

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.

# **Mastitis in New Zealand**

## **Dairy Herds:**

- I. Management, diagnosis, and treatment of subclinical mastitis*
- II. Phenotypic and genotypic characterisation of Streptococcus uberis isolates*

A thesis presented in partial  
fulfilment of the requirement for  
the degree of Doctor of Philosophy  
at Massey University

*Victoria Lynn Douglas*

*1999*

# **Mastitis in New Zealand**

## **Dairy Herds:**

- I. Management, diagnosis, and treatment of subclinical mastitis*
- II. Phenotypic and genotypic characterisation of Streptococcus uberis isolates*

*Victoria Lynn Douglas*

*1999*

## Abstract

Four scientific trials were undertaken to extend the scientific research on mastitis in New Zealand dairy herds, producing milk seasonally from grazed pastures.

### ***Trial 1:***

**Aim.** To compare the effect of drying-off techniques on the prevalence of new intramammary infections.

**Conclusions.** A 48-hour intermittent drying-off technique increased the quarter prevalence of mastitis caused by major and minor pathogens or major pathogens alone, at dry-off. There was no significant difference in the prevalence of mastitis in quarters dried-off with a 24-hour or 48-hour intermittent milking technique, during the first 12 days of the subsequent lactation. This may have resulted from higher culling (due to apparently unrelated causes) of infected cows from the 48 hour group.

### ***Trial 2:***

**Aims.** To assess the economic value of treating subclinical mastitis based on electrical conductance and/or rapid mastitis test results and to determine the cure rates and economic outcome to the dairy farmer.

**Conclusions.** The Rapid Mastitis Test or electrical conductance diagnosed 66% of the quarters infected by major pathogens. Cure rates were 58% in infected untreated quarters and 61% in infected quarters treated with a course of intramammary antibiotics. An assessment of costs and benefits from treating infected quarters, plus the inevitable cost of treatment of uninfected quarters, resulted in an overall net loss of approximately \$9.18 per quarter treated.

### ***Trial 3:***

**Aims.** To determine the antibiotic sensitivity patterns of 150 and 180 *Streptococcus uberis* isolates cultured from subclinical and clinical cases of mastitis respectively, in New Zealand dairy cattle from 15 different regions, using a disk diffusion assay. To assess the suitability of antibiograms for subtyping of *Streptococcus uberis* isolates for epidemiological studies.

**Conclusions.** The 330 isolates fit into 17 different antibiogram patterns. Ninety-five percent of the isolates followed one of five common antibiogram patterns. Eighty percent of the isolates were of

antibiogram pattern C. The only significant difference between the subclinical and clinical *Streptococcus uberis* isolates tested in this study was the clinical isolates were more sensitive to cloxacillin. Similarities in antibiograms between the study isolates and the isolates from the United States (McDonald *et al.*, 1976) were noted for cloxacillin, cephalothin, erythromycin, lincomycin, penicillin G, and streptomycin. The relatively low number of antibiogram patterns resolved from the 330 *Streptococcus uberis*, limits the usefulness of the technique as a solitary tool for epidemiological studies in New Zealand dairy herds.

#### **Trial 4:**

**Aims.** To determine and compare the restriction endonuclease fragment pattern of *Streptococcus uberis* isolates from subclinical and clinical cases of mastitis in New Zealand dairy cows, from 15 different farming regions in New Zealand. To compare the pulsed-field genotyping technique with antibiogram typing of *Streptococcus uberis* isolates within 8 different farms.

**Conclusions.** The 343 *Streptococcus uberis* isolates exhibited 330 different restriction endonuclease fragment patterns, indicating at least several hundred genetically different strains of *Streptococcus uberis* isolates in New Zealand capable of causing mastitis in dairy cattle. Genetically different and similar strains were identified within the same quarter of an individual cow, different quarters from the same cow, different cows within the same farm, and from different cows from the same or different districts, farming regions or islands. The high degree of dissimilarity among the isolates tested is an indication that *Streptococcus uberis* infections in New Zealand dairy cattle are largely due to the opportunistic nature of the organism in the cow's environment. Prevention and treatment of *Streptococcus uberis* mastitis will therefore need to be directed at a multitude of different strains present throughout the country as well as in individual herds.

Fewer antibiogram patterns were defined on each individual farm compared to the number of pulsed-field gel electrophoresis patterns on those farms. The pulsed-field gel electrophoresis typing technique appears to be a more discriminatory test for typing *Streptococcus uberis* isolates on 8 New Zealand dairy herds than antibiograms.

The information gained from these trials provides scientific research to strengthen the recommendations made in the Seasonal Approach to Managing Mastitis (SAMM) Plan, a mastitis prevention programme designed for seasonal dairy farmers in New Zealand.

## Acknowledgements

---

### THANKS

The acknowledgements are probably one of the last sections written. It is a difficult section because after many years of study you have to sit down and try to remember everyone who took a part in helping you through not only the successes and failures of your research, but just getting you through the day and night.

This thesis would not have been possible without the funding and support of the National Dairy Board and Livestock Improvement Corporation. Thank you. I thank my supervisors Drs. Colin Holmes, Norman Williamson (Chief Supervisor), Dirk Pfeiffer, Stan Fenwick, and Ian Steffert (retired) for helping me make things happen.

This thesis goes out to all the exceptional help I had along the way. Thanks for your help and/or advice: Magda Gwozdz, dairy farmers, especially Martin Chesterfield, Helen Crabb, Dr. John Kunkel, Daniel Russell, Dr. Geoffrey Gobert, Greg Gibbs, and Howard Wilson. Thanks to those in the microbacteriology/bacteriology laboratories at the Ministry of Agricultural and Fisheries laboratories who saved and sent me *Streptococcus uberis* samples.

Special thanks to my family and friends in the USA. Many have been there from the start. Thanks for the wonderful support: Casey and Geraldine Douglas, James and Cynthia Douglas, Joann Douglas, Casey Douglas III, Deborah Douglas (Stone), Courtney and Andre Robinson, Porscha and Damien Stone, Erik and Sarah Douglas, uncles, aunts, cousins, Bob and Nan Davis, Kimberley Klish (Perez), Jorge Cuevas, Stacy Nakashima, Maria and Rafael Verbera, Dr. James Cullor, Jon Dellinger, Rachid, Bala, Luc, Kei, Ranger, Randy, Erika, May, and the Eldridges.

---

Very special thanks to my friends whom tolerated my fits from thousands of miles away: Dr. Carol Su Adams, Dr. Monica S. Balfour, Dr. Emilio DeBess, Dr. William H.R. Davis and Drs. Earmie Edwards (Wayne).

I want to thank Dr. Davis again for introducing me to the South Island of New Zealand, during the fall of 1994. It was the beautiful land and people of New Zealand that made my stay most pleasurable. So I give another special thanks to those friends in New Zealand who welcomed me into their country and the “Kiwi” way of life and those foreign imports too: Barb Frey, Louise Craigie, Shelley Dansby, Tracey Caldwell, Iona Bichan, Col Wickes, Sandy McLachlan, Christine Leighton, Ash Sherwood, Dr. Kathy Gibson, Drs. Mike and Kathy Parton, Su and Todd Jenkins, Andrew Scuffham, Alan Appleton “Appleton”, Dr. Daniel and Bettina Laborde, and Jean Farqhar.

I would like to give a special thanks to Dr. Jeffery W. Tyler for all he has done for me through the years. He and Dr. Jenni Donald allowed me the time to put this thesis to rest.

I am sure there is more, but let’s get on with the thesis...

Many thanks to all

## Table of Contents

---

<b>Abstract</b> .....	<i>ii</i>
<b>Acknowledgements</b> .....	<i>iv</i>
<b>Table of Contents</b> .....	<i>vi</i>
<b>List of Figures</b> .....	<i>vii</i>
<b>List of Tables</b> .....	<i>ix</i>
<b>Chapter 1</b> Introduction .....	<i>1</i>
<b>Chapter 2</b> Literature review .....	<i>9</i>
<b>Chapter 3</b> Materials and methods .....	<i>112</i>
<b>Chapter 4</b> The influence of two different “intermittent” drying-off strategies on the prevalence of mammary infection in a New Zealand dairy herd .....	<i>125</i>
<b>Chapter 5</b> An economic assessment of lactational treatment of subclinical mastitis in individual quarters identified by the rapid mastitis test or the electrical conductivity test .....	<i>157</i>
<b>Chapter 6</b> Antibigrams of <i>Streptococcus uberis</i> isolates from subclinical and clinical cases of bovine mastitis .....	<i>186</i>
<b>Chapter 7</b> Genomic typing of <i>Streptococcus uberis</i> isolates from cases of mastitis, in New Zealand dairy cows, using pulsed-field gel electrophoresis .....	<i>212</i>
<b>Conclusion</b> .....	<i>257</i>
<b>Appendix I</b> .....	<i>270</i>
<b>Appendix II</b> .....	<i>275</i>
<b>Appendix III</b> .....	<i>277</i>



## List of Figures

---

### Chapter 2:

<b>Figure I</b>	Scoring and interpretation of the R/CMT .....	38
<b>Figure II</b>	Relationship between mastitis tests scores, bulk milk somatic cell counts (BMSCC), and herd milk production losses .....	39
<b>Figure III</b>	Percentage of milk production losses per quarter based on R/CMT results .....	42
<b>Figure IV</b>	The estimated (est.) infected animals in the herd relative to the BMSCC .....	45

### Chapter 7:

<b>Figure I</b>	Pulsed-field electrophoresis gel .....	228 228a
<b>Figure II</b>	The degree of dissimilarity of the banding patterns of the isolates from Farm 1 in the Manawatu district, Wellington Region .....	232
<b>Figure III</b>	The degree of dissimilarity of the banding patterns of the isolates from Farm 2 in the Manawatu district, Wellington Region .....	233
<b>Figure IV</b>	The degree of dissimilarity of the banding patterns of the isolates from Farm 3 in the Manawatu district, Wellington Region .....	234
<b>Figure V</b>	The degree of dissimilarity of the banding patterns of the isolates from Farm 4 in the Manawatu district, Wellington Region .....	235
<b>Figure VI</b>	The degree of dissimilarity of the banding patterns of the isolates from Farm 5 in the South Taranaki Region, Taranaki Region .....	236
<b>Figure VII</b>	The degree of dissimilarity of the banding patterns of the isolates from Farm 7 in the South Taranaki district, Taranaki Region .....	237
<b>Figure VIII</b>	The degree of dissimilarity of the banding patterns of the isolates from Farm 8 in the Horowhenua district, Wellington Region .....	238

<b>Figure IX</b>	The degree of dissimilarity of the banding patterns of the isolates from Farm 9 in the Manawatu district, Wellington Region .....	239
<b>Figure X</b>	The criteria used by Tenover <i>et al.</i> , 1995 for interpretation of DNA restriction patterns with regards to disease outbreaks ....	242
<b>Appendix I:</b>		
<b>Figure 1</b>	The 17 farming regions of New Zealand .....	271
<b>Figure 2</b>	The total number (n=342) of milk samples collected from both subclinical and clinical cases of <u>Streptococcus uberis</u> mastitis, from 15 farming regions in New Zealand .....	272
<b>Figure 3</b>	The total number (n=161) of milk samples collected from subclinical cases of <u>Streptococcus uberis</u> mastitis, from 5 farming regions in New Zealand .....	273
<b>Figure 4</b>	The total number (n=181) of milk samples collected from clinical cases of <u>Streptococcus uberis</u> mastitis, from 14 farming regions in New Zealand .....	274
<b>Appendix III:</b>		
<b>Figure 1</b>	Photograph of a PFGE gel containing isolates which were incubated for 16 and one for 24 hours at 37 °C .....	277 277a
<b>Figure 2</b>	Photograph of a PFGE gel containing isolates from different regions in New Zealand .....	278 278a
<b>Figure 3</b>	Photograph of a PFGE gel containing 18 isolates from Farm #3 and two base pair markers .....	279 279a

## List of Tables

---

### Chapter 1:

<b>Table I</b>	The main points to the 1994/95 SAMM Plan .....	4
----------------	--	---

### Chapter 4:

<b>Table I</b>	The variables used in the univariate and multivariate analysis ...	137
----------------	--	-----

<b>Table II</b>	The number of specific or mixtures of bacteria cultured from the positive cultures during the three periods .....	138
-----------------	---	-----

<b>Table III</b>	The number of quarters with subclinical mastitis infections caused by minor and major pathogens at before drying, at dry-off, and at lactation .....	139
------------------	--	-----

<b>Table IV</b>	Number of quarter infections caused by major and minor pathogens, in the two groups, at the different sample periods .....	140
-----------------	--	-----

<b>Table V</b>	Univariate logistic regression analysis (including random effect): Odds ratios (OR), 90% confidence limits (CL), describing the association between each of the independent variables and quarter mastitis at dry-off and within 12 days in lactation as well as intra-cluster correlations (ICC) .....	142
----------------	---	-----

<b>Table VI</b>	The sensitivity (Se), specificity (Sp), and the predictive value of a positive (PVP) and negative (PVN) test for RMT and EC test at drying off and lactation .....	143
-----------------	--	-----

<b>Table VII</b>	The multivariate logistic regression models (including random effect) for the dependent variable bacterial culture status (SCGP) at drying off and at lactation .....	143
------------------	---	-----

### Chapter 5:

<b>Table I</b>	The number (and percent) of specific bacteria that was cultured from pre treatment quarter milk samples from both the treatment and control groups.....	171
----------------	---	-----

<b>Table II</b>	The number of resolved (R) and persistent (P) infections by species of bacteria from pre quarter milk samples and the post sample new intramammary infections (NIMI).....	172
-----------------	---	-----

<b>Table III</b>	The number of specific organisms cultured from the post quarter milk samples that cultured negative on the pre sample ...	174
------------------	---	-----

<b>Table IV</b>	The sensitivity and specificity of the RMT and EC test for samples culturing positive for major and minor pathogens .....	176
<b>Table V</b>	Cost of mistakenly treating 44 “uninfected quarters” during September, due to misclassification .....	178
<b>Table VI</b>	Cost and benefit of treating 56 “infected” and 44 “uninfected” quarters during September .....	179
<b>Chapter 6:</b>		
<b>Table I</b>	Interpretation of the diameter (mm) of the zone of inhibition ...	195
<b>Table II</b>	The percentage of susceptible New Zealand subclinical and clinical <i>Streptococcus uberis</i> isolates to twelve antibiotics compared to other <i>Streptococcus uberis</i> isolates .....	196
<b>Table III</b>	The 17 antibiogram patterns of the 331 tested <i>Streptococcus uberis</i> isolates .....	197
<b>Chapter 7:</b>		
<b>Table I</b>	The number (#) of subclinical samples from districts and New Zealand Farming Regions .....	221
<b>Table II</b>	The number (#) of clinical samples from districts and New Zealand Farming Regions .....	222
<b>Table III</b>	Cluster of isolates with less than 4 band differences .....	230
<b>Table IV</b>	Cluster of isolates with 4 to 6 band differences .....	231
<b>Table V</b>	Antibiogram patterns of the isolates from Farm 1 in the Manawatu district, Wellington Region .....	232
<b>Table VI</b>	Antibiogram patterns of the isolates from Farm 2 in the Manawatu district, Wellington Region .....	233
<b>Table VII</b>	Antibiogram patterns of the isolates from Farm 3 in the Manawatu district, Wellington Region .....	234
<b>Table VIII</b>	Antibiogram patterns of the isolates from Farm 4 in the Manawatu district, Wellington Region .....	235
<b>Table IX</b>	Antibiogram patterns of the isolates from Farm 5 in the South Taranaki district, Taranaki Region .....	236
<b>Table X</b>	Antibiogram patterns of the isolates from Farm 7 in the South Taranaki district, Taranaki Region .....	237

<b>Table XI</b>	Antibiogram patterns of the isolates from Farm 8 in the Horowhenua district, Wellington Region .....	238
<b>Table XII</b>	Antibiogram patterns of the isolates from Farm 9 in the Manawatu district, Wellington Region .....	239
<b>Conclusion:</b>		
<b>Table I</b>	The main points to the 1994/95 SAMM Plan .....	259
<b>Table II</b>	Drying-off period control activities .....	260
<b>Table III</b>	Lactation period control activities .....	261
<b>Table IV</b>	Calving period control activities .....	266