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**An experimental challenge model in lactating dairy
cows using *Streptococcus uberis* for antibiotic
efficacy testing**

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Philosophy

In

Veterinary Science

At Massey University, Palmerston North,

New Zealand

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2013

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Abstract

The aim of this project was to develop a challenge model to test the efficacy of novel intramammary antimicrobial treatments for clinical mastitis. The use of the model, can reduce the costs of testing efficacy and accelerate the process of registration of new products. It provides controlled conditions which safeguard animal welfare.

The experimental challenge model using *Streptococcus uberis* developed in this thesis can provide the pharmaceutical industry and animal health research groups with a cost-effective method to test the efficacy of new antimicrobial products for treatment of mastitis in a safe and controlled environment. Two Cloxacillin-based antimicrobials with different formulations and treatment frequency were tested for their efficacy to cure *S. uberis* infections after infections were induced using the challenge model developed as described in the third chapter of this thesis.

The objective of the first study presented in this thesis was to choose one suitable strain from four strains of *S. uberis*, to be used in future challenge studies. Four strains were tested for their virulence and susceptibility to antibiotic therapy. A further study objective was to determine the dose (number of pathogens infused, expressed as colony forming units (CFU)) required for the tested strains to produce an acceptable proportion of clinical mastitis cases to enable future studies. The strain which accomplished the desired characteristics was then chosen and was utilised for experimental challenge in further studies (Chapters 4 and 5). The overall incidence of clinical mastitis obtained in this study at a quarter level was 54% (26/48). This study showed significant differences in the ability of different strains of *S. uberis* to cause clinical mastitis when inoculated via the intramammary route. However, only one of the four strains tested demonstrated favourable characteristics as a strain to be used in experimentally induced clinical mastitis studies.

Chapters 4 and 5 describe two challenge studies conducted using the experimental challenge model (Chapter 3) to test the efficacy of different antimicrobial drug formulations. In Chapter 4, the cure rate of one cloxacillin based product applied every 24 hr. was compared with the cure rate of a penicillin-based product applied every 12 hr. During the observation period of this investigation all challenged cows developed clinical

mastitis in at least one quarter. The incidence of clinical mastitis at the quarter level was high, with 91.25% (73/80) of challenged quarters being affected. After diagnosis of infections, the cows were randomly allocated to two treatment groups and treated accordingly. Clinical cases in which the quarter did not respond to three applications of the allocated antimicrobial product received an extended treatment of the same product. As the allocation to the extended treatment was not random, clinical and bacteriological cures were statistically evaluated for the short treatment only. Clinical cure rates for the short treatment (3 syringes) were 52.63% and 43.75% for the cloxacillin- and penicillin-based products, respectively. There was no significant difference between the treatments ($P = 0.8$) in their efficacy for the treatment of experimentally induced *S. uberis* clinical mastitis.

In Chapter 5, two long-acting cloxacillin containing products were compared in their efficacy to cure experimentally induced *S. uberis* infections. One commercially available product was compared with a novel long acting product (applied every 48 hr.). Out of 80 challenged quarters, 41 quarters developed clinical mastitis after inoculation (51.25%). Treatment with the novel product resulted in a total treatment success rate of 93.1% based on clinical examination, and 96.0% based on the bacteriological cure rate. Treatment with the control product resulted in total treatment success rate of 100% based on clinical and bacteriological cure rate. There was no significant difference between the products ($P=0.19$) in their efficacy for the treatment of experimentally induced *S. uberis* clinical mastitis.

Results in this thesis showed that experimental challenge models can be a useful tool in animal research to test the efficacy of new products in a safe and cost effective manner.

Acknowledgements

This page is to thank to all the people who accompanied me on this exciting journey as a postgraduate student, and also to thank all the people that made my journey possible.

I would like to thank my supervisors, Professor Norman B. Williamson (Chief Supervisor), Kiro Petrovski, Gina deNicolo and Alex Grinberg, for their patience and support. Thank you for understanding me, interpreting my ideas and improving my writing. Thanks also to Nicolas Lopez-Villalobos for his help with the statistical analysis of the results.

Thanks to J L Vet services director Jeremy Lind and his fantastic team: Sarah Poppleton, Steph Evans and Macey Waker for working so hard during the fields studies, for their great sense of humour and ability to transform hard work into an extremely enjoyable experience.

I really appreciate the help received from Liz Burrows and Tessie George from IVABS during my first steps in the laboratory.

This project would not have been possible without the financial support of Bayer Animal Health NZ.

To Massey University Dairy Farm Number 1 and 4 staff and managers for being there when needed, for taking care of the cows enrolled in the trials and for their smiles despite the extra work involved.

And finally, many thanks to my beloved husband Diego for his support and shared love, and my siblings Cintia and Fabio, without whose patience, effort and love in raising me up, I would not be here.

“Doing what you like is freedom,

Liking what you do is happiness”...

(Frank Tyger)

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List of Abbreviations

- ATB: Antibiotic
- BA: Blood Agar plates
- BAGG: Buffered Azide Glucose Glycerol Broth
- BSCC: Bulk somatic cell count
- CAMP: Christie Atkins and Munch Petersen
- CFU: Colony forming units
- CI: Confidence interval
- CLSI: Clinical Laboratory standards Institute
- CM: Clinical Mastitis
- CNS: *Coagulase Negative Staphylococci*
- CO₂: Carbon Dioxide
- DCT: Dry cow therapy
- FR/RL or FL/RR: Front-Right and Rear-Left or Front-Left and Rear-Right
- Hr.: Hours
- IVABS: Institute of Veterinary, Animal and Biomedical Sciences
- MIC: Minimum inhibition concentrations
- NEB: Negative energy balance
- NMC: National Mastitis Council
- PBS: Phosphate buffered saline
- PEB: Positive energy balance
- PFGE: Pulsed-field gel electrophoresis
- PMN: Polymorphonuclear cells
- rm ANOVA: Repeated measures analysis of variance
- RR: Relative risk
- SCC: Somatic cell count
- SCS: somatic cell scores
- S. uberis*: *Streptococcus uberis*
- Staph. aureus*: *Staphylococcus aureus*
- TS: time of sampling