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*coli* in another quarter and Coagulase-negative staphylococci in two other cases. The statistical calculations proved a significant correlation between cell counts and differential relationships of the four analysed cell types to each other. On the basis of the results of our study we detected inflammable reactions in the mammary glands of obviously healthy udders already at a level of 43,000 cells/ml.

**Key words:** differential cell count, low somatic cell count milk, differential cell pattern

#### 9 Understanding Mastitis Epidemiology on Farm - Determining the Importance of the Dry Period in Mastitis Control

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**Objectives:** Appropriately targeting mastitis controls on farm requires the clinician to determine the relative importance of the dry and lactating periods. The aim of this study was to investigate novel indices for clinical and sub-clinical mastitis, previously described by the authors, to categorise farms by the importance of the dry and lactating periods.

**Materials and Methods:** Using somatic cell counts (SCC) the importance of the dry period was investigated by calculating; the Fresh Calver Infection Rate (FCIR - proportion of cows with SCCs >200,000 cells/ml at the first recording post calving), the New Dry Period Infection Rate (DPNEW - proportion of cows with SCCs rising from < to > 200,000 cells/ml across the dry period), the Dry Period Cure Rate (DPCURE - proportion of cows with SCCs falling from > to <200,000 cells/ml across the dry period). Using clinical mastitis data, the proportion of the herd affected (IRCA) was calculated and cases were attributed either to the dry period or lactation on the basis of the timing of an "index" case (1<sup>st</sup> case in lactation) in an individual cow. Apparent Dry and Lactating Period Origin Clinical Mastitis Rates (DPORATE and LPORATE respectively) were then calculated, namely the 1st 30 day incidence rate (target: <1 in 12 cows affected) and the incidence rate for the remainder of lactation (target: <2 in 12 cows affected). Finally for each farm the relative contribution of the dry period (DPCONT) was calculated.

**Results:** Data is presented from 52 herds. Mean annual rates were calculated for each farm. These demonstrate significant variation in the indices between farms and within farms over time. The mean, median, min, max and target respectively, for each index is outlined below. FCIR: 0.23, 0.22, 0.10, 0.43, 0.10. DPNEW: 0.19, 0.19, 0.06, 0.42, 0.10. DPCURE: 0.71, 0.70, 0.51, 0.91, 0.85. ICRA: 0.44, 0.40, 0.14, 0.79, 0.25. DPORATE: 1.79, 1.74, 0.12, 4.94, 1.00. LPORATE: 3.00, 2.88, 0.97, 6.09, 2.00. DPCONT: 0.37, 0.35, 0.07, 0.75, 0.33.

**Conclusion:** The indices outlined in this study allow farms to be categorised according to the likely importance of the dry and lactating periods in mastitis control, thereby allowing the practitioner to select mastitis interventions likely to have the largest impact on farm. Continued monitoring allows

#### 10 Mammary Gland Immune Dysfunction in High Yielding Dairy Cows Immediately after Calving; can Dietary Vitamin C help?

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Neutrophils are the most vital part of the first line of immune defense against invading mastitis pathogens in the mammary gland of high yielding dairy cows. Their quality and quantity in the gland is important. Vitamin C is one of the most important water-soluble protective agents in mammalian cells. Although cows can synthesize vitamin C, substantial evidence suggests a link between vitamin C and immunity. We hypothesized that feeding vitamin C in dairy cows would improve milk neutrophil function via its antioxidant properties. This study was, therefore, aimed to determine whether supplemental dietary vitamin C affects on milk neutrophil quality and quantity immediately after calving. We fed Holstein cows supplemental vitamin C during the peri-partum period; at 10 d before anticipated calving, the cows were fed diets that provided 0 (n = 7) or 50 (n = 7) gm/d of supplemental vitamin C

(phosphorylated ascorbic acid). Treatments continued until approximately 15 days in milk. The viability of milk neutrophils and somatic cells were examined after calving. Compared with non-vitamin C supplemented group, feeding vitamin C during periparturient period resulted in significant increased neutrophils viability and decreased somatic cells in the mammary gland after calving. The reduced somatic cells in the gland from vitamin C treated cows suggest that it may reduce damage to the gland, milk and neutrophils caused by parturition-and-lactation-related stress. In our previous experiments we observed that the unnecessarily high number of neutrophils and excessive somatic cells in the milk may relate to udder and milk damage as free radicals and proteases released by neutrophils appear to be actively involved in the damage. Therefore, in the current study limited, but fairly enough, SCC with high quality of neutrophils observed in cows receiving vitamin C supplementation implies a more efficient neutrophil response compared with the response in non-supplemented cows. Overall, supplemental dietary vitamin C during the peri-partum period improves neutrophil function; this potentially results in faster elimination of pathogens in the gland during mastitis. Though still remains inconclusive, the application of vitamin C would be further examined in dairy cows for prevention and treatment of clinical mastitis; because it boosts milk neutrophil viability.

**Key words:** mastitis, milk, neutrophils, vitamin C

#### 41 Using Polymerase Chain Reaction Techniques in the Diagnosis of *Streptococcus Agalactiae*

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**Objectives of study:** Although *Streptococcus agalactiae* is commonly believed to be of decreasing importance in many dairy regions, infection with this pathogen is still suspected as being a cause of mastitis in many herds. Infection with *S. agalactiae* has serious implications for milk quality. Traditional methods of detecting *S. agalactiae* in herds have used bacteriological culture and phenotypic identification of isolates. These methods are time consuming and have practical limitations. PCR methods offer substantially improved sensitivity over culture. PCR methods also offer considerable technical advantages when used for testing bulk tank samples. The aim of this study was to develop a PCR methodology to detect *S. agalactiae* in vat milk samples.

**Materials and Methods:** Vat milk samples or individual cow milk samples were obtained from 26 Australian dairy herds, either directly from the herd owner or via the veterinarian advising the herd. Herds were selected because they had had recent problems with elevated bulk milk somatic cell counts, or increased numbers of subclinical or clinical mastitis cases. Submitted milk samples were incubated in Todd Hewitt broth overnight and then processed to extract the bacterial DNA. This DNA was then amplified by PCR using *S. agalactiae* specific primers spanning the 16S to 23S rRNA spacer region. These were subsequently separated and identified in polyacrylamide gels.

**Results:** Nine of 24 bulk tank samples were positive for *S. agalactiae*. For 7 herds, both bulk tank samples and individual cow samples were available. In only one of these herds did the results of the testing of the individual cow samples disagree with the result of the bulk tank test.

**Conclusions:** These early results suggest that, contrary to common thought, *S. agalactiae* continues to be an important mastitis pathogen in many herds. This pathogen represents a significant threat to efforts to control mastitis and to improve the quality of raw product supplied to processors. New methods such as PCR have the potential to offer an efficient method by which herds can be monitored for the presence of *S. agalactiae* using routinely collected bulk tank samples. This allows early identification of herds at risk of having high bulk milk somatic cell counts.

#### 42 Analytical Sensitivity of the PathoProof Mastitis PCR Assay Determined Using Two Different Experimental Approaches

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The PathoProof Mastitis PCR Assay is a novel polymerase chain reaction-based reagent kit for identification of all major mastitis-causing pathogens. Here, we report on the analytical sensitivity of the assay using two different experimental approaches. We first used purified DNA to prepare a standard dilution series from 50 to less than one genome copy of the assay's bacterial targets and determined how many copies of each target could be detected with 95% probability. In our second experiment,