

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.

EXPERIMENTAL PNEUMONIA  
INDUCED BY A **BORDETELLA PARAPERTUSSIS**-LIKE ORGANISM  
IN THE OVINE AND MURINE LUNG

A thesis presented in partial fulfillment (70%) of the  
requirements for the degree  
of Master of Philosophy  
in veterinary pathology at  
Massey University

CHEN WANGXUE

1987

## ABSTRACT

Thirty-four specific pathogen-free (SPF) Swiss mice were intranasally inoculated with a suspension containing about  $3 \times 10^7$  colony-forming units (CFU)/ml of a *B. parapertussis*-like organism isolated from pneumonic ovine lung. Eleven per cent of the animals died between 2 and 3 days of inoculation and over 90% of infected mice developed a subacute bronchopneumonia morphologically similar to early lesions of naturally-occurring ovine chronic non-progressive pneumonia (CNP). The sequential pulmonary changes were examined by light microscopy and transmission electronmicroscopy from 12 hr to 29 days after inoculation. The early stages were characterized by alveolar septal congestion and oedema, focal intra-alveolar haemorrhage, and intra-alveolar and septal infiltration by neutrophils and macrophages. Later, hyperplasia of perivascular and peribronchiolar lymphoid tissue and the deposition of collagen in the interalveolar septa were prominent. The bronchial and bronchiolar epithelium remained intact throughout the experiment, but bronchiolar lumina became occluded by inflammatory exudate at an early stage. Ultrastructural changes consisted of the degeneration of the alveolar type I and type II epithelial cells and marked degeneration of alveolar macrophages. Pure cultures of the *B. parapertussis*-like organism were consistently recovered from mouse lungs 12 hr to 6 days after inoculation. Both intact and degenerating organisms were found free in alveolar spaces and within phagocytic vacuoles of alveolar macrophages. However, replication of organisms was not observed at any stage of infection and no special association was observed between organisms and the ciliated or non-ciliated respiratory epithelium.

Injury to ovine respiratory tract was demonstrated when a similar bacterial suspension to that given to the mice was given by intratracheally to colostrum-deprived lambs. The lesions produced in the pulmonary parenchyma of the lambs were similar to those seen in both early naturally-occurring ovine CNP and the experimental infection with this organism in mice. They consisted of an acute

mild tracheobronchitis, severe alveolar collapse and acute bronchopneumonia which developed within 24 hr and was most severe at 1 to 3 days after inoculation. Ultrastructurally, the alveolar epithelium exhibited extensive degenerative changes and necrosis of individual epithelial cells. Topographical studies revealed extensive coverage of the infected tracheobronchial epithelium with a dense layer of inflammatory cells mixed with mucus, and focal extrusion of ciliated cells. Occasionally, moderate numbers of the **B. parapertussis**-like coccobacilli were seen closely associated with cilia. Inoculated lambs showed a marked elevation in the numbers of cells in bronchoalveolar lavage 24 hr after infection. Up to 93% of the cells in the lavage at 24 hr were neutrophils. However, no close interaction between phagocytic cells and the organisms was detected. Many of the macrophages in the lavage exhibited cytoplasmic vacuolation from five days after inoculation onwards. Blood leucocyte and neutrophil counts in infected lambs gradually rose to reach peaks at five and three days after inoculation, respectively. The **B. parapertussis**-like organism was recovered in pure culture from the nasal cavity of lambs killed on days one, three, five and nine. The viable bacterial count in bronchoalveolar lavage fluid decreased from 24 hr to 5 days with almost complete elimination of organisms nine days after inoculation.

The retention of the **B. parapertussis**-like organism in the mouse trachea was compared to that in the mouse lung from 0 to 48 hr after intranasal inoculation. Although there was greater bacterial deposition in the trachea than the lung there was a faster clearance from the trachea. At 48 hr after instillation, almost all organisms were eliminated from the trachea but about 45% of organisms were retained in the lung.

The current investigation has shown that the **B. parapertussis**-like organism can infect SPF mice and colostrum-deprived lambs and induce a subacute bronchopneumonia. The morphological changes seen suggest that this organism has the potential to predispose the ovine respiratory tract to further infection by other microorganisms and that the organism itself may also be able to cause severe pulmonary damage. The relevance of these observations to the problem of CNP in sheep in the field has yet to be determined.

## ACKNOWLEDGMENTS

I am grateful to the Department of Veterinary Pathology and Public Health, Massey University, for providing me with the opportunity to undertake this research project. I am particularly indebted to my chief supervisor Dr. M.R. Alley for his constant encouragement, and to my supervisor Professor B.W. Manktelow for his invaluable advice and helpful criticism during all phases of this work. My thanks are also due to Dr. A. Baskerville, Dr. R.B. Marshall and Dr. A. Al-Kaissi for their valuable suggestions.

This study could not have been fully undertaken without the technical assistance of a number of people whose help I gratefully acknowledge. Skilled assistance was provided by:

Mrs. P. Slack and Mrs. P. Davey for histological preparations and other technical help whenever needed;

Miss L. Cullinane in the bacteriological studies;

Mr. D. Hopcroft in the preparation of tissues for scanning electron microscopy;

Mr. P. Wildbore in technical administration;

Miss E. Davies in the haematological studies;

Mrs. J. Schrama in media preparation;

Mrs. L. Dickson and Mr. F. Sharpe for their help in arranging animals for the transmission experiments.

Special thanks are also due to Mrs. A. Scott for her skilful guidance and invaluable assistance in the preparation of the final copy of this manuscript.

Parts of tables in this thesis were typed by Mrs. E.M. Wake. The photographs were printed by the staff at the Central Photographic Unit and Mr. T. Law. Their efforts are greatly appreciated.

I also need to thank my colleagues in the Veterinary Science Department, at Zhejiang Agricultural University, People's Republic of China, for supporting my leave extension.

Finally, I would like to express my thanks to my friends and family for their understanding and forbearance, but most of all I would like to thank my wife Su Dairu for her love, support and willingness to share in all aspects of my study. This thesis is dedicated to her and my son.

Financially, this work was jointly supported by a Chinese Visiting Scholarship, the Department of Veterinary Pathology & Public Health, a Graduate Study Award from Massey University and a BNZ Postgraduate Study Bursary in Veterinary Science. I would like to express my thanks to my supervisors for their support in helping me to obtain this financial assistance.

## TABLE OF CONTENTS

	Page
ABSTRACT	
ACKNOWLEDGEMENTS	
LIST OF FIGURES	
LIST OF TABLES	
INTRODUCTION	
CHAPTER 1 GENERAL LITERATURE REVIEW	1
Section 1 Pathology of naturally-occurring ovine pneumonia	2
1.1 Acute fibrinous pneumonia (AFP)	3
1.1.1 Macroscopic lesions	3
1.1.2 Microscopic lesions	5
1.1.3 Ultrastructural changes	6
1.2 Chronic non-progressive pneumonia (CNP)	6
1.2.1 Macroscopic lesions	8
1.2.2 Microscopic lesions	9
1.2.3 Ultrastructural changes	12
Section 2 The role of bacteria in pneumonia of sheep	15
2.1 Epidemiological evidence of bacterial involvement	16
2.1.1 <i>Pasteurella haemolytica</i>	16
2.1.2 <i>Pasteurella multocida</i>	20
2.1.3 Other bacteria	21
2.2 Experimental bacterial infection <u>in vivo</u>	24
2.3 Experimental bacterial infection <u>in vitro</u>	26
2.4 Possible importance of bacterial cytotoxins	30
2.4.1 Exotoxin (Leucotoxin)	31
2.4.2 Endotoxin (Lipopolysaccharide, LPS)	33
2.5 Conclusions	34
Section 3 Biology of <i>Bordetella parapertussis</i>	36
3.1 General description of <i>B. parapertussis</i>	37
3.2 Epidemiology	40
3.3 Pathogenesis	42
3.4 Pathology	45
3.4.1 Human infection	46
3.4.2 Animal models	48
3.5 Conclusions	50

	Page
CHAPTER 2    INDUCTION OF PNEUMONIA IN MICE WITH A <b>BORDETELLA</b> <b>PARAPERTUSSIS-LIKE ORGANISM</b>	52
CHAPTER 3    EXPERIMENTAL INFECTION OF LAMBS WITH A <b>BORDETELLA</b> <b>PARAPERTUSSIS-LIKE ORGANISM</b>	77
CHAPTER 4    TRACHEAL VERSUS PULMONARY CLEARANCE OF A <b>BORDETELLA</b> <b>PARAPERTUSSIS-LIKE ORGANISM IN MICE</b>	108
CHAPTER 5    GENERAL DISCUSSION	116
BIBLIOGRAPHY	122
APPENDIX	



## LIST OF FIGURES

Figure		Following page
1.1	<b>Bordetella pertussis</b> components putatively involved in the pathogenesis of pertussis.	43
2.1	Right: Lungs from a mouse killed three days after inoculation of the <b>B. parapertussis</b> -like organism. There is dull red consolidation involving the entire left lobe of the lung. Left: Lungs from a control mouse killed at the same time.	57
2.2	I: Pulmonary consolidation in a mouse killed five days after inoculation of the <b>B. parapertussis</b> -like organism. The lungs are enlarged and show dull red consolidation in the anterior and middle parts of the left lobe and focal consolidation in the right lobe. C: Lungs from a control mouse killed at the same time.	57
2.3	I: Gross appearance of mouse lung showing marked enlargement two days after inoculation with the <b>B. parapertussis</b> -like organism. Red areas of consolidation extend through almost the entire lungs. C: Lungs from control mouse killed at the same time.	58
2.4	Extensive pulmonary congestion and oedema in a mouse which died three days after inoculation of the <b>B. parapertussis</b> -like organism.	58
2.5	Mouse lung 12 hr after inoculation of the <b>B. parapertussis</b> -like organism showing patchy pneumonia surrounding small bronchioles. H.E. x 12.5	59
2.6	A focal area of pneumonia in mouse lung 12 hr after inoculation. There is moderate alveolar collapse and interstitial hypercellularity. H.E. x 50 Inset: Higher power photomicrograph from the same field. H.E. x 250	59

- 2.7 Peribronchiolar infiltration by neutrophils in a mouse killed 12 hr after inoculation. The surrounding alveoli are congested and also infiltrated by many neutrophils. H.E. x 125 59
- 2.8 The dense homogenous appearance of pulmonary tissue in a mouse killed 24 hr after inoculation. Note the marked infiltration of neutrophils and severe alveolar collapse. H.E. x 125 59
- 2.9 Confluence of focal pneumonia in a mouse killed two days after inoculation. H.E. x 50 59
- 2.10 An intense neutrophilic exudate has accumulated in a bronchiole and surrounding alveoli of a mouse killed two days after inoculation with the **B. parapertussis**-like organism. H.E. x 250 59
- 2.11 Lung from mouse killed two days after inoculation showing focal intra-alveolar haemorrhage. H.E. x 250 59
- 2.12 Mouse lung four days after inoculation with the **B. parapertussis**-like organism showing an increase in numbers of macrophages in some alveolar spaces. Neutrophils are still present at this time. H.E. x 250 59
- 2.13 Mouse lung five days after inoculation. Besides the marked infiltration of neutrophils and macrophages into the bronchiolar lumen and alveolar spaces, a perivascular and very mild peribronchiolar lymphoid hyperplasia is present in some areas. H.E. x 125 59
- 2.14 Lung from a mouse which died two days after inoculation showing extensive pulmonary congestion and moderate oedema. Many small bronchioles and their surrounding alveoli are necrotic (arrows). H.E. x 50 60

- 2.15 Severe extensive pulmonary congestion and oedema in a mouse which died three days after inoculation of the **B. parapertussis**-like organism. The perivascular lymphatics are markedly dilated and contained much fibrinous exudate. H.E. x 125 60
- 2.16 Many alveoli in the lungs of mice which died three days after inoculation contained densely packed fibrinous material and small numbers of mixed inflammatory cells. H.E. x 250 60
- 2.17 Mouse lung six days after inoculation. Neutrophil infiltration is still a prominent feature in many bronchioles. The numbers of macrophages in alveoli are significantly increased. H.E. x 125 60
- 2.18 Infiltration of large numbers of macrophages into the alveolar spaces in a mouse killed eight days after inoculation with the **B. parapertussis**-like organism. These cells are very large in size and have a foamy cytoplasm and a relative small nucleus. H.E. x 250 60
- 2.19 Perivascular and peribronchiolar lymphoid aggregations. Note that lymphoid hyperplasia is more severe in areas surrounding blood vessels than around bronchioles. The adjacent alveoli contain small numbers of macrophages. Mouse killed eight days after inoculation of the **B. parapertussis**-like organism. H.E. x 125 60
- 2.20 The occlusion of a bronchiolar lumen by mucus and cellular debris in a mouse killed 14 days after inoculation of the **B. parapertussis**-like organism. The bronchiolar epithelium is moderately hyperplastic and the surrounding alveoli are collapsed. H.E. x 50 61

- 2.21 A mouse lung in the resolution stage. Apart from a few alveoli containing proteinaceous exudate, the majority of alveoli are free of exudate and re-aerated. The only remaining pneumonic feature is the presence of perivascular and peribronchiolar lymphoid cuffs. Mouse killed 24 days after inoculation. H.E. x 50 61
- 2.22 Early proliferation of alveolar type II cells can be seen in some alveoli. Mouse killed 29 days after inoculation. Epoxy embedded. Tb x 500 61
- 2.23 Bronchiolar mucosa from a mouse killed 12 hr after inoculation of the *B. parapertussis*-like organism. A ciliated cell shows loss of ciliary shafts and internalization of basal bodies (arrows). TEM x 7,800 62
- 2.24 The contents of a bronchiole from a mouse killed five days after inoculation. There are large numbers of neutrophils (N), a few macrophages (M) and necrotic cellular debris. TEM x 3,400 62
- 2.25 A bronchiolar lumen densely packed with cellular debris from degenerating macrophages (M), mixed with amorphous material. From a mouse 20 days after inoculation. TEM x 5200 62
- 2.26 A bronchiole from the mouse killed five days after inoculation showing a focal cytoplasmic projection (arrows) on the apex of a ciliated cell. The cell itself has fewer cilia than normal. TEM x 11,200 62
- 2.27 Mild infiltration of inflammatory cells into the bronchiolar mucosa in a mouse killed six days after inoculation. A neutrophil (N) and a small mononuclear cell (M) are migrating between epithelial cells and large number of neutrophils are present in the submucosa. TEM x 3,400 62

- 2.28 A bronchiole showing marked increase in numbers of Clara cells. Mouse killed 26 days after inoculation with the **B. parapertussis**-like organism. TEM x 3,400 62
- 2.29 Diffuse swelling of an alveolar type I cell (I) in a mouse killed one day after inoculation of the **B. parapertussis**-like organism. TEM x 11,200 63
- 2.30 An alveolus from the lung of a mouse which died three days after inoculation. The alveolar type I (I) and type II (E) epithelial cells show moderate degenerative changes. There is accumulation of electron-dense proteinaceous fluid and necrotic debris in the alveolar spaces (A). The alveolar capillaries (c) contain erythrocytes, neutrophils and macrophages. TEM x 5,200 63
- 2.31 An alveolus from a mouse killed 11 days after inoculation. The alveolar epithelium shows areas of thickening of plasma membrane (arrows) in both types of cell. TEM x 5,200 63
- 2.32 Alveolar wall showing focal sloughing of a type I epithelial cell (arrow) leaving a denuded basement membrane. A macrophage (M) is closely adherent to the underlying capillary endothelium. Mouse killed three days after inoculation. TEM x 7,800 63
- 2.33 Degenerative changes in an alveolar type II cell three days after inoculation. The cell (E) has swollen mitochondria (m), increase in ribosomes, severe decrease in number of lamellated bodies, loss of microvilli (arrow) and migration of chromatin to the periphery of the nucleus (n). TEM x 7,800 63
- 2.34 Early degenerative changes in an alveolar type II cell consisting of severe swollen and disrupted mitochondria and distention of the rough endoplasmic reticulum (arrow). Mouse which died three days after inoculation. TEM x 15,300 63

- 2.35 A type II alveolar epithelial cell (E) of a mouse killed five days after inoculation having an electron-dense plasma membrane (arrow) on its outer surface. The alveolar space (A) contains inflammatory exudate which is composed of many macrophages and a few neutrophils. The alveolar septum (S) shows infiltration of macrophages and neutrophils into the interstitium. TEM x 11,200 63
- 2.36 Interalveolar septum (S) and alveolar spaces (A) from a mouse which died three days after inoculation. The alveolar space contains a desquamated type II cell (E) and extensive amorphous material. There are aggregations of platelets and monocytes in a dilated capillary lumen (c). TEM x 7,800 63
- 2.37 Alveolar spaces (A) from the lung of a mouse killed one day after inoculation. Neutrophils are predominant in the intra-alveolar exudate. The alveolar type I cells (I) show extensive vesiculation of the cytoplasm. The interalveolar septa contain leucocyte-packed capillaries (c) and infiltrating macrophages (M). TEM x 5,200 64
- 2.38 Typical intra-alveolar cellular population in a mouse killed one day after inoculation. The neutrophils have numerous digestion vacuoles (v) in the cytoplasm and these contain much phagocytosed material and occasional **B. parapertussis**-like organisms (arrow). TEM x 7,800 64
- 2.39 An alveolar macrophage from a mouse killed three days after inoculation showing the cytoplasm almost completely occupied by numerous empty vacuoles. TEM x 5,200 64

- 2.40 An alveolar macrophage from a mouse killed five days after inoculation. The cell has many phagocytic vacuoles (v) which contain large quantities of cellular debris. The nucleus (n) has been displaced to the periphery of the cell. TEM x 7,800 64
- 2.41 An alveolar macrophage containing cytoplasmic aggregations of glycogen-like particles (G). Mouse killed five days after inoculation of the **B. parapertussis**-like organism. TEM x 7,800 64
- 2.42 Pulmonary macrophages (M) in the alveoli of a mouse killed 11 days after inoculation. There are many electron-dense granules (g) and other electron-dense material (arrow) in the cytoplasm as well as numerous pseudopodia. TEM x 5,200 65
- 2.43 A macrophage containing large amounts of presumed degenerate pulmonary surfactant material (s) in phagocytic vacuoles. Mouse killed 29 days after inoculation. TEM x 11,200 65
- 2.44 The interalveolar septum (S) of a mouse killed one day after inoculation. The alveolar wall is moderately thickened by the infiltration of neutrophils (N) and mononuclear cells (M). The alveolar epithelium shows areas of swelling (arrows). TEM x 5,200 65
- 2.45 Thickening of interalveolar septa (S) in a mouse killed eight days after inoculation. There is proliferation of collagen (C) and infiltration of mononuclear cells (M). The alveoli (A) contained occasional inflammatory cells. TEM x 3,400 65
- 2.46 The alveolar basement membrane (arrows) is separated by proliferating collagen (C) from the underlying interstitial tissue. A neutrophil (N) can be seen closely associated with an alveolar type I cell (I). Mouse killed 11 days after inoculation. TEM x 5,200 65

- 2.47 Alveoli from a mouse killed 11 days after inoculation. 65  
There is severe collapse, congestion and leucocytes in  
alveolar capillaries (c) as well as interstitial  
infiltration of inflammatory cells and early fibrosis  
(F). TEM x 5,200
- 2.48 Alveoli from a mouse killed 26 days after inoculation. 65  
The interalveolar septa are markedly thickened by the  
proliferation of collagen (C). The alveolar epithelium  
at this stage appears to be normal. TEM x 11,200
- 2.49 **Bordetella parapertussis**-like organisms in the alveolar 66  
spaces of a mouse which died two days after inoculation.  
Many intact organisms (arrows) are trapped in the  
necrotic debris and exudate in alveolar spaces.  
The organism has a furrowed cell wall with abundant  
ribosomes in its cytoplasm. The nucleoplasm of the cell  
is whorled and rarefied. TEM x 48,600
- 2.50 **Bordetella parapertussis**-like organisms (arrows) in 66  
the phagocytic vacuoles of alveolar macrophages showing  
varying degrees of degeneration. From a mouse killed  
six days after inoculation. TEM x 31,800
- 3.1 Average daily rectal temperature of lambs inoculated 83  
with either sterile PBS or the **B. parapertussis**-like  
organism (BPLO).
- 3.2 Comparison of total blood leucocyte counts between 83  
groups of lambs after intratracheal instillation of  
either PBS or the **B. parapertussis**-like organism (BPLO).
- 3.3 Comparison of blood neutrophil counts between 83  
groups of lambs after intratracheal instillation of  
either PBS or the **B. parapertussis**-like organism (BPLO).



3.4	Distribution of gross lung lesions in infected and control lambs.	84
3.5	Lung from a lamb 24 hr after inoculation of sterile PBS showing a normal appearance.	84
3.6	Lungs from a lamb 24 hr after inoculation of the <b>B. parapertussis</b> -like organism. A thick dull red band (arrow) of consolidation is present in the right lung roughly parallel with the midline. It extends from the posterior part of the cranial lobe to the mid ventral region of the caudal lobe.	84
3.7	Three days after inoculation of the <b>B. parapertussis</b> -like organism. Several small irregular foci of dull red pulmonary consolidation and patchy areas of congestion are present on the ventral margins of the right middle and caudal lobes.	85
3.8	Nine days after inoculation of the <b>B. parapertussis</b> -like organism. Several dull red areas of collapse of varying size persist in the ventral margin of right middle and caudal lobes of the lung.	85
3.9	Comparison of total bronchoalveolar lavage cell counts between groups of receiving either sterile PBS or the <b>B. parapertussis</b> -like organism (BPLO).	85
3.10	Bronchoalveolar lavage cell from a control lamb killed nine days after inoculation consisting mainly of macrophages. Giemsa stain, x 500	85
3.11	Bronchoalveolar cells from a lamb inoculated with the <b>B. parapertussis</b> -like organism and killed 24 hr later. Note that a large proportion of cells are neutrophils. Giemsa stain, x 500	85

- 3.12 Alveolar macrophages recovered from an animal killed nine days after inoculation showing extensive cytoplasmic vacuolation. Giemsa stain, x 250 85
- 3.13 An alveolar macrophage recovered from a control lamb given sterile PBS intratracheally and killed 24 hr later. The cytoplasm of the cell is relatively electron-dense and contains numerous characteristic phagolysosomes (p), endoplasmic reticulum (r) and mitochondria (m). TEM x 7,800 86
- 3.14 Another type of alveolar macrophage (M) recovered from a control lamb given sterile PBS intratracheally and killed 24 hr later. The cytoplasm is less electron-dense but contains high numbers of phagolysosomes (p). Organelles are often distended, and numerous pseudopods (P) are present on the cell surface. TEM x 11,200 86
- 3.15 A bronchoalveolar macrophage of lamb inoculated with the *B. parapertussis*-like organism and killed 24 hr later. The cell has a similar morphology to a normal macrophage but contains a degenerate neutrophil (D) in a phagocytic vacuole. TEM x 7,800 86
- 3.16 A macrophage from bronchoalveolar lavage fluid from a lamb inoculated with the *B. parapertussis*-like organism, killed 24 hr later. Two phagocytic vacuoles containing degenerating organisms (arrows) are visible. TEM x 48,600 86
- 3.17 Bronchoalveolar macrophages from a lamb inoculated with the *B. parapertussis*-like organism and killed at p.i.d. 5. Most macrophages have undergone varying stages of cellular disruption. The degenerate cells contain a rounded pyknotic nucleus (n) with thickened nuclear membrane (arrow). The cytoplasm is sparse and contains many vacuoles. TEM x 5,200 86

- 3.18 A degenerate alveolar macrophage (M) from a lamb killed nine days after inoculation. The cytoplasm is electron-lucent, shows severe vacuolation of the rough endoplasmic reticulum (arrow) and swelling of mitochondria (m). It has decreased numbers of phagolysosomes (p). The nuclear membrane is separating from the nuclear contents (arrowhead) and the cell surface has lost its pseudopodia. TEM x 7,800 86
- 3.19 Exudate in the trachea of a lamb killed 24 hr after inoculation. Large numbers of neutrophils, macrophages, and sloughed epithelial cells mixed with mucus are present in the lumen. H.E. x 250 87
- 3.20 Acute focal tracheitis in a lamb killed at 24 hr after inoculation of the **B. parapertussis**-like organism. Large numbers of neutrophils are present between the epithelial cells of the tracheal mucosa. The lamina propria is infiltrated by many mononuclear cells and a few neutrophils. H.E. x 250 87
- 3.21 Extensive infiltration of neutrophils into bronchi, bronchioles and surrounding alveoli, many of which are collapsed. Lamb killed 24 hr after inoculation with the **B. parapertussis**-like organism. H.E. x 50 87
- 3.22 Bronchus from a lamb killed 24 hr after inoculation showing early lymphoid aggregation (arrows) in the lamina propria with large numbers of neutrophils in the bronchial lumen. H.E. x 250 87
- 3.23 Extensive areas of alveolar collapse with the accumulation of neutrophils in terminal bronchioles. They are well-demarcated from the less affected surrounding areas. Lung from lamb killed 24 hr after inoculation. H.E. x 50 88

- 3.24 Extensive infiltration of neutrophils into terminal bronchioles and collapsed alveolar spaces in a lamb killed 24 hr after inoculation. H.E. x 125 88
- 3.25 Fibrinous exudate mixed with numerous neutrophils and a few macrophages in the alveoli of a lamb killed 24 hr after inoculation. H.E. x 125 88
- 3.26 Focal intra-alveolar haemorrhage (arrows) with pronounced inflammatory exudation into alveoli of a lamb killed 24 hr after inoculation. H.E. x 250 88
- 3.27 The alveoli at the periphery of the pneumonic areas containing moderate numbers of macrophages and a few neutrophils. Lamb killed 24 hr after infection. Epoxy embedded, Tb x 250 88
- 3.28 A severe tracheitis in a lamb killed three days after inoculation. Numerous neutrophils have infiltrated between the epithelial cells, and into the lamina propria and submucosa. H.E. x 250 88
- 3.29 Tracheal mucosa of a lamb killed three days after inoculation. There are moderate numbers of lymphoid aggregations immediately beneath the epithelial layer. H.E. x 250 88
- 3.30 Lung from a lamb killed three days after inoculation showing accumulation of neutrophils in the lumen and neutrophilic infiltration of the peribronchiolar alveoli. H.E. x 250 88
- 3.31 Extensive areas of alveolar collapse with the accumulation of moderate numbers of neutrophils in terminal bronchioles and alveolar spaces. Lamb killed three days after inoculation of the **B. parapertussis**-like organism. H.E. x 50 89

- 3.32 Intensive infiltration of numerous neutrophils and a few macrophages into the alveoli. Lamb killed three days after inoculation. H.E. x 125 89
- 3.33 Focal severe fibrinous exudate in the alveoli at three days after inoculation. Moderate numbers of neutrophils are embedded in the exudate. H.E. x 250 89
- 3.34 A perivascular cuff in a lamb 24 hr after inoculation composed mainly of mononuclear cells. These cuffs were usually associated with bronchioles containing inflammatory exudate. H.E. x 250 89
- 3.35 The lung from a lamb five days after inoculation with the **B. parapertussis**-like organism. The collapsed alveoli present a featureless appearance and only very mild inflammatory change can be seen at low magnification. H.E. x 125 89
- 3.36 Alveoli from the lung of a lamb killed five days after inoculation. The hypercellularity of alveolar septa is mainly due to infiltrating mononuclear cells. The alveoli contain many macrophages and a few neutrophils. H.E. x 250 89
- 3.37 Lung from lamb killed five days after inoculation of the **B. parapertussis**-like organism showing mild proliferation of alveolar type II cells. Almost every alveolar space in the section contains a prominent type II cell. The alveoli in the less severely collapsed areas showed infiltration of small numbers of macrophages. Epoxy embedded, Tb x 250 89
- 3.38 Lung nine days after inoculation showing re-aeration of most alveoli. Mild to moderate perivascular and peribronchiolar lymphoid cuffs were invariably present. H.E. x 50 89

- 3.39 The epithelial surface of the trachea from a control lamb composed almost entirely of ciliated cells. Inset: Higher magnification showing the normal feature of cilia. SEM Bar 1 $\mu$ m 90
- 3.40 The epithelial surface of a bronchus from a control lamb. Ciliated cells cover the majority of the luminal surface but many non-ciliated cells are also present in some areas. SEM Bar 1 $\mu$ m 90
- 3.41 The epithelial surface of a bronchus from control lambs containing occasional non-ciliated cells, openings of goblet cells and submucosal glands. The figure illustrates the discharging secretion of a goblet cell fixed in situ (arrow). SEM Bar 1 $\mu$ m 90
- 3.42 Mucosal surface of the trachea of a lamb 24 hr after inoculation of the **B. parapertussis**-like organism. The tracheal ciliated epithelium is almost completely covered by a dense layer of inflammatory cells, probably neutrophils, together with some mucous strands and granules. SEM Bar 1 $\mu$ m 90
- 3.43 The luminal surface of a bronchus from a lamb 24 hr after inoculation showing a thick layer of mucus mixed with neutrophils (N) and macrophages (M) covering areas of the ciliated surface. A variable number of coccobacilli (arrows) are trapped in the mucus. SEM Bar 1 $\mu$ m 90
- 3.44 Tracheal mucosa in a lamb 24 hr after inoculation showing many cilia forming conglomerations with adjacent cilia (arrow). Inset: High magnification showing coccobacilli (arrow) on the surface of the cilia. SEM Bar 1 $\mu$ m 90

- 3.45 Ciliated cell damage in the bronchial mucosa three days after inoculation with the **B. parapertussis**-like organism. Severe swelling and extrusion of ciliated cells were noted (arrow). There are only few cilia retained on the surface of these disrupted cells. SEM Bar 1 $\mu$ m 90
- 3.46 Bronchial epithelial surface of a lamb three days after inoculation showing partial exfoliation of the affected ciliated epithelium. A ciliated columnar epithelial cell (CC) with an attached organism is shown extruding from the luminal surface. Inset: Higher magnification to show an organism attached to a cilium of the extruded cell. SEM Bar 1 $\mu$ m 91
- 3.47 Tracheal mucosal surface three days after inoculation showing a decrease in inflammatory cell exudate and the amount of mucus. SEM Bar 1 $\mu$ m 91
- 3.48 The bronchial mucosa at five days after inoculation. It is covered with a little exudate and very small numbers of bacteria (arrows) randomly scattered over the tops of cilia. The ciliary density has decreased slightly. SEM Bar 1 $\mu$ m 91
- 3.49 Tracheal epithelial cells from a lamb three days after inoculation with the **B. parapertussis**-like organism. The ciliated cells show severe reduction in ciliary density. The cytoplasm contains swollen mitochondria (m) and enlarged endoplasmic reticulum (r) which has markedly dilated cisterni. TEM x 11,200 92
- 3.50 The tracheal epithelium of a lamb killed nine days after inoculation showing only a few retained cilia (arrows) and marked distention of cellular organelles. TEM x 15,300 92

- 3.51 An atypical ciliary structure (arrow) observed in the tracheal epithelium of a lamb nine days after inoculation. Multiple true cilia have fused to form a giant, bizarre structure which is enveloped by the outer cell membrane. TEM x 11,200 92
- 3.52 Cytoplasmic vacuolation in bronchi of a lamb killed 24 hr after inoculation. Note the vacuoles present in the cytoplasm of goblet cells (GC) and a brush cell (BC). TEM x 7,800 92
- 3.53 A bronchiole from a lamb killed 24 hr after inoculation of the **B. parapertussis**-like organism. There are several neutrophils, and some cellular debris in the lumen. Note a neutrophil (N) infiltrating between the epithelial cells. The submucosa is oedematous. TEM x 3,400 92
- 3.54 The bronchiole of lamb killed 24 hr after inoculation showing marked decrease in numbers of cilia. Instead, the microvilli on the luminal surface are higher (a) and thicker (b) than normal, and densely distributed. TEM x 11,200 92
- 3.55 Bronchiolar epithelium three days after inoculation of the **B. parapertussis**-like organism showing cytoplasmic projections from the ciliated cells. TEM x 15,300 92
- 3.56 An alveolus of a lamb killed 24 hr after inoculation of the **B. parapertussis**-like organism. The type I cells show an increase in density of cytoplasmic extensions (arrow). The alveolar capillary (c) is distended by erythrocytes and leucocytes. There is marked oedema in the interalveolar septa (S). TEM x 5,200 93



- 3.57 Alveoli from a lamb killed 24 hr after inoculation. 93  
Part of a type I cell has sloughed (arrow) exposing the underlying basement membrane. Elsewhere the type I cells have abnormally dense cytoplasm. A mononuclear cell (M) has infiltrated into the moderately oedematous interstitium. TEM x 5,200
- 3.58 Degenerative changes in alveolar type I cells three 93  
days after inoculation. The cells show diffuse swelling of the cytoplasmic extensions lining the alveolar space. The underlying capillary is packed with erythrocytes. TEM x 7,800
- 3.59 Early degeneration of a type II cell (E). The 93  
degenerative cell shows generalised enlargement, swelling of mitochondria, distention of endoplasmic reticulum and loss of microvilli. The alveolar interstitium (S) is moderately thickened by proteinaceous and cellular exudate. Lamb killed 24 hr after inoculation. TEM x 5,200
- 3.60 Desquamation of type II cell (E) in a lamb killed 93  
24 hr after infection. The desquamating cell exhibits early degenerative change consisting of mild pyknosis (n) and enlargement of lamellated bodies (b). The alveolar capillaries are severely congested. An erythrocyte (R) has been phagocytosed by a macrophage (M) which is closely associated with the alveolar epithelium. TEM x 5,200
- 3.61 Desquamation of alveolar type II cell E) in a lamb 93  
three days after inoculation of the **B. parapertussis**-like organism. The sloughed cell shows advanced degeneration with karyolysis (n). The alveolar space (A) contains proteinaceous exudate and underlying capillaries (c) are densely packed with erythrocytes. TEM x 7,800

- 3.62 Advanced degeneration of a type II cell (E) in a lamb killed three days after inoculation. The cell has few microvilli and shows severe dilation of endoplasmic reticulum with migration of chromatin in the nucleus. The cellular membrane has disintegrated (arrow). TEM x 7,800 93
- 3.63 High magnification of degenerate type II cell in an alveolus. The cell shows swollen mitochondria, distended endoplasmic reticulum, distended nuclear envelope (e) and reduced numbers of microvilli. Lamb killed three days after receiving the **B. parapertussis**-like organism. TEM x 15,300 93
- 3.64 A severely degenerating type II cell (E). The cell shows severe distension of most cellular organelles as well as karyorrhexis (n). Large segments of the plasma membrane show severe thickening (arrow). TEM x 11,200 94
- 3.65 Early proliferation of alveolar type II cells (E) in the corner of an alveolar space (A). The cells contain large lamellated bodies (b) and have relatively few microvilli. Lamb killed 24 hr after inoculation. TEM x 5,200 94
- 3.66 Alveolus of a lamb 24 hr after inoculation of the **B. parapertussis**-like organism. The alveolar capillaries are congested and contain neutrophils (N). A macrophage (M) can be seen in the interstitium. The alveolar space (A) contains some proteinaceous material and a type II cell (E) shows loss of microvilli and abnormally dense cytoplasm. TEM x 5,200 95
- 3.67 A small bronchiolar vein (V) in a lamb killed five days after inoculation of the **B. parapertussis**-like organism. There are numerous aggregated platelets and erythrocytes occluding the lumen. TEM x 5,200 95

- 3.68 Moderately severe alveolar collapse in a lamb killed nine days after inoculation. The alveolar interstitium (S) is moderately thickened and contains several migrating mononuclear cells (M). The alveolar capillaries (c) are congested. The cytoplasmic extensions of alveolar type I cells are swollen in some areas (arrow). TEM x 3,400 95
- 3.69 Early interalveolar fibrosis (F) in a lamb killed nine days after inoculation. TEM x 5,200 95
- 3.70 Number of viable **B. parapertussis**-like organisms in the bronchoalveolar lavage fluid of infected lambs. 95
- 4.1 Pulmonary and tracheal retention of the **B. parapertussis**-like organism in mice sacrificed at different time intervals after inoculation. 111

## LIST OF TABLES

Table	Following page
1.1 Bacterial species isolated from the pneumonic ovine respiratory tract	15
1.2 Characteristics of <b>Pasteurella haemolytica</b> A and T biotypes	16
1.3 Differential characteristics within the genus <b>Bordetella</b>	37
1.4 Antigenic factors of the <b>Bordetella</b> species	39
2.1 Experimental design: Schedule of inoculation and killing of mice inoculated with <b>B. parapertussis</b> -like organism (BPLO)	55
2.2 Results of intranasal inoculation of mice with <b>B. parapertussis</b> -like organism	57
4.1 Colony forming units of <b>B. parapertussis</b> -like organism in the lungs and trachea of mice at various times after inoculation ( $x \pm SD$ )	111

## INTRODUCTION

Pneumonia is one of the most important infectious diseases of sheep, especially feedlot lambs, in sheep-raising countries throughout the world. As New Zealand is one of the largest exporters of lamb in the world, ovine pneumonia is of special economic importance in this country. The disease can infect animals whether they are fattened intensively indoors, are grazed extensively on pasture for all or part of the year, or are reared under nomadic conditions (Davies, 1985).

Ovine pneumonia in New Zealand is usually divided into two forms (Alley, 1975a). One is an acute fibrinous pneumonia (AFP) as has been described by Salisbury in 1957, and the other more common form is chronic non-progressive pneumonia (CNP) which was described initially by Alley (1975a) and comprehensively studied in vivo and in vitro by Alley (1975a & b) and Al-Kaissi (1986).

Fortunately, outbreaks involving mortalities from acute pneumonia in sheep are sporadic in New Zealand, so overall losses are low (Manktelow, 1984) although in some individual flocks mortality may reach up to 47% (Sorenson, 1976). The disease was second only to pregnancy toxemia as a cause of death in a survey conducted in 1974 (Davies, 1974) and caused 1-8% mortalities of sheep 3 years and older according to surveys by Salisbury (1957) and Downey (1957). The surviving animals may lose an average of 1.4 kg body weight per animal and 0.28 kg wool per animal (Nikitin et al., 1981).

The most common form of ovine pneumonia in this country is CNP and up to 70 to 80% of the lambs in some flocks may be affected (Alley, 1975a). Since CNP is usually subclinical, it is difficult to estimate its real economic importance. The initial studies in this country showed that when lambs were affected at an early age, survivors were likely to take longer to reach a predetermined weaning weight (Kirton et al., 1976). More recent weight gain trials

have shown that under pastoral conditions, lambs affected by CNP had a mean liveweight gain of 1.74 kg less than controls after 30 days, and 2.19 kg after 60 days (Alley, 1987). In poor growing conditions, some affected animals even lost weight. A significant linear relationship was established between liveweight gain and the extent of the pneumonic lesions.

Another important indirect economic loss is the development of pleural adhesions, a common sequela in both AFP (Alley, 1975a) and CNP (Jones & Gilmour, 1983). Carcasses with pleural adhesions, are unacceptable in several major overseas markets such as the U.S.A., Canada, and European Economic Community (EEC), and are therefore downgraded (Brain, 1980). The pleural adhesions may sometimes account for 31.4% of the carcass defects, second only to sarcocystis (Central Districts Farmer, 1985). The annual loss attributed to pleurisy alone in the industry in New Zealand, excluding the cost of treatment or preventive measures, has been estimated at 1.8 million New Zealand dollars in 1974/1975 season (Dysart, 1976), and 26 million in 1983 (Alley, 1983).

The aetiology and details of the pathogenesis of CNP have however, not been unequivocally determined. It is currently believed that the disease is multifactorial, and that the presence of bacteria is essential for the development of the lesions (Alley & Clarke, 1979; Jones & Gilmour, 1983; Davies, 1985). A wide range of bacteria have been isolated from pneumonic ovine lungs (Steveson, 1969; Alley, 1975b; Robinson, 1983; Davies, 1985), but the aetiological importance of many of them is doubtful. Recently, a **Bordetella parapertussis**-like organism was isolated from both pneumonic and healthy ovine lungs as well as the nasal cavities of healthy ewes (Manktelow, 1984; Alley, 1986; Cullinane et al., 1987). The organism was demonstrated to be able to attach and damage the ovine ciliary epithelium in tracheal organ culture (Al-Kaissi, 1986) and it was proposed that this organism may have a role in initiating or prolonging CNP in sheep in New Zealand (Al-Kaissi et al., 1986).

The objective of the current research was to study the possible role and pathogenesis of the **B. parapertussis**-like organism in CNP and to accumulate preliminary information on its pathogenicity. A

three-step project was designed, aimed at firstly establishing a laboratory animal model to study the pathogenesis of any disease caused by the organism. Secondly, to determine the pathogenicity of the organism for colostrum-deprived lambs and finally to investigate the persistence of the organism in the trachea and lungs of SPF mouse.