TECHNOTE

21 Managing introductions to your herd

Assess risk through use of records and testing at the herd and individual cow level

DRY PERIOD

Buying Cows- Check cell count and treatment histories, examine udders.

Confidence – High

Many outbreaks of mastitis are seen to follow the introduction of infected cows.

Research priority – Low

The Food and Agriculture Organisation of the United Nations defines biosecurity as 'the implementation of measures that reduce the risk of the introduction and spread of disease agents'. In most cases, the emphasis should be on preventive biosecurity to decrease the risk of introduction (bioexclusion), although limiting spread (biocontainment) must also be considered. For the purposes of this Technote, bioexclusion is the primary focus.

Radostits et al (1999) recommend three biosecurity measures to reduce the risk of introducing mastitis pathogens into a herd (bioexclusion):

- assessing the mastitis status of the herd of origin;
- assessing the mastitis status of the individual cows; and
- protecting the home herd until the new introductions are deemed "safe".

In Australian herds the major contagious, or cow-associated, mastitis pathogens are

- *Staphylococcus aureus*
- *Streptococcus agalactiae*
- *Mycoplasma bovis.*

While *Strep. uberis* and *Strep. dysgalactiae* are usually classified as environmental pathogens, they can be spread from cow to cow by mechanisms that we would traditionally classify as contagious.

Different strains of *Strep. uberis* have been shown to differ markedly in their ability to cause intramammary infections (IMIs) and cause clinical mastitis (Hill 1988). While this suggests that similar biosecurity practices, as listed above, should be practiced with *Strep. uberis*, in order to avoid the possibility of introducing more pathogenic strains, our ability to classify their pathogenicity is at this stage limited.

Other *Mycoplasma spp.* have been shown to cause IMIs in Australian dairy herds and it could be argued that they should also be included in the list of major contagious pathogens.

Rational biosecurity practices allow us to assess the risk associated with the introduction of new animals in to the herd. Each farm manager will have a different attitude to risk and will assess that risk in light of the perceived benefits of introduction.

In some situations, a comprehensive approach will be taken. By using more than one test, the risk of introduction of a source of infection can be reduced. This of course comes at a cost, both in time and dollars and requires an increased level of expertise. In other situations, a more minimal approach could be justified. This is easier to implement, less costly but of course is associated with an increased risk of introduction of pathogens.

Assessing the mastitis status of the herd of origin and the status of individual cows.

21.1 Conduct a herd screen for the major contagious organisms

Bulk milk samples can be collected for examination using culture, PCR, antibody testing or bacterial sensitivity to common antibacterial agents.

Culture:

Microbiological culture of a bulk milk sample has been used for the detection of contagious pathogens. Gonzalez and Wilson (2002) reported a study where a single culture of the bulk milk was taken at the same time as culture of all lactating cows. Herds were classified as being infected if a single individual sample was positive. The sensitivity of a single bulk milk sample as a method to classify a herd's status, was calculated as 70.6% for *Strep. agalactiae,* 59.1% for *Staph. aureus* and 33% for *M. bovis.* 3 samples collected 3 to 4 days apart increases the predictive value of a negative culture, but not to 100%. While culture has been largely superseded by PCR testing, there are situations where the use of both methods may increase the sensitivity of detection. There is currently some interest in using on farm rapid culture techniques to monitor bulk milk samples. There is not yet any published research on their use in this situation.

PCR:

The use of PCR testing of bulk milk samples to detect contagious pathogens has become widespread and there are currently several laboratories that offer this service.

The specificity of the test is very high, close to 100%. While it is commonly believed that sensitivity is also close to 100% there are some situations where this may not be the case.

Firstly, a bulk milk sample only contains milk from cows that are contributing to the vat. Cows that have been detected with clinical mastitis are likely to be in the hospital herd while they are being treated and during the milk withholding period. During that time, they will not be contributing to the bulk milk sample.

Sampling of the hospital herd milk is a useful strategy to increase the likelihood of detecting active infection in a herd. This is particularly the case with Mycoplasma infections.

Again, by increasing the frequency of sample collection, the sensitivity of the test will increase. However it is difficult to obtain consent to sample a vendor's hospital herd on a regular basis and purchases are often done relatively quickly. In most cases there would be other buyers who would not require this level of scrutiny and the opportunity to purchase would be lost.

Secondly, when a PCR is used to determine the presence of infection with *Mycoplasma bovis,* particular attention must be given to the peculiarities of this organism and its interaction with the host. Infected cows are known to shed intermittently. (Biddle et al., 2003). Parker et al (2017) collected 186 bulk milk samples from 19 known infected herds over time. Only 7 of the 186 samples were positive. This could be due to intermittent shedding; infected cows being milked in the hospital herd and because infections may be in sites other than the udder. Cows infected with *Strep. agalactiae* tend to shed continuously so the sensitivity of a single sample as an indication of herd status is higher.

Thirdly, in herds with a low prevalence of infected cows, the number of organisms may be below the level of detection. This may be a more significant issue when using multiplex PCRs as opposed to an assay that detects only a single organism. Multiplex PCR tests allow the detection of more than one species of pathogen in a single sample. Parker et al (2017) used a 3 agent PCR (M. bovis,M. californicum and M. bovigenitalium) to detect samples with a known concentration of organisms. When all three target species were present within a milk sample, the ability to detect each organism decreased by 100-1000 fold. This is due to the competition for reagents that are used to amplify the target organism.

Interpretation of milk samples using a multiplex PCR, where more than a single PCR target organism is present, should consider this observation. If you require greater confidence that a PCR target organism is not present in a sample, consider using a single agent PCR test.

In summary, a single negative PCR on a bulk vat or waste milk sample is not an indication that the herd is free of mycoplasma species.

Antibody testing

When assessing the Mycoplasma status of a herd, the use of a single PCR or culture has limitations as discussed above.

The detection of antibodies, and hence, evidence of previous exposure, may be a more sensitive method of detecting infected herds than PCR or culture. (Parker et al., 2017).

A feature of herd outbreaks of mycoplasma is the widespread seroconversion in the absence of clinical disease (Hazelton et al., 2018).

There are currently two ELISA kits that have been used in Australian herds. Wawegama et al., unpublished, tested 5190 bulk milk samples in 2017 from herds across Australia using the MilA ELISA. Definitive cutoffs for the use of the test in this manner have yet to be established but this study suggested that the apparent prevalence of infected herds was significantly higher than previously thought.

Parker et al., 2017 used the Bio-X Bio K 302 kit. Their study examined the potential of the ELISA kit to identify herds with past exposure to M.bovis. The bulk tank milk optical density coefficient (BTM ODC%) was higher in herds 5 to 8 weeks after calving and highest sooner after M. bovis clinical disease outbreaks. There was also a significant association with the proportion of the cows in the herd that were ELISA positive. However, the coefficient of determination value was low (16%) indicating that within herd seroprevalence explained little of the variation in BTM ODC% between herds.
Peterson et al (2016) on the other hand observed a much stronger association with an increase of 9 ODC% per 10% increase in the proportion of cows that were ELISA positive for M.bovis compared with the Parker et al study, in which the estimated increase was just 1 ODC% per 10% increase.
It was suggested by Parker et al that that the low coefficient of determination value and weaker association in their study may have been due, in part, to imperfect specificity of the ELISA in Australian dairy production systems.

Considerably more work needs to be done to define how we use these tests and in quantifying the risk of disease transmission in antibody positive cows and herds.

Antibiogram

McDougall et al described the development and implementation of a system that could determine the minimum inhibitory concentrations (MIC's) to various antibiotics of two common mastitis bacteria. *Staph. aureus* and *Strep. uberis*' after isolation from the bulk milk.

The Dairy Antibiogram is an assay on a bulk milk tank sample that produces reliable data on antibiotic resistance patterns in Staph. aureus and Strep. uberis.

After culture of the bulk milk sample, several colonies of these organisms are selected and using broth microdilution, the MIC of an antimicrobial agent can be calculated.

It is possible that by defining a farms antimicrobial sensitivity pattern over time, a rational decision could be made on the risk of introducing bacteria that may have a different, less susceptible pattern.

21.2 Examine the Bulk Milk Cell Count (BMCC) records.

BMCC records are readily available from the milk processor for every herd. Request details covering the last 2 years to allow a more complete assessment of the herd's mastitis history.

While BMCC can be influenced by clinical mastitis detection ability or policy, it is a reflection of the prevalence of IMIs in cows, both clinical and subclinical, that are contributing to the vat.

Herds with a high prevalence of *Strep. agalactiae* or *Staph. aureus* tend to have a BMCC that is greater than 200,000 cells/mL.

Seasonal calving herds with poorly controlled contagious mastitis will tend to have a gradual increase in BMCC throughout lactation, a drop in BMCC at calving, reflecting the introduction of clean heifers and the results of antibiotic Dry Cow Treatment and then another increase during the subsequent lactation.

See Technote 12 for ICCC analysis

21.3 Examine the clinical case records

Countdown recommends targets of 5 clinical cases per 100 cows at calving and 2 per 100 cows per month during lactation. In herds where calving dates are not available this target could be expressed as <25 cases per 100 cows per year. Herds with rates greater than this require further evaluation.

Look for evidence of multiple quarter clinical mastitis and an increased number of cases that do not respond to treatment. This may indicate a risk that *Mycoplasma bovis* is active in the herd.

Cows with multiple cases of clinical mastitis are at higher risk of chronic infection.

21.4 Ask about Dry Cow Treatment (DCT) history

Determine the strategy that was used for DCT and if it was a suitable strategy for this farm. For example, part herd antibiotic DCT may have been a suitable strategy, but now poses a risk due to the need to transport cows to a new farm.

The use of whole herd Internal Teat Sealant (ITS) may partially mitigate this risk.

If antibiotic DCT has been used, dates of treatment and the details of the product used is essential information to minimise/eliminate the risk of antibiotic residues in milk and meat.

21.5 Examine and analyse Individual Cow Cell Counts (ICCC).

Analysis of ICCC data allows calculation of apparent new infection rates and apparent cure rates. ICCC's also give us good information about the infection status of individual cows.

All of the data that allows us to make rational culling decisions is also relevant when purchasing cows. Factors which reduce the likelihood of a cure at drying off should be considered when purchasing cattle. These include age, ICCC, number of clinical cases, organism likely to be involved and previous lactation history.

Cows with ICCC's greater than 250,000, multiple clinical cases, and elevated ICCC's that have persisted from the previous lactation, despite antibiotic DCT, are at higher risk of chronic infection.

See Technote: 12

21.6 Examine the udder of cows intended for purchase.

Look for cows with uneven quarters which may indicate chronic infection. In the absence of ICCC data and if the cow is still lactating a Rapid Mastitis Test will indicate the likelihood that a subclinical infection is present.

Palpation of udders will detect the presence of scarring, abscess formation or a nonfunctional quarter.

21.7 Introduce maiden heifers rather than older cows.

Age is a significant risk factor for mastitis. Older cows have higher rates of mastitis than younger cows because:

- they have had more exposure to mastitis bacteria and the milking process
- they are more likely to have had mastitis in a previous lactation
- they may have existing udder tissue damage (Buddle et al., 1987)

Heifers that have not previously been milked in a herd are more likely to be free of the major contagious mastitis bacteria.

21.8 Protect the home herd until introduced cows have been assessed more thoroughly.

Introduced animals should ideally be isolated from the home herd and/or milked last until appropriate further assessment is undertaken.

Further assessment could include:

- observation for clinical signs
- examination of individual milk samples using Rapid Mastitis Test, ICCC, culture or PCR
- examination of a composite sample of all introduced cows, using either PCR, cell count or culture
- monitoring of pooled hospital milk

Milk from introduced cows could pose a risk to calves until assessed for mycoplasma.

Introductions can arrive in ways other than planned purchases. Milking a neighbour's cow/s has at least the same and sometimes a greater risk of introducing infection to a herd.

Ensure that fences are maintained to reduce the opportunity for stock to enter the property through this route.

Natural disasters such as bushfire, flooding and power supply failures may create a situation where it is necessary to milk a neighbours' cows. All efforts should be made to minimise the risk to both herds. This could mean running the herds separately, washing the plant between herds and paying extra attention to hygiene and fomites in the dairy.

21.9 Make an assessment of the biosecurity practices of the farm of origin.

Does the herd have a history of introductions or has it remained "closed".

Bibliography

Biddle MK, Fox LK, Hancock DD. 2003. Patterns of mycoplasma shedding in the milk of dairy cows with intramammary mycoplasma infection. Journal of the American Veterinary Medicine Association 223: 1163–1166.

Hill AW. Pathogenicity of two strains of *Streptococcus uberis* infused into lactating and non-lactating bovine mammary glands. Research in Veterinary Science 1988; 45, 400-404

Gonzalez R, Wilson D. Realistic Milk Culture Programs for Herd Expansion. National Mastitis Council Annual Meeting Proceedings 2002; 118-124

Parker AM, House JK, Hazelton,MS, Bosward KL, Morton, JM & Sheehy PA. 2017. Bulk tank milk antibody ELISA as a biosecurity tool for detecting dairy herds with past exposure to *mycoplasma bovis*, Journal of Dairy Science 100: 8296–8309

Penry J, Malmo J, Mein G, Morton J. 2014. Molecular testing of milk: interpretation and application in Australian dairy herds. NMC Annual Meeting Proceedings, pp 133-146.

Hazelton MS, Morton JM, Bosward KL, Sheehy PA, Parker AM, Dwyer CJ, Niven PG and House JK. 2018. Isolation of *Mycoplasma spp.* And serological responses in bulls prior to and following their introduction into *Mycoplasma bovis* – infected dairy herds, Journal of Dairy Science 101:7412 – 7424

Wawegama, Browning, Watt, Tivendale, Firestone and Mansell, (unpublished).

McDougall S, Castle R, MacPherson Y, Karkaba A, Graham L. The Dairy Antibiogram; a novel way to monitor antimicrobial sensitivities of mastitis pathogens in dairy herds. 2018 Conference Proceedings of the Society of Dairy Cattle Veterinarians of the NZVA

Buddle BM, Herceg M, Ralston MJ, Pulford HD. Reinfection of bovine mammary glands following dry-cow antibiotic therapy. Vet Microbiol 1987;15:191-199.

Dinsmore RP, English PB, Gonzalez RN et al. Evaluation of methods for the diagnosis of Streptococcus agalactiae intramammary infections in dairy cattle. J Dairy Sci 1991;74:1521-1526.

FAO 2008. 'Biosecurity for highly pathogenic avian influenza: issues and options', Animal Production and Health Paper 165.

Radostits OM, Blood DC, Gay CC. Mastitis. In: Veterinary Medicine, Chapter 15, 9th edition. Bailliere Tindall, London, 1999:676.

Petersen, M. B., K. Krogh, and L. R. Nielsen. 2016. Factors associated with variation in bulk tank milk Mycoplasma bovis antibodyELISA results in dairy herds. J. Dairy Sci. 99:3815–3823.