3.2</td>
<td>Isolates design according to the source of isolation</td>
<td>54</td>
</tr>
<tr>
<td>3.3</td>
<td>PCR analysis of pure cultures and foot swabs of <em>D. nodosus</em></td>
<td>56</td>
</tr>
<tr>
<td>3.4</td>
<td><em>Dichelobacter nodosus</em> serogroups and number of isolates in different sheep farms</td>
<td>57</td>
</tr>
<tr>
<td>3.5</td>
<td>Serotypes of <em>D. nodosus</em> isolates in all infected farms</td>
<td>58</td>
</tr>
<tr>
<td>3.6</td>
<td>Microtitre agglutination reaction between <em>D. nodosus</em> isolates and specific rabbit antisera</td>
<td>59</td>
</tr>
<tr>
<td>3.7</td>
<td>Elastase and gelatin gel tests of 293 <em>D. nodosus</em></td>
<td>61</td>
</tr>
<tr>
<td>3.8</td>
<td>Virulence of 293 isolates of <em>D. nodosus</em> serogroups by elastase and gelatin gel tests</td>
<td>63</td>
</tr>
<tr>
<td>4.1</td>
<td>Preliminary experimental to induce the footrot in crossbred sheep</td>
<td>82</td>
</tr>
<tr>
<td>4.2</td>
<td>Gram-staining and direct culture of <em>D. nodosus</em> from all feet of sheep in all groups</td>
<td>86</td>
</tr>
<tr>
<td>4.3</td>
<td>Number of inoculated feet, number of infected feet and score lesion at each inspection following infection</td>
<td>88</td>
</tr>
<tr>
<td>5.1</td>
<td>Positive and negative control assessment of immunohistochemistry</td>
<td>129</td>
</tr>
<tr>
<td>6.1</td>
<td>Number of inspected animals, number of samples, breed, age and sex of sheep at MARDI Serdang farm</td>
<td>152</td>
</tr>
<tr>
<td>6.2</td>
<td>Number of flocks, number of inspected animals, number of samples, breed, age and sex of sheep at MARDI Kluang</td>
<td>154</td>
</tr>
<tr>
<td>6.3</td>
<td>Number of inspected animals, number of samples and number of clinical and culture cases in MARDI Serdang and Kluang</td>
<td></td>
</tr>
</tbody>
</table>
6.4 The prevalence of ovine footrot in four flocks in IHK farm from June to 1999 to May 2000.

6.5 Ovine footot cases in relation to the total monthly rainfall and mean daily temperature at IHK farm during the study period.

6.6 Prevalence of ovine footrot by age of sheep at IHK farm.

6.7 Prevalence of ovine footrot by sex of sheep at IHK farm.

6.8 Prevalence of ovine footrot by breed at IHK farm.

6.9 Prevalence of ovine footrot in five sheep farms.

6.10 Distribution of footrot lesions by the scoring system in four farms positive for ovine footrot.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>A Gram-stained smear from a footrot lesion. Arrow shows large Gram-negative rod bacterium with swollen ends resembling <em>D. nodosus</em>.</td>
<td>51</td>
</tr>
<tr>
<td>3.2</td>
<td>Agarose gel electrophoresis of <em>D. nodosus</em> genomic DNA amplification production using the Ac and C primer combination of 780 base pairs. Samples were prepared from lesion materials that showed negative culture on HA using rapid DNA extraction, lanes 6-8 and 10, <em>D. nodosus</em>. C, reference strain, A1001, (M) marker 100 bp. lanes 1-5 and 9 were no band, resembling negative footrot cases.</td>
<td>56</td>
</tr>
<tr>
<td>3.3</td>
<td>Clear zone of elastin particles in TSA agar medium was produced by positive elastase <em>D. nodosus</em> virulent strain IHK 5 (v), no clear zone with the benign strain IHK 9 (b).</td>
<td>60</td>
</tr>
<tr>
<td>3.4</td>
<td>Stable and unstable proteases in a complete gelatin gel test of virulent strain IHK 5 (V), intermediate strain IHK 8 (I), benign IHK 9 (B) of <em>D. nodosus</em> and control benign strain G1674 (C). Benign strain 10 (F) (fail isolate to give complete stable and unstable protease in gelatin gel at 8 or 16 minutes heated sample).</td>
<td>62</td>
</tr>
<tr>
<td>3.5</td>
<td>A transmission electron photomicrograph showing the <em>D. nodosus</em> virulent strain IHK 5 grown onto 4% HA. Note the number of fimbriae attached to the cell surface (arrow) and polar region, methylamine tungstate X 28,600.</td>
<td>65</td>
</tr>
<tr>
<td>3.6</td>
<td>A transmission electron photomicrograph showing the <em>D. nodosus</em> intermediate strain IHK 8 grown onto 4% HA. Note the number of fimbriae attached to the cell surface (arrow), methylamine tungstate X 26,670.</td>
<td>65</td>
</tr>
<tr>
<td>3.7</td>
<td>A transmission electron photomicrograph showing <em>D. nodosus</em> benign strain IHK 9 grown onto 4% HA solid media. Note no fimbriae attached bacterial cell surface (arrow) methylamine tungstate X 26,670.</td>
<td>66</td>
</tr>
<tr>
<td>3.8</td>
<td>Transmission electron photomicrographs showing the longitudinal thin section of virulent <em>D. nodosus</em> strain IHK 5 grown on a 4% HA. Note the plasma membrane (pm), intracytoplasmic membrane (im), peptidoglycan layer (pl), outer membrane (om), additional layer (a), nucleoid region (n) and ribosome (r). Lead citrate and uranyl</td>
<td></td>
</tr>
</tbody>
</table>
Foot of sheep infected with virulent strain of the *D. nodosus* indicating a footrot score of 1 at day 7 p.i. characterised by a slight to moderate moistness and hyperaemia of interdigital skin with some erosion (arrow).

Foot of sheep infected with virulent strain of *D. nodosus* indicating a footrot score of 2 at day 14 p.i. characterised by erosion and ulceration of interdigital skin covered with moist necrotic material with alopecia (arrow).

Foot of sheep infected with virulent strain of *D. nodosus* indicating a footrot score of 3a at day 14 p.i characterised by erosion and ulceration of interdigital skin. The inflammation and underrunning cross the skin-horn junction at about 1 cm (arrow).

Foot of sheep infected with virulent strain of the *D. nodosus* indicating a footrot score of 3b at day 21 p.i characterised by erosion and ulceration of interdigital skin. The inflammation and underrunning cross skin-horn junction at about 3 cm (arrow).

Foot of sheep infected with virulent strain of the *D. nodosus* indicating a footrot score 3c at day 21 p.i characterised by ulceration of interdigital skin. The inflammation and complete underrunning cross the skin-horn junction at about more than half distance between the skin-horn junction and the outside edge of the sole of the claw (arrow).

Foot of sheep infected with virulent strain of the *D. nodosus* indicating a footrot score of 4 at day 25 p.i., the inflammation and ulceration of interdigital skin. The complete underrunning of the hoof extended to the abaxial edges of the sole of the claws (arrow).

Foot of sheep infected with virulent strain of the *D. nodosus* indicating a footrot score of 5 at day 25 p.i., characterised by inflammation and ulceration of interdigital skin. The inflammation and complete underrunning of the hoof extended to the abaxial edges of the sole of the claws (arrow).
4.8 Foot of sheep infected with virulent strain of the *D. nodosus* indicating a footrot score of 5 at day 28 p.i showing a complete separation of the hoof from one digit................................. 93

4.9 Foot of sheep with footrot lesion score of 5 showed bleeding and granulation tissue................................. 94

4.10 Sheep infected with virulent strain of *D. nodosus* showing the affected animal hanging the infected right front leg................................. 94

4.11 Skin of sheep infected with virulent strain. Acute dermatitis a characterised by congested blood vessels (thin arrow), focal necrotic dermis (thick arrow) and slight hyperkeratosis consistent with a score of 1. H&E x 10................................. 96

4.11 Skin of sheep infected with virulent strain. Acute dermatitis b characterised by mild hyperkeratosis with necrotic debris (thin arrow), parakeratosis, hair follicular degeneration and alopecia, consistent with a score of 1 (thick arrow), H&E x 4................................. 96

4.12 Skin of sheep infected with virulent strain. Advance case of dermatitis, hyperkeratosis, parakeratosis (thin arrow), acanthosis and scanty leukocytic infiltration, massive necrosis and microabscess in the epidermal papillary, consistent with a score of 2 (thick arrow), H&E x 10................................. 97

4.13 Skin of sheep infected with virulent strain. Edema, neutrophils, macrophages and lymphocytes infiltration and folliculitis, H&E x 20................................. 97

4.14 Skin of sheep infected with virulent strain. Vasculitis and perivasculary cuffing, H&E x 40................................. 98

4.15 Skin of sheep infected with virulent strain. Lymphangitis and leukocytic infiltration of sweat glands, H&E x 20................................. 98

4.16 Skin of sheep infected with virulent strain. Light photomicroscopy of a sheep at day 25 p. i. consistency with a score of 4 showing infiltration of mast cells, H&E x 40................................. 99

4.17 Skin of sheep infected with benign strain. Light photomicroscopy showing new vascularization and