Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author. STUDIES ON Mycoplasma ovipneumoniae IN NEW ZEALAND SHEEP: EPIDEMIOLOGY AND COMPARISON OF ISOLATES

A THESIS PRESENTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MICROBIOLOGY AT MASSEY UNIVERSITY, NEW ZEALAND.

GEORGE IONAS

1983

83.07136

MASSEY UNIVERSITY

1.*	(a)	I give permission for my thesis, entitled
		Studies on Mycoplasma ovipneumoniae in New Zealand Sheep:
		Epidemiology and Comparison of Isolates
		to be made available to readers in the Library under the conditions determined by the Librarian.
	(b)	I agree to my thesis, if asked for by another institution, being sent away on temporary loan under conditions determined by the Librarian.
	(c)	I also agree that my thesis may be copied for Library use.
2. *		k da noz wish nyxthesix xeritled x
		ик bec xnade sozi lable xto xiz adexs ar ax be sen xto xother ioxitix in ess x with out xn x xnixten x consent xnixbin x bec xnxxxxxxx x x Signed
		Signed
		Date
*		Strike out the sentence or phrase which does not apply.
The I	library	
	y Unive rston N	ersity lorth, N.Z.
the sp		t of this thesis belongs to the author. Readers must sign their name in ow to show that they recognise this. They are asked to add their ldress.
Name	and A	ddress Date

.....

ABSTRACT

As part of a larger study to examine the role of M, ovipneumoniae in chronic non-progressive pneumonia (CNP) of sheep, the colonisation of the respiratory tract by mycoplasmas was examined in two flocks of lambs over a nine month period.

In both farms M. ovipneumoniae was detected in the ewes at the time of first swabbing of the lambs. The two flocks of lambs differed in the time when the nasal cavity first became colonised by M. ovipneumoniae: thus in Flock 2 M. ovipneumoniae was detected in the nasal cavity relatively early and also became disseminated throughout the flock some months earlier than occurred in Flock Nevertheless, M. ovipneumoniae was 1. widespread in both flocks of lambs by March i.e. at or before peak seasonal prevalence of CNP. At slaughter in May, Flock 2 (colonised early) had a much higher prevalence of CNP than Flock 1.

These findings are consistant with the hypothesis that \mathcal{M} . *ovipneumoniae* colonises the nasal tract of lambs and subsequently invades the lung possibly in response to the stress of exposure to hot dry weather.

The second part of this thesis is concerned with the adaptation of Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to distinguish strains of *M. ovipneumoniae* with the ultimate objective of comparing *M. ovipneumoniae* strains isolated from pneumonic lungs to nasal isolates and isolates from apparently normal lungs.

Isolates from different sources were heterogeneous when examined by SDS-PAGE, but comparisons were made difficult because of the excessive number of protein bands. In response to this problem fractions of *M. ovipneumoniae* were examined. Membrane preparations conserved the unique protein bands which in principle allow discrimination between strains, but because the number of protein bands was still excessive we examined surface proteins by labelling intact cells with Fluorescein isothiocyanate (FITC). This approach still gave gels with too many protein bands for convenient comparisons to be made, but had the advantage of allowing the identification of surface proteins, some of which were unique to individual isolates.

This encouraged us to combine SDS-PAGE with a classic immunological approach to strain identification i.e. we investigated the possibility of excising protein bands from gels for subsequent use as immunising antigens. One common protein band was excised, it was found to be antigenic and the antisera crossreacted with a single line of identity in gel precipititin tests with all the strains tested. While within the time limit available we have examined only one common protein band, the result suggests that the excision of individual strain-specific protein bands from SDS-PAGE for use as immunising antigens will provide strain-specific antisera which should allow the development of a simple approach to strain identification.

iii

ACKNOWLEDGEMENTS

I am indebted to the Department of Microbiology and Genetics, for providing the opportunity and facilities for these studies.

Department of Microbiology and Genetics

In particular, I would like to thank: My supervisor Dr J.K. Clarke for his excellent guidance and advice throughout the course of this study.

Professor D.F. Bacon, Dr E. Terzaghi, Mr P.C. Calder, Mr R.H. Tucker, Mrs A.J. Mew, Ms A. Lee and all other members of the academic and technical staff.

Department of Veterinary Pathology and Public Health

I would like to thank Dr M.R. Alley for the collection of specimens and pathological examination of the sheep lungs.

I would like also to thank:

The staff at the engineering workshop for the construction of the gel apparatus.

The staff of the Central Photographic Unit.

Mrs V. Fieldsend for the excellent typing of this thesis.

And finally, my parents for their encouragement throughout this project.

iv

CONTENTS

. . .

TITLE PAGE		i
ABSTRACT		ii
ACKNOWLEDGE	MENTS	iv
LIST OF CON	TENTS	Ņ
LIST OF FIG	URES	ix
LIST OF TAB	LES	xiv
INTRODUCTIO	N	1
CHAPTER 1:	HISTORICAL REVIEW	3
1.1	Nomenclature of the Disease	3
	1.1.1 Hogget Pneumonia	4
	1.1.2 Summer Pneumonia	4
	1.1.3 Enzootic Pneumonia	4
	1.1.4 Atypical Pneumonia	4
	1.1.5 Proliferative Exudative Pneumonia	5
	1.1.6 Proliferative Interstitial Pneumonia	5
	1.1.7 Sheep Pulmonary Adenomatosis	5
1.2	Economoic Importance of Chronic Non- Progressive Pneumonia	5
	1.2.1 Diminished Weight Gain	6
	1.2.2 Pleural Adhesions	7
1.3	Epidemiology of M. ovipneumoniae	8
. 1.4	Transmission of Chronic Non-Progressive Pneumonia	9
1.5	Distinguishing Strains of M. ovipneumoniae	11
	1.5.1 Serology	11

1.5.2 Restriction Endonuclease Analysis

1.5.3 SDS-PAGE

PAGE

13

13

v

vi

ÿ

1 /	Discharing Disconting of Musculasman	1 1
1.6	Biochemical Dissection of Mycoplasmas	14
	1.6.1 Separation of Internal and Membrane Proteins	14
	1.6.2 Identification of Surface	
	Proteins by Labelling	16
	1.6.3 Detection of Adsorbed Media Constituents	17
CHAPTER 2:	A Study of the Colonisation of the	
	Respiratory Tract of Lambs by Mycoplasmas	18
2.1	Introduction	18
2.2	Materials and Methods	19
	Materials and Methods	19
2.3	Results	25
2.4	Discussion	38
CHAPTER 3:	Comparison of M. ovipneumoniae Isolates	
	by SDS-PAGE	41
3.1	Estimation of Total Protein of M. ovipneumon.	iae
	by the use of a Protein-Dye Binding Technique	41
	3.1.1 Introduction	41
	3.1.2 Materials and Methods	42
	3.1.3 Results	43
	3.1.4 Discussion	44
3.2	Selection of a Gel System and Protein Concentration for Maximum Resolution	45
	3.2.1 Introduction	45
	3.2.2 Materials and Methods	45
	3.2.3 Results	65
	3.2.4 Discussion	71
3.3	Reproducibility of SDS-PAGE Separation of Proteins from Replicate Culture of an <i>M. ovipneumoniae</i> Strain	72

vii

		PAGE
	3.3.1 Introduction	72
•	3.3.2 Materials and Methods	72
	3.3.3 Results	72
	3.3.4 Discussion	74
3.4	The Effect of Trichloroacetic Acid Precipitation and Sonic Disruption on the Electrophoretic Banding Pattern of M. ovipneumoniae	75
	3.4.1 Introduction	75
	3.4.2 Materials and Methods	75
	3.4.3 Results	76
	3.4.4 Discussion	78
3.5	Comparison of Different Isolates of M. ovipneumoniae by SDS-PAGE	79
	3.5.1 Introduction	79
	3.5.2 Materials and Methods	79
	3.5.3 Results	81
	3.5.4 Discussion	86
CHAPTER 4:	Fractionation of M. ovipneumoniae	87
4.1	Separation of Internal Proteins from Membrane Proteins	87
	4.1.1 Introduction	87
	4.1.2 Materials and Methods	89
	4.1.3 Results	92
	4.1.4 Discussion	96
4.2	The Use of Fluorescein Isothiocyanate to Preferentially Label Surface Proteins of M. ovipneumoniae	97
	4.2.1 Introduction	97
	4.2.2 Materials and Methods	97
	4.2.3 Results	100
	4.2.4 Discussion	104

DACE

viii

n

PAGE

	4.3	A Comparison of 3 Strains of <i>M. ovipneumonia</i> by Labelling Intact Cells with FITC	ue 106
		4.3.1 Introduction	106
		4.3.2 Materials and Methods	106
		4.3.3 Results	106
		4.3.4 Discussion	108
	4.4	Detection of Medium Constituents Adsorbed to the Surface of <i>M. ovipneumoniae</i>	109
		4.4.1 Introduction	109
		4.4.2 Materials and Methods	110
		4.4.3 Results	112
	3	4.4.4 Discussion	114
	4.5	Determination of the Molecular Weight and Antigenicity of a Low Molecular Weight Protein	115
		4.5.1 Introduction	115
		4.5.2 Materials and Methods	115
		4.5.3 Results	117
		4.5.4 Discussion	123
CHAPTER	5 :	General Discussion	124
BIBLIOG	RAPH	Y	131

LIST OF FIGURES

Figure

1 (i) The (cummulative) proportion of Perendale lambs which showed nasal carriage of *M. ovipneumoniae* on at least one occasion.

(ii) The proportion of lambs from which*M. ovipneumoniae* was recovered from thelungs at slaughter

2 (i) The (cummulative) proportion of Suffolk lambs which showed nasal carriage of *M. ovipneumoniae* on at least one occasion.

(ii) The proportion of lambs from which *M. ovipneumoniae* was recovered from the
lungs at slaughter

- 3a The cummulative proportion of Suffolk lambs (Flock 2) from which *M. anginini* was recovered from the nasal cavity.
- 3b The cummulative proportion of Perendale lambs (Flock 1) from which *M. anginini* was recovered from the nasal cavity
- 4 *M. ovipneumoniae* (Strain 5), 7 days growth on FM4 agar. Photographed by oblique light (x 56)
- 5 *M. anginini* (isolate 37E), 7 days growth on FM4A agar. Photographed by oblique light (x 56)

ix

PAGE

28

30

31

31

33

А

6	Colonies of M . arginini at 7 days growth, viewed with transmitted light (x 56)	35
7	Colonies of <i>M. ovipneumoniae</i> exhibiting enhanced growth beside a cotton fibre (this was frequently seed and was reproduceably observed with a proportion of isolates. Magnification (x 56)	36
8	CNP lesions consisting of dull red consol- idation on the right cardiac lobe in a $8\frac{1}{2}$ month old lamb	37
9	Standard curves for protein assay: (i) Bradford's technique (ii) Modified Bradford technique	44a
100	The apparatus used for SDS-PAGE electro- phoresis of Mycoplasma proteins	56
11	Two rectangular pieces of plate glass measur- ing 5.5 x 170 x 130mm were cut. From one of these plates a section of glass was removed from one end	57
12	Assembly of the glass plates	58
13	Pouring the gel	59
14	Preparation of gradients gels	60
15	Stacking gel and wells	61
16	Assembly of the gel apparatus	62

х

FIGUR	E	PAGE
17	Gel drying apparatus	63
18	Total protein of <i>M. ovipneumoniae</i> (strain 5) electrophoresed through a 5% acrylamide gel	66,
19	Total protein of <i>M. ovipneumoniae</i> (strain 5) electrophoresed through a 7.5% acrylamide gel	67
20	Total protein of <i>M. ovipneumoniae</i> (strain 5) electrophoresed through a 10% acrylamide gel'	68
21	Total protein of <i>M. ovipneumoniae</i> (strain 5) electrophoresed through a 15% acrylamide gel	69
22	Total protein of <i>M. ovipneumoniae</i> (strain 5) electrophoresed through a 7.5-15% acrylamide gradient gel	70
23	Reproducibility of protein separation on a 10% SDS-PAGE gel	73
24	Some experiments require that proteins be precipitated by TCA. This figure invest- igates any effect which this step may have on subsequent protein separations by electro-	

phoresis

25Six isolates from the nasal track of lambsof one flock are examined by SDS-PAGE83

xi

Ю

PAGE FIGURE 26 Six isolates from the nasal track of lambs of one flock are examined by SDS-PAGE an extension of Figure 25 84 27 protein of eight isolates The total of M. ovipneumoniae all derived from sheep on different farms are examined by SDS-PAGE 85 28 Inactivation of M. ovipneumoniae (strain 5) by Dicyclohexylcarbodiimide 93 29 The decrease in the optical density of M. ovipneumoniae (strain 5) following the addition of Dicyclohexylcarbodiimide 94 30 Α comparison of total protein, membraneassociated protein and cytoplasmic proteins of *M. ovipneumoniae*(strain 5) 95 The surface proteins 31 detection of of M. ovigneumoniae (strain 5) by FITC labelling 102 32 This is a repeat of the experiment shown in Figure 31, except that a 15% acrylamide gel (instead of the usual 10% acrylamide gel) was used 103 33 A comparison of the surface proteins of 3 strains of M. ovipneumoniae. Intact cells were labelled with FITC 107 34 Detection of medium constituents adsorbed to the surface of *M. ovipneumoniae* 113 35 Determination of the molecular weight of

the leading protein band 119

xii

xiii

FIGUR	PA	GE
36	Gel precipitation test for antibody to low molecular weight protein	120
37	Gel precipitation test of 3 <i>M. ovipneumoniae</i> strains against antibody to the low molecular weight protein	121
38	Gel precipitation test to determine if the low molecular weight protein is a major	

antigen or not

LIST OF TABLES

TABLE PAGE Ι Recovery of M. ovipneumoniae or M. anginini from: (i) The nasal cavity of Perendale lambs swabbed at approximately monthly intervals (ii) The lungs at slaughter 27 II Recovery of M. ovipneumoniae or M. anginini from: (i) The nasal cavity of Suffolk lambs swabbed at approximately monthly intervals. (ii) The lungs at slaughter The nasal carriage of M. ovipneumoniae and III M. anginini of flock 1 (Perendale) and flock 2 (Suffolk) ewes at the time of weaning 32 of their lambs IV The prevalence of lung lesions in: (i) Flock 1 (Perendale lambs) located adjacent to the Pahiatua Track; and 2 (Suffolk (ii) Flock lambs) located at Massey University Sheep Unit No 1 32 V. Recipe for gel preparation using the SDS-50 discontinuous buffer system

xiv

- VI 6 isolates of *M. ovipneumoniae* from one flock. These consist of 3 pairs. The isolates within a pair are indistinguishable by REA. The isolates of different pairs were substantially or totally different by REA
- VII 6 isolates of *M. ovipneumoniae* from one flock. These consist of 3 pairs. The isolates within a pair are indistinguishable by REA. The isolates of different pairs were substantially or totally different by REA
- VIII 8 independent isolates of *M. ovipneumoniae*, examined by SDS-PAGE
 - IX Summary of labelling procedures

PAGE

xv

80

81

99