

# Technical Series

## Issue 19

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Mastitis in dairy cows causes significant losses on every farm in New Zealand, with industry-wide costs estimated at \$280M per year. This *Technical Series* looks at what's new in the world of mastitis research and a review of the Mastitis Tools project.

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DairyNZ

# What's new in the world of mastitis research?



Sally-Anne Turner, DairyNZ Scientist – Lactation Biology & Mastitis

*Mastitis in dairy cows causes significant losses on every farm in New Zealand, with industry-wide costs estimated at \$280M per year. Mastitis due to *Streptococcus uberis* is the most significant problem, as approximately 10% of cows suffer clinical mastitis at calving and about 75% of these cases are due to *Str. uberis*.*

This issue of the *Technical Series* reviews the programme of work 'Novel tools to prevent *Streptococcus uberis* mastitis in dairy cows', more commonly referred to as the Mastitis Tools project.

Led by DairyNZ scientists, in collaboration with researchers from AgResearch (Hopkirk Institute), ViaLactia (a Fonterra subsidiary) and LIC, the project has aimed to develop effective 'tools' (genetic markers and/or a vaccine) that could help New Zealand dairy farmers reduce the incidence of clinical mastitis by 50%, increase herd productivity and improve milk quality.

The research has been jointly funded by the Ministry of Business, Innovation and Employment (formerly Foundation for Research, Science, and Technology) and by New Zealand dairy farmers through DairyNZ Inc.

Split into two broad objectives, the first has focused on determining the genetic basis for differences in mastitis resistance between cows. Many farmers know cows in their herd that have remained free of infection for years on end, while others are culled due to repeated or persistent infections.

This issue of the *Technical Series* contains two articles that report results of studies on phenotypic differences (observable traits) between cows with different susceptibilities to *Str. uberis* mastitis and research to find genetic markers of resistance to mastitis.

The second objective was sub-contracted to AgResearch and focused on the search for a vaccine for *Str. uberis* mastitis. Progress made in this direction is summarised in commentary by Eric Hillerton, DairyNZ Chief Scientist (page 12). This article also presents a review of current mastitis control strategies for minimising and preventing mastitis. It explores the issues around the use of some of these 'old technologies' and identifies new technologies that are becoming available overseas and their uptake in New Zealand.

Additionally, there is a review by Nicole Steele, MSc Student (pg 15), which describes a new technology that may improve the speed and sensitivity of identifying bacteria responsible for mastitis in New Zealand herds.



# Factors affecting *Streptococcus uberis* mastitis



Sally-Anne Turner, DairyNZ Scientist; John Williamson, DairyNZ Lead Research Technician; Eric Hillerton, Chief Scientist; and Jane Lacy-Hulbert, DairyNZ Senior Scientist

## Key findings

- Studies with twin cows reveal that the rate of *Streptococcus uberis* mastitis is more similar within a set of twins than between unrelated animals.
- Cows that do not develop mastitis differ in phenotypic characteristics (observable traits) from cows that develop mastitis.
- A past history of no mastitis may not mean a future with no mastitis – especially if the teat canal is breached.
- Changing the genetics of a herd could improve resistance to *Str. uberis* mastitis.



**Mastitis in dairy cows causes significant cow losses on every farm, with costs estimated in New Zealand at \$280M per year<sup>1</sup>.**

Mastitis due to *Streptococcus uberis* is the most significant cow health problem for dairy farmers in New Zealand, and also affects other pasture-based dairy systems around the world<sup>2,3</sup>. In New Zealand, approximately 10% of cows suffer clinical mastitis at calving, and about 75% of these cases are due to *Str. uberis*<sup>4</sup>.

Some cows appear more able to resist becoming infected. In most herds, farmers recognise cows that remain free of mastitis throughout their lifetime. Closer inspection of such cows within research herds has highlighted some important indicators of apparent resistance.

## Why worry about *Str. uberis* mastitis?

Mastitis caused by *Str. uberis*, an environmental pathogen, affects all herds but noticeably those that have good controls in place for preventing contagious mastitis.

Historical records on more than 1500 cows within the DairyNZ mastitis database reveal that around 14% of the herd (26% of heifers and 11% of cows) had an intramammary infection, due to *Str. uberis* after calving. Of these infections, 54% went on to become clinical within the same lactation, often within the first week after calving. Studies on commercial farms suggest similar results. Between 10-30% of intramammary infections at calving in heifers were found to be due to *Str. uberis*<sup>5</sup> and about 40-50% of intramammary infections due to streptococci progressed to clinical mastitis<sup>6</sup>.

Using the DairyNZ identical twin herd, milk production was examined in 29 pairs of twins, where one twin had a *Str. uberis* intramammary infection at calving and the other did not<sup>7</sup>. Heifers infected with a *Str. uberis* intramammary infection at calving produced 7% less milk during their first 200 days in milk. So preventing heifers from getting an infection in the first place could deliver returns of an extra 19.5 kg MS per heifer (based on average production of 280 kg MS<sup>5</sup>).

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### What is the role of genetics?

Examining the DairyNZ identical twin herd records has also revealed that a twin member is twice as likely to develop a *Str. uberis* IMI if her twin mate has been infected. Figure 1 compares the prevalence of infection in both members of a twin set (related) with the prevalence expected for unrelated but matched pairs of cows (unrelated). The difference in prevalence of infection is statistically highly significant<sup>8</sup> and confirms the possibility of a genetic basis to resistance to *Str. uberis* mastitis.

Using information from a genetic study (FJXB trial, see pg 10), a genetic influence on mastitis resistance was also found. The number of infections was different between the daughters of the six sires (Figure 2). Daughters of sire B had 25% of quarters infected with *Str. uberis* at calving, whereas only 8% of quarters of daughters of sire C were infected. Sire also affected the likelihood of clinical mastitis, with infection case rates ranging from 4 to 16% between the different groups (Figure 3).

### What are phenotypes and why measure them?

Determining if there is a genetic basis to the risk of mastitis requires phenotypes to be defined. A phenotype is the observable characteristic or trait resulting from the interaction of the animal's genes (genetics) with the environment. Cows have many defence mechanisms to prevent mastitis. Many are associated with the teat orifice (the opening) and the teat canal; others are associated with the immune response that occurs when bacteria enters the udder. In our research, the main phenotype of interest was whether cows differed in the amount of mastitis they developed.

### Do cows really differ in resistance to mastitis?

To characterise cows that get less mastitis, the DairyNZ mastitis database (see pg 7) was examined to find cows with either a history of mastitis or no recorded mastitis. 'Resistant' cows were those that had no recorded isolation of a major mastitis pathogen (*Str. uberis*, *Staphylococcus aureus*, *Streptococcus dysgalactiae*) during a minimum of one and a half lactations and including two calving periods.

'Susceptible' cows had one or more recorded cases of clinical mastitis due to *Str. uberis*, or two or more samples from which *Str. uberis* was isolated. While 9% of all cows in the database were positive for a major pathogen at some point during their infection histories, 12% of cows had no recorded infection.

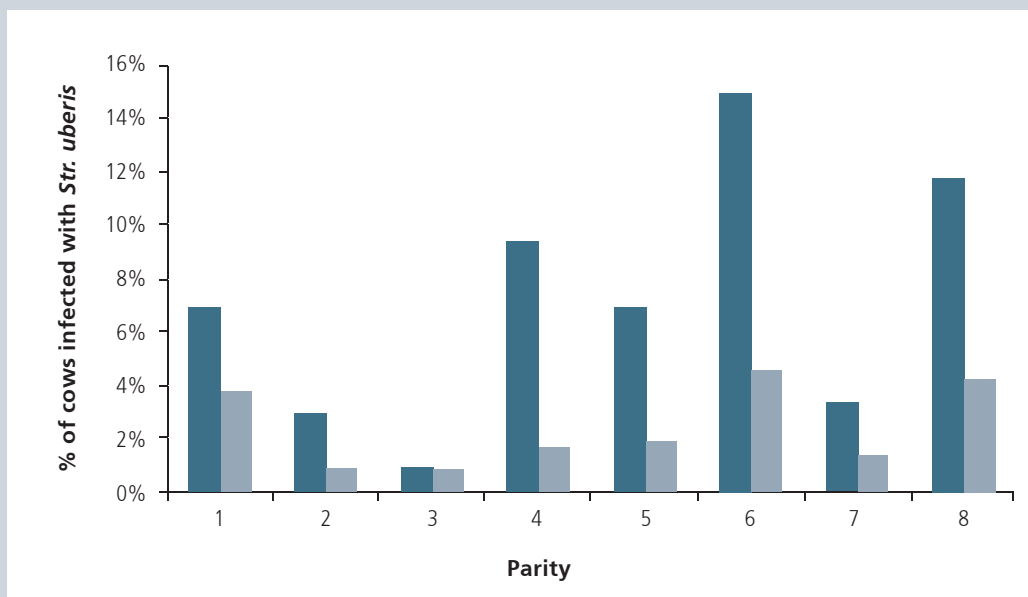
Twenty-six 'resistant' and 27 'susceptible' cows were selected for further examination of phenotypic characteristics. Measurements included phenotypes of possible significance published elsewhere: teat length, height of the teat tip from the ground<sup>9</sup>, teat skin pigmentation<sup>10</sup>, teat canal length, the amount of keratin that could be removed from each teat canal<sup>11</sup>, milk yield and composition including somatic cell count, peak and average milk flow rates<sup>11</sup> and the afternoon milking duration. Cows with a history of intramammary infections ('susceptible') produced more milk fat (1.22 vs. 1.07 kg/d;  $P < 0.01$ ) and had slightly shorter teat canals (9.9 vs. 10.8 mm;  $P = 0.07$ ) than 'resistant' cows<sup>12</sup>. No other differences were found.

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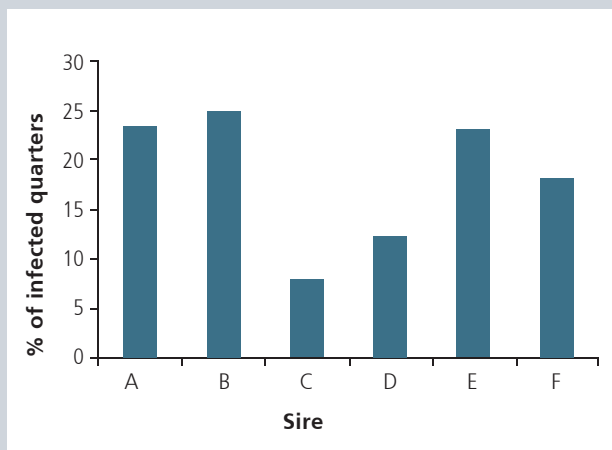


**Figure 1.**

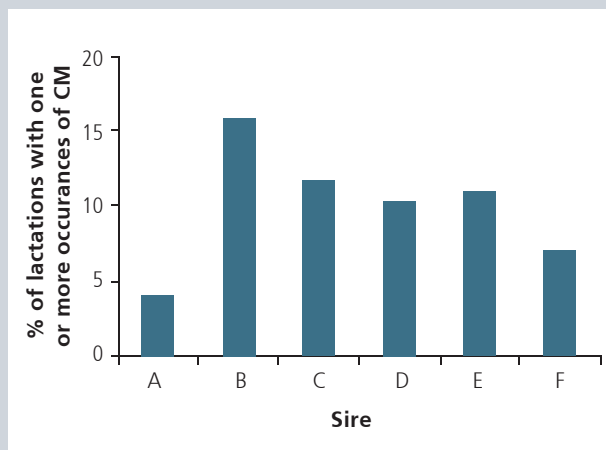
Prevalence of infection in both members of a twin set (dark bars) compared with the prevalence expected in unrelated cows (pale bars)



**Figure 2.** Differences between daughters of the six sires (A-F) for the proportion of quarters infected with *Str. uberis* at calving in their first lactation.



**Figure 3.** Differences between daughters of the six sires (A-F) for the proportion of cow lactations with one or more cases of clinical mastitis.



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### Is a resistant cow always resistant?

These cows were then subjected to an infection challenge to examine the connection between a cow's history of infection and her ability to resist an infection in the future.

Three challenge methods were used sequentially: dipping the teats in bacteria (DIP), inoculating bacteria into the teat canal (TC) and inoculating bacteria inside the udder (intramammary; IM), to examine whether the protective characteristics were operating at the teat orifice, teat canal or immunological level (influenced by the immune system).

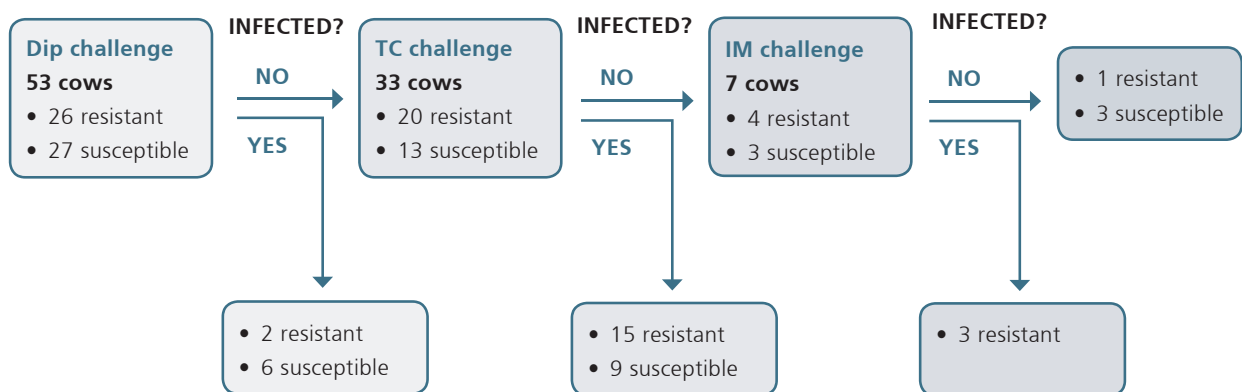
Challenge one (DIP) involved dipping all four teat ends of the cows in skim milk containing *Str. uberis* after milking, for a six-week period (Figure 4). For challenge two (TC), which occurred 16 weeks after challenge one, those cows unaffected following the DIP challenge and remaining uninfected at the beginning of the TC challenge had *Str. uberis* inoculated about 3 mm into the teat canal. Challenge three (IM) involved inoculating *Str. uberis* into the mammary gland, via the teat canal.

Initially, 'resistant' cows tended to 'resist' infection during the DIP challenge. But later, once the teat canal was breached in challenges two and three, 'all bets were off' and their earlier histories did not predict the outcome. So, while genetics are likely to play a role, managing the condition of the teats is very important. Additionally, the results indicate that there is a minority of animals which have highly resistant phenotypes. These cows are of interest for genetic studies of *Str. uberis* mastitis.

### Conclusions

While breeding approaches may help deliver the 'right cow' to New Zealand dairy farmers, the day-to-day management of the cow in her environment will remain important for reducing the impact of mastitis on dairy farms. So it pays to follow the saying "manage the right cow and manage the cow right".

**Figure 4.** Summary of the outcomes of a challenge series in which 53 cows with 'resistant' or 'susceptible' mastitis histories were subjected to a series of *Str. uberis* challenges. DIP = teat ends dipped in *Str. uberis* broth; TC = *Str. uberis* inoculated into the teat canal; IM = *Str. uberis* inoculated into the mammary gland via the teat canal.



## The DairyNZ mastitis database – a unique resource

The DairyNZ mastitis database is unique. Containing nearly 78,000 quarter milk sample records across 16 seasons from 1997-2013, it provides valuable information on mastitis incidence and prevalence on a Waikato dairy farm.

Cows are sampled on an individual quarter basis at the first milking after calving, once in peak lactation,

once in mid/late lactation and again before drying off. Extra samples are collected from clinical cases prior to antibiotic treatment, if the milk secretion looks abnormal or for research purposes. Foremilk samples are collected and analysed for presence of bacteria according to recommended guidelines.

### Pathogens in milk samples collected from DairyNZ cows between 1997 and 2013.

	# of samples	% of positive growths	% of clinical mastitis cases <sup>3</sup>
<b>No growth</b>	66810	-	17%
<b><i>Corynebacterium spp</i></b>	3355	33%	1.5%
<b>Coagulase negative staphylococci</b>	2963	29%	4.6%
<b><i>Streptococcus uberis</i></b>	2435	24%	40%
<b><i>Staphylococcus aureus</i></b>	602	5.9%	7.2%
<b><i>Escherichia coli</i></b>	251	2.5%	10%
<b>Other pathogens<sup>1</sup></b>	562	5.5%	12%
<b>Other<sup>2</sup></b>	1119	-	8.0%

<sup>1</sup>Includes all other pathogens not classified above

<sup>2</sup>Includes blind quarters & contaminated samples

<sup>3</sup>Pathogen isolated as a percent of the 1546 identified clinical cases.



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# Genetic markers of mastitis resistance



Sally-Anne Turner, DairyNZ Scientist; John Williamson, DairyNZ Lead Research Technician; Eric Hillerton, DairyNZ Chief Scientist; and Jane Lacy-Hulbert, DairyNZ Senior Scientist

## Key findings

- A herd of Friesian X Jersey cross-bred (FJXB) cows was used to look for genetic markers associated with mastitis resistance.
- A genetic marker has been identified that is associated with resistance to an intramammary challenge with *Streptococcus uberis*.
- Validation of the marker in a large, independent population will confirm the value of the marker for New Zealand dairy farmers.
- Genetic selection for mastitis resistance could result in significant financial benefits to dairy farmers.



**Mastitis in dairy cows compromises animal welfare and results in significant economic loss. The likelihood of mastitis occurring is influenced by environmental and genetic factors.**

As discussed in the previous article on mastitis phenotypes (see pg 3), DairyNZ studies<sup>1</sup> show clear links between mastitis resistance and a cow's genetic background.

Clinical mastitis can be identified in cows at milking time, but subclinical mastitis is much harder to detect. It relies on regular herd testing, the laborious process of testing every cow with a rapid mastitis test (RMT) or the expensive bacterial culturing of milk samples. Subclinical mastitis is dynamic; cows can self-cure and become re-infected between herd tests.

Subclinical infections affect milk production. For every doubling of the somatic cell count above 100,000 cells/mL, a cow loses 2% of her production<sup>2</sup>. Also, heifers infected with a *Str. uberis* intramammary infection at calving have been shown to produce 7% less milk during their first 200 days in milk<sup>3</sup>. Cows that are genetically more resistant to mastitis would deliver substantial benefits to New Zealand dairy farmers.

## The search for genetic markers

Identifying any component of the genetic basis of mastitis resistance involves sophisticated tools and techniques, and collaborations between science organisations. A successful collaboration between LIC, ViaLactia and DairyNZ enabled access to the phenotypic (observable characteristics) and genotypic (gene) information from the BoviQuest FJXB trial. Bioinformatic (analysis of biological data) investigations produced additional information on the associations between these data.

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## The BoviQuest FJXB Trial

The FJXB (Friesian X Jersey cross-bred) trial was designed to allow the discovery of genes and genetic variations responsible for economically important dairy traits. Pure-bred Jersey and pure-bred Holstein-Friesian cows and bulls were mated to produce first cross (F1) FJXB cows and bulls of high genetic merit. Over two seasons, 864 second cross (F2) cows were produced to form the F2 pedigree herd<sup>4,5</sup>, through the mating of six of the F1 bulls with F1 cows. Cows were managed on a single farm in Taranaki, under typical dairy farming practices using a seasonal, pasture-based system.

## Collecting the phenotypes

To find genetic markers, phenotypes (including those associated with mastitis occurrence or absence) are associated with genomic information. Phenotypes measured in the FJXB cows included: incidence of clinical mastitis; prevalence of intramammary infections (measured four times during each of three successive lactations); and response to a *Str. uberis* challenge (once in their third lactation).

Differences in cow response to a challenge with *Str. uberis* were investigated following a single intramammary infusion of bacteria after a milking. Cows were monitored for mastitis for two weeks following the challenge. When clinical mastitis was detected, cows were sampled and then treated with antibiotics. Within seven days of the challenge, 71% of the herd had developed clinical mastitis.

## How does a mastitis challenge study lead to a genomic marker?

Phenotypes collected from the FJXB cows were used to find regions of DNA associated with desirable traits, known as quantitative trait loci (QTL). Several QTL were found on different chromosomes. The most significant was associated with whether or not a cow developed clinical mastitis after the *Str. uberis* challenge.

Using DNA from the six sires, genes in the QTL regions were tested for SNP (single nucleotide polymorphisms). SNP is a single nucleotide difference in the DNA sequence, at a particular point in the gene, between paired chromosomes. SNP are important as they can be responsible for differences in gene function, in this case response to infection.

A total of 485 SNPs were identified in the sires for further investigation in the FJXB cows. The DNA of the FJXB cows was then sequenced to look for these SNP. When compared with the responses to challenge, one group of SNP in an area of one bovine chromosome was associated with 'resistance' to experimentally induced mammary infection (Littlejohn et al 2013, unpublished).

The frequency of one particular SNP was compared with the infection rates in the cows. Cows can have two copies of the 'desirable' version of the SNP (one on each paired chromosome), two copies of the 'undesirable' version or one of each. Having two copies of the 'undesirable' version of the SNP increased the likelihood of developing clinical mastitis under challenge conditions from 50% to more than 80% ( $P < 0.001$ , Table 1).

## Could a genetic marker for mastitis be financially valuable?

Because large differences in the rate of clinical infection between cows carrying two different versions of the SNP were apparent, a basic economic prediction was carried out using the SmartSamm gap calculator ([smartsamm.co.nz](http://smartsamm.co.nz)), to determine if changes in the clinical mastitis rate would be financially valuable if achieved under normal farming practice.

Estimates suggest that reducing the incidence of clinical mastitis from 15 cases to 10 cases per 100 cows (performance of median herds compared to top 25% of herds<sup>6</sup>) could lead to savings of around \$2500 per herd (for an average 390 cow herd) or \$29M for the New Zealand dairy industry.

It is now necessary to determine if cows that carry the more desirable version of the SNP respond similarly to natural infection and if the marker is important for subclinical infections.

**Table 1.** Association between single nucleotide polymorphism (SNP) versions and the response to an intramammary challenge with *Str. uberis*.

Version of SNP	% cows with clinical mastitis
2 'undesirable' copies	82
1 'desirable', 1 'undesirable' copy	73
2 'desirable' copies	50

If it is, then another benefit could be a lower bulk milk somatic cell count and an affect on milk yield.

### So what's next?

A marker of significant interest has been found in a population of FJXB cows, in response to an experimental mastitis challenge with *Str. uberis*. Investigation and validation of the marker in a large independent population is required before the marker can be considered for release to the dairy industry.

To achieve this, approximately 3000 heifers have been monitored and characterised during their first lactation since 2010. Enrolled to the study from a total of 11 herds, these heifers have been monitored for key phenotypic traits associated with mastitis resistance. Heifers provide the basis of a large independent population, as this ensures the validity of the historical mastitis data.

Additionally, heifers were used as there was no influence of pre-calving treatments (e.g. teat sealants or dry cow therapy antibiotics) on the mastitis phenotype. Information on infection history (as assessed by routine milk sample collection for bacterial analysis), production history (milk yields and composition) and other relevant data such as calving dates, treatment histories and breeding worth have been collected. To provide material for genotyping, blood samples were collected from the heifers for DNA extraction.

During the 2013/14 season, the DNA samples from the heifers are being genotyped for the marker discovered in the FJXB cows. Productivity, fertility and survival traits will also be examined to determine if there are any negative traits associated

with resistance to mastitis. This will help ensure that the markers are appropriate for use in breeding schemes to maximise lifetime productivity of dairy cows. A thorough financial analysis will be conducted if the validation of the genotypes in the heifers proves successful.

In future, the potential benefits may not only be derived from farmers 'managing the cow right'. The whole industry may benefit from 'breeding the right cow' to reduce clinical mastitis.

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# Pills, potions and powders in mastitis management



Eric Hillerton, DairyNZ Chief Scientist

*Well not strictly pills, potions and powders, but a whole range of physical, chemical and biological tools have been, are or may be used to prevent or treat intramammary infections in cows and other species.*

Infection of the mammary gland is inevitable. Every organ of every species has a fauna and/or flora – in this context bugs! Some organisms may be pathogens and affect the well-being of the host; many others are facultative (not of any real consequence), and have only a minor influence on the host.

Some are truly commensal and may benefit the host, and the majority are probably transitory – here today and gone tomorrow. This is either because they cannot survive in the mammary gland, or the immune system ensures they don't! An uninfected mammary gland is rare and essentially a myth.

We identify what our technology allows and do so only when we have a need to. This is usually when the presence of a pathogen changes the function or product of the gland and, therefore, its value. As an example, we rarely culture anaerobic organisms (those that don't like too much oxygen and need special lab methods to find them). And we never try to culture viruses when considering milk quality or udder health. We don't know what we don't know.

The rest of this article focuses on some key methods and technologies used to manage what we do know.

## *Teat disinfectants*

It is a basic theory of hygiene, from the days of Pasteur and his peers, that minimising or protecting exposure to microorganisms helps keep food pure and safe, and animals healthy. Moak in 1917 first reported attempts to do this for mastitis by dipping teats in a disinfectant after milking. He was successful but the product tainted the milk. Better products have since been evolved. Perhaps the simplest and cheapest is a 2-4% solution of bleach.

Indeed, it was common for a long time in the south-east of the United States for farmers to buy and dilute supermarket bleach as a teat dip, or in the United Kingdom to dilute agricultural grade sodium hypochlorite (concentrated bleach) bought as a milking machine cleaning neutraliser.

More sophisticated halogen teat disinfectants became adopted. These are iodine-based products that can be combined with emollients (like glycerol) to protect skin, surfactants (wetting agents) and humectants (anti-drying agents) to keep them active for longer. Chlorhexidine products also have become popular as they avoid some of the problems of iodine products, such as

over-use tainting milk and allergic reactions of a few cows and milkers. However, that is where New Zealand experience largely stops.

Alternative products are rare in our market; some swimming pool disinfectant-type products have existed but are not really effective or cheap enough. North America and Europe have a much wider range of newer technologies, including powders and complicated potions. Alternative active ingredients abound, including sodium chlorite, lactic acid, chlorine dioxide, chlorous acid and hydrogen peroxide. Alternative actions are available, including more persistent or barrier products that act for longer between milkings.

Unfortunately, New Zealand has very limited access to these technologies. The value of the New Zealand market means potential suppliers usually cannot recover the costs of registration in New Zealand as overseas data are apparently not allowed. Also, the companies do not want to spend money on a test, required for registration here, that was replaced as outdated and misleading elsewhere a decade ago. DairyNZ is working with the suppliers' association to help our dairy farmers access more effective and affordable teat disinfectant technologies.

The means of applying a teat disinfectant also requires comment because every teat should be disinfected after every milking – a premise known since 1962. Some readers may remember Woody Pankey, a United States researcher who made a couple of trips of several months duration to New Zealand in the 1990s. He told many dairy audiences that if the teat was not clean enough to put in your mouth it was not clean enough to drink the milk from it – words of wisdom for purveyors of unpasteurised milk!

Disinfectant spraying is used 95% of the time in New Zealand whilst teat dipping is used 95% of the time in the United States. Manual spraying uses twice as much disinfectant but is quicker. I doubt the argument will ever be sorted on which is better. Spraying is supposedly quicker but less reliable. Auto sprayers are growing in popularity after a chequered history of unreliability. Whatever the method, it is the outcome that is important and mastitis will not be properly controlled unless an effective disinfectant covers the entire teat.

## *Mastitis treatments in lactation*

### **Milking**

Older readers may recall a story from James Herriot, the Yorkshire veterinary author of the 1930s. He gently told the farmer that the cow would likely not make it and maybe she should be put down peacefully to prevent her suffering.

The farmer told him to come back in the morning as he couldn't bear to let her go just yet. When Mr Herriot returned in the morning, he found an exhausted farmer and a chipper cow. To save his cow, the farmer had sat up all night milking her and saved her life. Frequent milking and cold water were the farmer's only tools before antibiotics. They were useful then and still can be now.

### **Penicillin**

In 1944 Paul Stuart obtained an early supply of penicillin (how he did so in the middle of World War II Britain is another story) and found that a simple solution put into the mammary gland could cure clinical mastitis.

In early post-war years, new and better formulations were quickly developed, packaged in lead eye ointment tubes, and used with great success to cure many cows and eliminate *Streptococcus agalactiae* completely from herds. Animal health companies have continued to develop better products for more mastitis problems, and better treatment strategies.

Probably all infections can be cured, but for the commercial problems of treatment cost and, especially, the amount of milk not allowed to be sold due to antibiotic residues. The most useful products are applied directly into the mammary gland. Gideon Ziv, a therapy guru of 40 years ago, showed clearly this was the best approach.

### **Residues**

Drug residues have been partly avoided by treating using intramuscular injections of antibiotics. That has raised the spectrum of exposing non target bacteria in other parts of the body to antibiotics and inducing antibiotic resistance. The human medicine jury has found veterinary medicine guilty of rendering some antibiotics no longer useful for people.

*(cont'd pg 14)*



The veterinary jury differs significantly in its opinion, not least because the eminent Sanger Institute in the United Kingdom recently reported that strains of the same species of bacteria found in humans and animals differ significantly. The battle rages, with mastitis specialists adamant there is no evidence of antibiotic resistance changing in response to treating mastitis with antibiotics. However, veterinary medicine and mastitis treatment will inevitably be barred from the use of new antibiotics and are likely to be restricted in the use of antibiotics already available. An astute veterinarian is a valuable friend.

### **Dry cow treatment**

The biggest pressure is on use of dry cow treatment (DCT) – special long-acting antibiotic formulations applied intramammarily after the last milking of the lactation. It is of clear value when treating existing infections, indeed it is the most appropriate method. Political pressure aims to minimise or ban its use to prevent new dry period infections. The dry period is when 50% of all new infections occur.

The case for DCT is being defended stoutly. Fortunately, internal teat sealants applied after the last milking are effective in preventing most new infections and actually last longer so are of benefit when the dry period is greater than 8-10 weeks. This technology is probably still in its infancy but its value is already immeasurable. It will give extended life to use of the limited and older antibiotics accessible in the future for animal care.

### **Antibiotics**

Many countries are forming multidisciplinary groups for Responsible Use of Medicines in Animals (e.g. RUMA in the UK), campaigning to protect access to antibiotics to safeguard the health of all animals. Several people in the New Zealand dairy industry are becoming engaged in this work. Failure to treat mastitis is a welfare issue and antibiotics will remain essential tools in its management.

Not all antibiotics are equal. The vast majority of mastitis cases in New Zealand are caused by bacteria sensitive to some form of penicillin or cephalosporin. Changing farm systems, with more starch-based supplements being fed and/or stand-off and barn systems, means cows will have much more exposure to coliform bacteria. These require different antibiotics and different forms of treatment. A rethink of mastitis management has to be started.

Alternatives to antibiotics have many advocates. The problem is that support is largely based on anecdotes and not controlled studies. A recent publication from DairyNZ adds to growing international literature that the only proven treatments are antibiotic based.

### **Vaccines**

Appreciation quite some time ago that restrictions on chemical (antibiotic) treatment of disease would increase meant that most

animal health companies started to develop biological controls, i.e. manipulating the immune system by vaccination. Many highly-effective vaccines are available for viral infections but fewer for bacterial infections.

Several vaccines to control mastitis have come and gone over the past three decades. Those available now are J5 products in northern hemisphere countries, which limit the severity of coliform clinical mastitis but do not reduce the incidence. A broader spectrum vaccine is also available in several countries, but it does not cover *Streptococcus uberis* – the cause of 75% of New Zealand clinical mastitis.

The Mastitis Tools research programme (see pg 2) has included a project led by AgResearch to examine a new concept of mastitis vaccine, not inducing antibodies but enhancing immune cell defences. Initial work has been promising, sufficient for commercial company involvement in the next stages. Creation of an antibacterial product will not be easy and development programmes commonly take 10 years or so to get a product to market. This is one reason why a commercial partner is so important.

During the wait, close adoption of SmartSAMM is essential. The programme has been developed by DairyNZ combining excellent resources and best practice methods from international partners in Australia, Ireland and the United Kingdom. SmartSAMM has many components tailored and tested for New Zealand farming conditions. It is easily accessed at [smartsamm.co.nz](http://smartsamm.co.nz)

### ***Progress to date and onwards***

The impact of mastitis is now so much less than in the days of James Herriot. Both the rate of clinical mastitis and milk cell count are 60-90% lower. These improvements are due to the actions of dairy farmers in caring for their cows and the tools available for management. This progress has taken place when the likelihood of mastitis has probably increased significantly in higher yielding and faster milking cows.

It is often hard to see progress as it is virtually never rapid, but every decade for the past 60 years has seen major changes – including disinfectants, antibiotics, DCT formulations and teat sealants.

Today's political background will start to demand tools other than antibiotics. The discovery of parts of the genetic basis of resistance to mastitis, as described earlier in this *Technical Series*, opens one door to a more sustainable means of preventing mastitis. New Zealand dairy farming still needs access to better versions of disinfectants and products as innovative as the teat sealants, as no one silver bullet will ever exist.

# Identifying mastitis pathogens using polymerase chain reaction (PCR)



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## Summary

- Identifying the bacteria responsible for an intramammary infection is important for mastitis management.
- Various polymerase chain reaction (PCR) kits are used overseas as mastitis pathogen diagnostic tests.
- PCR is quick and detects lower levels of bacteria compared with bacterial culture.
- Interpretation of PCR results can be difficult due to detection of both live and dead bacteria and lack of clear guidelines to define contaminated samples.
- An assessment on the application and practicality of PCR for diagnosing mastitis in New Zealand is underway.

## Why do we want to identify bacteria?

Mastitis-causing pathogens fall into two categories: contagious or environmental. Knowing the type and species helps determine the best mastitis management for a herd.

## What mastitis diagnostic tests are available?

Bacterial culture of milk is the traditional test to detect the cause of mastitis. Milk is spread on a sterile agar plate and incubated to allow bacterial growth<sup>1</sup>. Available through veterinarians and diagnostic laboratories, the test takes two to four days to perform.





Molecular technology can provide results much faster than this – typically within 24 hours. Polymerase chain reaction (PCR) is one such technology which locates specific parts of DNA that are unique to each bacterial species and amplifies these to confirm their presence in a sample. PCR tests for diagnosing mastitis pathogens are commercially available and used by various milk testing laboratories in Europe, North America and Australia, but are not currently used in New Zealand.

Neither method is perfect. Their advantages and disadvantages are shown in Table 1.

(cont'd pg 16)



**Table 1.** Comparison of bacterial culture and PCR methods for identification of mastitis pathogens in milk.

	Bacterial culture	Polymerase chain reaction
		<p>Extract bacterial DNA from milk </p> <p>Run real-time PCR </p> <p>Analyse results </p>
<b>Time</b>	Minimum = 18 hours, often 3-4 days	Minimum = 4 hours, usually 1 day
<b>Range of detection</b>	<ul style="list-style-type: none"> <li>• Medium sensitivity – can only detect 100 bacterial cells per mL of milk.</li> <li>• Detects a range of bacteria that can grow in culture conditions.</li> <li>• Separate test required to determine antibiotic resistance.</li> <li>• Detects live bacteria only.</li> </ul>	<ul style="list-style-type: none"> <li>• High sensitivity – can detect as few as 15 bacterial cells per mL of milk.</li> <li>• Detects only specific groups of bacteria, targeted by the test.</li> <li>• Can detect antimicrobial resistance genes.</li> <li>• Detects live and dead bacteria.</li> </ul>
<b>Interpretation</b>	<ul style="list-style-type: none"> <li>• Clear guidelines available on how to interpret contaminated samples.</li> </ul>	<ul style="list-style-type: none"> <li>• Issues in defining contaminated samples.</li> </ul>
<b>Cost (NZD)</b>	About \$25-\$30 per sample.	About \$55-\$65 per sample*.

\*Using overseas data as a price guide

## What are the advantages of PCR?

### PCR is faster than culture

PCR can provide results within the same day, unlike bacterial culture. This allows farmers and veterinarians to make rapid and more informed treatment decisions, which could improve cure rates and shorten recovery times.

### PCR is more sensitive than culture

PCR requires the presence of only a small amount of DNA. Overseas studies show that the commercial tests are highly sensitive<sup>2, 3</sup>, particularly for *Staphylococcus aureus* which can be detected as low as 15 bacterial cells per mL of milk<sup>4</sup>.

PCR is slightly less sensitive to *Streptococcus uberis* but can still detect 40 cells per mL<sup>4</sup>.

The flipside of this sensitivity is the enhanced detection of other common bacteria which may have little relevance for udder health.

PCR often detects several different pathogens within single milk samples<sup>2, 3</sup>. In culture, where three or more different species are isolated, a sample is regarded as contaminated, and resampling is recommended<sup>1</sup>. No such guidelines exist for PCR, so it is harder to determine which pathogen is causing the infection, if any. Improving the aseptic conditions for collecting the original milk sample can help reduce this problem.



## Potential implications of PCR

### PCR targets specific bacteria only

Commercial kits target up to 11 of the most common mastitis-causing species<sup>4</sup>, but cannot detect any pathogens outside the range specified by the kit. This means a rare cause of mastitis may go undiagnosed. Therefore, test results require careful interpretation to prevent mistreatment.

### PCR detects both live and dead bacteria

DNA is the target of the PCR test, but this may be from live or dead bacteria, as DNA can survive for weeks following the death of bacteria<sup>5</sup>. The current mastitis PCR tests cannot distinguish between live and dead bacteria, which may confuse interpretation. Improved technology (not yet available in mastitis diagnostics) can determine the proportions of live and dead bacteria, which would provide useful information when confirming the cause of infection or checking the success of treatment.

## How might PCR be used in New Zealand?

The PCR method can be used to identify mastitis pathogens at the quarter, cow and bulk tank level. Quarter level information assists treatment decisions. Cow level information helps culling decisions or selection for dry cow antibiotics. Bulk tank information enables screening of the herd for significant udder-associated bacteria. In Australia, PCR is now used to screen groups of cows and bulk milk for major pathogens.

The use of PCR technology in New Zealand is currently under investigation, in collaboration with LIC. Milk samples collected from DairyNZ cows are being examined for presence of *S. aureus* and *Str. uberis* using traditional culture methods and a commercial mastitis PCR kit. Recommendations for the use of PCR will be made in the next few months.

For the foreseeable future, PCR will not replace culture, but it will become a useful addition to the toolkit, at a cost. With greater access to this technology, veterinarians and diagnostic laboratories will be better able to help dairy farmers manage mastitis and produce high quality milk.

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# What can DNA tell us?



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### From previous articles:

- DNA is the recipe book for life, containing the genetic code for all biological functions and structures.
- The genetic code is a DNA sequence compiled from an alphabet of only four letters, which are called bases.
- DNA bases are organised into genes or recipes, which each have a particular purpose.
- Differences in the base sequence in a gene produce 'gene variants'. These variations are what make individuals different.



### *Elite cows in your herd, and sires that produce the most profitable daughters, have the best combination of genetic variants in their DNA.*

A sire or cow's DNA can be extracted, usually from a blood, skin or hair sample, and the DNA sequence can be determined. The DNA sequence of an individual is referred to as its genotype. The physical traits of an individual cow, such as liveweight, milk protein yield or fertility, are collectively referred to as its phenotype.

The same genotype does not always produce exactly the same phenotype, due to the influence of environmental conditions. By comparing the genotypes and phenotypes of a population of cows or sires, it is possible to determine if a sire or cow has a collection of good, average or poor DNA variants in its genotype. Breeding according to this information is commonly known as genomic selection.

### *Breeding worth (BW)*

New Zealand Animal Evaluation Limited (NZAEL) uses genetic information based on the comparative performance of related dairy cows and, in particular, daughters of a sire in New Zealand, parentage and overseas information.

Sires at the top of the ranking of active sires (RAS) lists have daughters who consistently outperform other animals within the same herd, age and season, with traits such as milk protein content and fertility in New Zealand.

These sires are widely used for breeding as they have an excellent combination of DNA variants which are passed onto their progeny, and this is reflected in the high rating of their breeding values and breeding worth (BW).

Genetic selection has been used to develop domestic animals and plants for thousands of years directly or indirectly. By choosing to breed from animals with desirable traits, the traits are being selected.

Breeding to select for one specific trait or individual DNA variant can generate fast genetic progress for a trait, but often comes at the expense of genetic progress in other traits.

It is far better to focus on an overall breeding objective such as BW (profit per 5 tonne of dry matter fed), where genetic information and performance data are both taken into account. In BW, seven breeding values for individual traits, including milkfat, fertility and liveweight, are multiplied by their respective economic values and summed.

The traits with most economic relevance for farm profit have the greatest influence on a cow or sire's BW. Elite dairy cattle in New Zealand are selected for overall BW based on their DNA.

Visit [nzael.co.nz/all-about-bw](http://nzael.co.nz/all-about-bw) for more information.

### Summary

- Differences in the genetic sequence of an animal's DNA and its relationship with animal performance can be detected.
- This information is used in breeding programmes to breed from animals with the most desirable genetic traits.
- In New Zealand, genotypic information is included in the evaluation of breeding worth (BW) for an individual sire or cow.



# Focus on international research

DairyNZ comments on three recently published key science papers

## **Real and others (2012) Breeding of an early-flowering and drought-tolerant *Lotus corniculatus* L. variety for the high-rainfall zone of southern Australia.**

*Crop & Pasture Science* 63: 848-857

Birdsfoot trefoil (*Lotus corniculatus* L.) is a valuable summer-autumn forage; providing more feed than white clover, particularly in dryland environments. A breeding programme was undertaken to develop varieties of *L. corniculatus* suitable for the high rain zone (>600 mm) of southern Australia and, therefore, extend the adaptation of *L. corniculatus* to drier areas and/or lower altitudes.

**DairyNZ comment:** DairyNZ data have shown increased milk production by cows fed indoors with increasing proportion of *L. corniculatus* (15-45%) in the diet, compared with perennial ryegrass. The condensed tannins in *L. corniculatus* lowered urinary nitrogen but the uptake of *L. corniculatus* in dairy systems is limited by its poor agronomic performance.

## **Smith and Western (2013) Predicting nitrogen dynamics in a dairy farming catchment using systems synthesis modelling**

*Agricultural Systems* 115: 144-154

This Australian study modelled scenarios that included changes to fertiliser use, stocking rate and feeding practices as options for farmers to constrain nitrogen (N) losses while intensifying milk production. There were notable differences in total N loss, even for scenarios with the same level of milk production, and N loss did not increase in proportion to N consumed. The scenario with the smallest N loss over the baseline system increased N leaching by 44%. Intensive systems were more efficient at converting ingested N to milk than lower input systems, but the N loss per unit of milk production was higher.

**DairyNZ comment:** DairyNZ's five-point plan includes a lower stocking rate, use of high genetic merit cows, less N fertiliser, and standing cows off pasture during high-risk periods. Five-year farmlet trials are underway in the Waikato and Canterbury, comparing a future system with the common system in each region. Data on the production, profitability and environmental footprint are being recorded.

## **Spek and others (2013) Prediction of urinary nitrogen and urinary urea nitrogen excretion by lactating dairy cattle in northwestern Europe and North America: a meta-analysis**

*Journal of Dairy Science* 96: 4310-4322

A meta-analysis comprising 47 trials in Europe and North America evaluated the effect of dietary and animal factors on the excretion of total urinary nitrogen (N) and urinary urea N. Milk urea nitrogen (MUN) and dietary crude protein (CP) concentration were the best predictors of N excretion in urine. The relationships between urinary N, MUN and CP were more robust when predictions were based on urinary N data collected from a total collection of urine rather than from urine spot samples, or assuming a zero N balance. Inclusion of dry matter intake improved predictions for urinary N but only for the European dataset.

**DairyNZ comment:** in contrast with dairy systems of the northern hemisphere, perennial ryegrass pastures in New Zealand are characterised by high but variable concentrations of N, meaning the relationship between dietary CP and urinary N is complex. Work is planned with DairyNZ as a partner in an international collaboration, that will investigate methodologies for predicting urinary N.

