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

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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 reduces 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.

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"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*

CO_2: 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