

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.

*Pasteurella multocida*

---

A Study on the Isolation, Identification and  
Characterization of New Zealand Strains.

A Thesis Presented in Partial Fulfilment of the  
Requirements for the Degree of Master of Science  
in Microbiology at Massey University, New Zealand.

Rhys John Jones

1988

### ABSTRACT

*Pasteurella multocida* is a small Gram negative bacillus which causes disease in many animal species. Its pathogenicity is attributed to two mechanisms: invasiveness and toxin production. The proportion of strains which produce toxin is low but such strains cause severe atrophic rhinitis in swine. Invasiveness is the predominant mechanism of pathogenicity and is largely dependant on the possession of a capsule.

*P. multocida* is divided into five serotypes (A, B, D, E and F) based on the antigenic structure of the capsule and a correlation exists between serotype, disease and the animal species affected. However, many isolates are non-typable due to the possession of a non-antigenic (hyaluronic acid) capsule. The presence of an antigenic capsule and a hyaluronic acid capsule are independant. ie one, both or neither may be present.

To facilitate the isolation of *P. multocida* we assessed the selective medium of Smith and Baskerville(1983) and found that many New Zealand isolates of *P. multocida* grew poorly in the absence of blood (not present in Smith and Baskerville's medium). Furthermore, many isolates were inhibited by Polymixin and the high alkalinity (pH 8.6) of the medium.

On the basis of these observations we formulated a modified selective medium which omitted Polymixin and was composed of a blood agar base at neutral pH. However, it contained three antibiotics (Gentamycin, Bacitracin and Mycostatin) present in the original medium of Smith and Baskerville. This modified medium propagated all our test isolates of *P. multocida* which were derived from seven

animal species (fowl, sheep, goat, cattle, rabbit, dog, cat). It also suppressed the background flora present in swabs taken from the nasal cavity of rabbits.

Both the traditional isolation technique of plating specimens on blood agar and the modified selective medium were used to obtain isolates of *P. multocida* from several species of domestic animal. These were: pigs (15 isolates), rabbits (25), cats (10), dogs (21) and deer (1). Isolates were examined in some detail.

Isolates were serotyped using the indirect haemagglutination assay (IHA). They were found to be Type A (7%), B (1%), D (9%) or untypable (83%). This is similar to overseas findings.

The IHA assay is a laborious technique so two possible alternatives were examined. *viz* sodium dodecyl sulphate polyacrylamide-gel analysis (SDS-PAGE) of proteins and restriction endonuclease analysis (REA) of DNA. Both techniques failed to distinguish between *P. multocida* serotypes in the sense that isolates were extremely heterogeneous and most gave a unique pattern both with SDS-PAGE and REA.

This heterogeneity allowed the identification of individual strains of *P. multocida* and consequently SDS-PAGE was used in an epidemiological investigation which traced the origin of an outbreak of respiratory disease in rabbits and provided evidence that the outbreak was not (as was earlier believed) due to the introduction of rabbits from overseas into a New Zealand colony. Furthermore, using this approach we were able to show that strains which cause severe pneumonia were not confined to the lower respiratory tract but could also be carried in the nasal cavity. This is consistent with the accepted hypothesis that *P. multocida* is an opportunistic pathogen.

Severe atrophic rhinitis is a disease of pigs which is caused by toxin-producing strains of *P. multocida*. The disease is responsible for much economic loss overseas but has not been reported in New Zealand. We obtained a toxigenic strain of *P. multocida* and used it to validate an *in vitro* (mammalian cell culture) method for detecting toxin-producing strains. This method was then used to examine New Zealand isolates of *P. multocida*. None of these were shown to be toxigenic. This suggests that toxigenic strains, if present in New Zealand, are not common.

Diseases due to *P. multocida* are commonly treated with antimicrobial agents such as the Penicillins, Aminoglycosides and Sulphonamides. Strains which are resistant to these agents are present in overseas countries and resistance is associated with the presence of plasmids.

We determined the minimum inhibitory concentrations (MIC), of four antimicrobials (Penicillin G, Streptomycin, Tetracycline and Sulphadiazine) for New Zealand isolates. Considerable variation was found between isolates but no isolate was of sufficiently high resistance to be considered "resistant". This was despite the fact that 12 of 73 (16%) of the field isolates possessed plasmids of a similar size (1Mdal to 5Mdal) to plasmids known to carry antibiotic resistance markers.

Despite its importance as a pathogen of swine, cattle, sheep, fowl and rabbits, *P. multocida* has been little studied in New Zealand. This thesis represents a preliminary stage to a fuller understanding of the importance of *P. multocida* in this country and the best means of control. The possibility of outbreaks of disease due to *P. multocida*, eg atrophic rhinitis, may well stimulate further work on this organism.

### ACKNOWLEDGMENTS

I am indebted to the Department of Microbiology and Genetics for providing the facilities for this project.

I wish to thank my supervisor, John Clarke, for his contribution to this work. His zeal and grammatical dexterity were most appreciated.

Thanks go to Ron Tucker and Paul Hocquard, for their efforts in supplying materials, and to the following people and organizations for donating materials and providing financial assistance:

The Leonard Condell Trust

Ministry of Agriculture and Fisheries

Rex Patterson (Gibco NZ Ltd)

Drs J.M. Rutter and P.D. Luther (ARC Institute, United Kingdom)

I would like to thank George Ionas for generously providing technical advice. I am also indebted to him for his philosophical teachings, especially his balanced concept of a utopian society.

Finally I thank my classmates Mike, Nicky, Sally and Steve for their friendship and to the technical staff for entertaining us all.

TABLE OF CONTENTS

	Page
TITLE PAGE.. . . . .	i
ABSTRACT .. . . . .	ii
ACKNOWLEDGEMENTS.. . . . .	v
TABLE OF CONTENTS .. . . . .	vi
LIST OF FIGURES .. . . . .	xi
LIST OF TABLES .. . . . .	xiii
INTRODUCTION .. . . . .	1
CHAPTER 1: <u>Historical Review</u>	
1.1 Classification and Nomenclature .. . . . .	4
1.2 Serological Characterisation of <i>P. multocida</i>	
1.2.1 Early Attempts at Grouping.. . . . .	9
1.2.2 Discovery of Capsular Types .. . . . .	12
1.2.3 Discovery of Somatic Antigenic Groups ..	16
1.2.4 Agreement between Typing Methods.. . . . .	19
1.2.5 Bacteriophage Typing.. . . . .	20
1.3 Antigenic Structure of <i>P. multocida</i> .. . . . .	20
1.4 Toxigenic Strains of <i>P. multocida</i> .. . . . .	26
1.5 Disease Associations and Epidemiology of <i>P. multocida</i>	
1.5.1 General .. . . . .	29
1.5.2 Cattle .. . . . .	31
1.5.3 Sheep .. . . . .	33
1.5.4 Goats.. . . . .	35
1.5.5 Rabbits .. . . . .	37
1.5.6 Swine.. . . . .	38
1.5.7 Deer .. . . . .	39
1.5.8 Cats and Dogs .. . . . .	39
1.5.9 Man .. . . . .	40





CHAPTER 3:	<u>Isolation of <i>P. multocida</i>, Serotyping of Isolates and Comparison of These by SDS-PAGE and Restriction Endonuclease Analysis (REA)</u>	
3.1	Introduction.. . . . .	72
3.2	Materials and Methods.. . . . .	72
3.2.1	Isolation and Identification of <i>P. multocida</i> .. . . . .	73
3.2.11	Materials .. . . . .	74
3.2.12	Methods .. . . . .	73
3.2.2	Procedures for the IHA Assay	
3.2.21	Materials .. . . . .	74
3.2.22	Methods .. . . . .	76
3.2.3	Procedures for SDS-PAGE Analysis	
3.2.31	Materials .. . . . .	84
3.2.32	Methods .. . . . .	88
3.2.4	Procedures for Restriction Endonuclease Analysis (REA)	
3.2.41	Materials .. . . . .	92
3.2.42	Methods .. . . . .	95
3.3	Results	
3.3.1	Isolation of <i>P. multocida</i> and Some Epidemiological Aspects .. . . . .	99
3.3.2	Serotyping: Validation of IHA Assay and its Application to the Typing of Field Strains	
3.3.21	Validating the IHA Assay .. . . . .	103
3.3.22	Serotyping of Field Isolates .. . . . .	104
3.3.3	SDS-PAGE Analysis of Strains of <i>P. multocida</i> .. . . . .	105
3.3.4	Epidemiological Tracing of <i>P. multocida</i> by SDS-PAGE.. . . . .	107

3.3.5	The Source of a <i>P. multocida</i> Isolate Associated with an Epidemic of Snuffles .. .. .	113
3.3.6	Comparison of <i>P. multocida</i> Serotypes with SDS-PAGE and REA.. .. .	116
3.4	Discussion .. .. .	119
CHAPTER 4:	<b><u>The Detection of Toxigenic Strains of <i>P. multocida</i> Derived from New Zealand Animals</u></b>	
4.1	Introduction.. .. .	120
4.2	Materials and Methods	
4.2.1	Preparation of Reagents and Media.. .. .	121
4.2.2	Methods .. .. .	124
4.3	Results	
4.3.1	Validation of the EBL Assay.. .. .	128
4.3.2	Assay of Field Isolates .. .. .	130
4.4	Discussion .. .. .	131
CHAPTER 5:	<b><u>Comparison of New Zealand Isolates of <i>P. multocida</i> With Particular Reference to Plasmids</u></b>	
5.1	Introduction.. .. .	132
5.2	Materials and Methods.. .. .	132
5.2.1	Examination of <i>P. multocida</i> for Plasmid Carriage	
5.2.11	Materials .. .. .	133
5.2.12	Methods .. .. .	133
5.2.2	<i>P. multocida</i> : Determination of the Minimum Inhibitory Concentrations of Antibiotics	
5.2.21	Materials .. .. .	134
5.2.22	Methods .. .. .	135
5.2.3	Procedures for SDS-PAGE Analysis .. .. .	136

5.3	Results .. .. .	136
5.3.1	The Antibiotic Sensitivity of Strains of <i>P. multocida</i> .. .. .	139
5.3.2	Comparison of Proteins of <i>P. multocida</i> . Plasmid Carrying Strains Versus Non- Plasmid Carrying Strains.. .. .	147
5.4	Discussion .. .. .	147
CHAPTER 6:	<u>General Discussion</u> .. .. .	151
6.1	The Development of a Selective Medium for the Isolation of <i>P. multocida</i> in New Zealand ..	152
6.2	Isolation and Serotyping of <i>P. multocida</i> from New Zealand Animals.. .. .	152
6.3	SDS-PAGE Analysis of Field Isolates of <i>P. multocida</i> .. .. .	154
6.4	Comparison of <i>P. multocida</i> Serotypes by Restriction Endonuclease Analysis (REA).. .. .	155
6.5	Assay for Exotoxigenic Strains of <i>P. multocida</i> .. .. .	156
6.6	Antibiotic Resistance and its Relationship to Plasmids in New Zealand Strains of <i>P. multocida</i> .. .. .	157
6.7	Outlook .. .. .	158
APPENDIX	.. .. .	159
BIBLIOGRAPHY	.. .. .	164

LIST OF FIGURES

Figure	Page
1.1 Gram Stain of <i>P. multocida</i> .. . . . . .	5
1.2 Antigenic Characteristics of <i>P. multocida</i> .. . . . . .	18
1.3 Type A Colonies of <i>P. multocida</i> .. . . . . .	22
1.4 Type D Colonies of <i>P. multocida</i> .. . . . . .	22
2.1 The Suppression of Nasal Bacterial Flora by a Selective Medium (SM) .. . . . . .	67
3.1 SDS-PAGE Protein Analysis of <i>P. multocida</i> Isolated from Several Animal Species .. . . . . .	106
3.2 SDS-PAGE Analysis of <i>P. multocida</i> Isolates from Swine .. . . . . .	108
3.3 SDS-PAGE Analysis of <i>P. multocida</i> Isolates from Dogs.. . . . . .	109
3.4 SDS-PAGE Analysis of <i>P. multocida</i> Isolates from Cats.. . . . . .	110
3.5 SDS-PAGE Analysis of <i>P. multocida</i> Isolates from Rabbits.. . . . . .	111
3.6 SDS-PAGE Analysis of <i>P. multocida</i> from Rabbits.. . . . . .	112
3.7 SDS-PAGE Analysis of <i>P. multocida</i> Isolates from Rabbits :A comparison of lung and nasal tract isolates.. . . . . .	114

3.8	SDS-PAGE Analysis of <i>P. multocida</i> Isolates from Rabbits :A comparison of isolates obtained before and after the introduction of animals, from overseas, to a rabbit colony .. .. .	115
3.9	SDS-PAGE Analysis of <i>P. multocida</i> serotypes A, B and D ..	117
3.10	Restriction Endonuclease Analysis of <i>P. multocida</i> :A comparison of serotypes A and D.. .. .	118
4.1	Cell Culture Monolayer of Embryonic Bovine Lung Cells ..	129
4.2	Cell Culture Monolayer of Embryonic Bovine Lung Cells :The cytopathic effect of <i>P. multocida</i> exotoxin .. .. .	129
5.1	Agarose-Gel Electrophoresis of DNA from Plasmid- Containing Feline Isolates of <i>P. multocida</i> .. .. .	137
5.2	Agarose-Gel Electrophoresis of DNA from Plasmid- Containing Canine Isolates of <i>P. multocida</i> .. .. .	138
5.3	Variation of MICs Between Strains of <i>P. multocida</i> .. .. .	142
5.4	SDS-PAGE Analysis of <i>P. multocida</i> Isolates from Dogs :A comparison of plasmid-containing isolates with isolates which do not contain plasmids .. .. .	148
5.5	SDS-PAGE Analysis of <i>P. multocida</i> Isolates from Cats :A comparison of plasmid-containing isolates with isolates which do not contain plasmids .. .. .	149



3.3	Serotyping Prototype Strains of <i>P. multocida</i> by IHA	
	Assay .. .. .	103
3.4	Serotypes of Field Isolates as Determined by the IHA	
	Assay .. .. .	104
4.1	Assay of Prototype Strains of <i>P. multocida</i> for	
	Exotoxin Production.. .. .	130
4.2	Assay of Field Isolates of <i>P. multocida</i> for Exotoxin	
	Production .. .. .	131
5.1	Assay of Strains of <i>P. multocida</i> for Plasmids .. .. .	136
5.2	An Evaluation of Plasmids Carried by Strains of	
	<i>P. multocida</i> .. .. .	139
5.3	Minimum Inhibitory Concentrations of Antibiotics For	
	<i>P. multocida</i> .. .. .	140