Mastitis and On-Farm Milk Cultures - A Field Study - Part 1

This two-part article discusses the results of a research project undertaken by Dr. Tim Olchowy, Senior Lecturer in Livestock Medicine, School of Veterinary Science University of Queensland, Gatton. The project was funded by Subtropical Dairy and was endorsed by the South-east Queensland Regional Group.

Introduction

Clinical and subclinical mastitis has been, and will continue to be, a significant challenge to the management of dairy cows. Each dairy property is different (cows, feeding programs, topography of the land, shade structures, weather patterns), may have different types of mastitis causing bacteria, and will likely have unique treatment protocols for clinical mastitis cases.

Traditionally, milk samples are collected from a quarter with mastitis and sent off-farm to a microbiology laboratory. Turn-around time for culture results is usually several days. If the actions of the dairy producer associated with the culture result are not time sensitive, then this approach provides the best quality data for decision making. If the actions of the dairy producer are time sensitive, then the laboratory results may be received too late to be of maximum value.

The management and care of cows affected by clinical mastitis may involve administration of antimicrobials, anti-inflammatory drugs and, in some severely affected cows, intravenous or oral fluid therapy. All properties will have similar issues associated with the use of medications. One such issue is the question of “to use” or “not to use” antimicrobial drugs. This decision is often based on knowing the type of bacteria (gram positive or gram negative) responsible for the clinical disease. A common treatment recommendation is to only use antimicrobials to treat cows with clinical mastitis caused by gram positive bacteria. The implementation of this recommendation is dependent on receipt of the milk culture result and therefore is a time sensitive decision.

A study was conducted on a southeast Queensland dairy property to explore the use of on-farm milk cultures in the therapeutic management of clinical mastitis. The study compared the results of on-farm cultures against those from a microbiology laboratory, determined the ease/difficulty of the on-farm culturing technique, and estimated the potential benefits of an on-farm milk culture program.

Methods

The study was conducted on a year-round calving dairy milking approximately 250 predominantly Friesian cows. Milk samples were collected during the period of November 2016 to May 2017.

A short training program was run on the study farm with the participating producer and herdsman prior to the start of the study. The training covered the proper

collection and handling of milk samples, use of culture plates, use of the milk sample applicator (Figure 1), and interpretation of culture results.

Milk samples were collected from quarters of cows with signs of clinical mastitis. For this study clinical mastitis was defined as either abnormal appearance of milk or abnormal appearance of both milk and quarter. Disposable gloves were worn to prepare teats (remove debris, scrub with alcohol swabs) and collect milk samples into sterile containers. Samples were kept cool (refrigerated, cool box) until cultured. Milk samples were cultured on-farm using culture bi-plates containing selective bacterial growth media (Figure 2) and in a microbiology laboratory (School of Veterinary Science, University of Queensland, Gatton, QLD) using both standard microbiology laboratory methods and the same culture bi-plates as used on the study farm.

All culture bi-plates were examined and reported after 24 hours of incubation. Samples processed by the microbiology laboratory using standard microbiological methods were examined and reported after 24 hours (preliminary results) and again after 48 to 72 hours (final results) incubation.

The culture results from the bi-plates were interpreted as growing gram positive bacteria (such as *Streptococcus*, *Staphylococcus*, *Enterococcus*), gram negative bacteria (such as *E. coli*), or having no bacterial growth (Figures 3a 3b). The culture results from the microbiology laboratory identified the specific bacteria type (such as *Streptococcus dysgalactiae*). Comparisons were made between the on-farm culture results and the microbiology laboratory culture results.

Simple models were created to compare the effect of using on-farm milk cultures on the costs of treating clinical cases of mastitis. These models considered the labour costs associated with the culturing of the milk sample and the administration of treatments to the affected cows, and the cost of medications used to treat the affected cows.

Results

A total of 135 milk samples were used to compare the culture results of the microbiology laboratory and the on-farm culture bi-plate system (Tables 1 and 2). Two samples identified using the standard laboratory methods of the microbiology laboratory as either ‘no bacterial growth’ or ‘light mixed growth’ were classified as ‘gram positive bacterial growth’ by the on-farm culture bi-plate system. Five samples identified by the microbiology laboratory as ‘gram positive growth’ were identified by the on-farm culture bi-plate system as ‘no bacterial growth’. However, two of these five samples required 48 hours of incubation in the microbiology laboratory before bacterial organisms were identified. Two samples identified by the microbiology laboratory as ‘gram positive’ growth were identified by the on-farm cultures as ‘gram negative’ growth. Four samples identified by the microbiology laboratory as ‘gram positive’ growth were identified as having both ‘gram positive’ and ‘gram negative’ growth by the on-farm culture system.

The on-farm bi-plate culture results were compared with the bi-plate culture results from the microbiology laboratory. Results from a total of 98 milk samples were compared (Table 3). Of the 63 samples identified as either 'gram negative' or 'no bacterial growth' using the standard microbiology methods of the microbiology laboratory, 3 were identified as 'gram positive' by the on-farm cultures. Of the 33 samples identified as having 'gram positive' growth by the microbiology laboratory, 1 was identified as 'no growth' by the on-farm cultures. One of the two samples producing 'mixed growth' cultures in the microbiology laboratory produced 'gram positive' growth in the on-farm cultures.

The results produced by the standard laboratory methods of the microbiology laboratory were compared to the on-farm bi-plate culture results to determine the effect on the selection of a treatment protocol. For this comparison, milk cultures which resulted growth of gram positive bacteria justified the use of antimicrobials. The microbiology laboratory identified samples from 92 cows with clinical mastitis ('gram negative', 'no growth', 'non-significant/mixed growth') which would not have required the administration of antimicrobials (Table 4). The on-farm bi-plate cultures identified 2 of these 92 milk samples as having 'gram positive' growth and as such would have resulted in two additional cows receiving antimicrobial treatments.

There were 43 milk cultures from cases of clinical mastitis which resulted in growth of gram positive bacteria in the microbiology laboratory (Table 5). Two of these samples required more than 48 hours before any growth was evident. Using the on-farm bi-plate culture system, 7 of the 43 samples were identified as either 'gram negative' or 'no growth' and would not have received antimicrobial treatments.

Discussion

The results from the on-farm bi-plate culture system differed from the results produced by the standard microbiology methods used by the microbiology laboratory. A small number of cultures were presumed to be misclassified using the on-farm system. The difference in the results could be caused by several factors.

- The on-farm culture system of this study has a fixed incubation period of 24 hours. The microbiology laboratory routinely continues cultures which have no apparent bacterial growth at 24 hours for a further 48 to 72 hours to determine if slow growing bacteria are present in the sample. Slow growing bacteria resulting in no bacterial colonies, or colonies too small to be readily seen, at 24 hours may have been missed by the on-farm culture system.

- The volume of milk applied to the culture plates differed. A larger amount was applied on-farm than in the microbiology laboratory due differences in technique. The on-farm culture technique used a larger volume cotton bud style applicator (Figure 1) because the plastic loop applicator (Figure 1) used in the microbiology laboratory was too difficult to be of practical use in the on-farm setting. When used on-farm, the small 0.1 mL volume plastic loop was difficult to slide along the surface of the culture media and often cut directly into the media in the culture plate. The use of the cotton bud applicator eliminated this problem.

- The time delay between sample collection and application of the milk to the culture plate was much shorter on-farm (a few hours or less) compared to the time delay of samples delivered to the microbiology laboratory (often several days). Even though the milk sample was kept cool (refrigerated, cool box), bacteria present in the sample on farm may not have survived the delay in adequate numbers to be detected in the microbiology laboratory. Conversely bacteria present in undetectable very small numbers at the time of on-farm culture may have multiplied and become detectable in the microbiology laboratory.

- The microbiologist in the laboratory is able to inspect and select suspicious appearing individual bacterial colonies from a mixed growth culture and perform additional testing to determine if the colonies are mastitis causing bacteria or are contaminants. The on-farm culture system did not allow this selection and testing procedure. This may allow identification of bacterial pathogens in the laboratory setting which were missed by the on-farm culture system.

- By its nature, on-farm milk culturing is at high risk of contamination as few dairies have a room dedicated to only laboratory analyses. There were very few of the on-farm milk cultures classified as contaminated. This suggests a high level of laboratory skill can be mastered in very short time period and the implementation of a simple on-farm milk culturing program is relatively easy for a dairy producer.

There are a number of advantages and disadvantages of the simple on-farm milk culture program used in this study.

- An additional milk sample can easily be collected and re-cultured if the results of the initial culture are suspicious, such as mixed growth cultures which may be the result of sample contamination. The large number of mixed growth cultures in the microbiology laboratory may represent a greater sensitivity of the laboratory methodology than the on-farm culture system. However, an on-farm culture program would allow repeated sample collection and re-culturing of the new sample with only a 24 hour delay. Results would be available in 48 hours instead of the usual 24 hours. The logistics of repeat sampling and shipping the new sample to a microbiology laboratory would result in a considerably longer delay.

- The 24 hour incubation of the on-farm system was selected because of the limited time period within which a treatment protocol must be selected. Longer incubation periods may allow the detection of slow growing bacteria but most of the common mastitis causing bacteria will produce growth within 24 hours. This 24 hour limitation of the on-farm system is unlikely to be significant in most cases of clinical mastitis.

- An on-farm culture program provides opportunities to improve treatment decisions. When compared to the microbiology laboratory bi-plate culture results, the on-farm culture results would have resulted in the use of an antimicrobial in a few additional cases. These cases could be considered as ‘false positive’ on-farm culture results. However, this conclusion assumes bacteria present in the sample when cultured on-farm were still present in the sample at the same concentration when it was cultured in the microbiology laboratory. It is unlikely,

but possible that death of bacteria (or reduction in bacterial numbers below culture detection levels) in the milk sample between the time the sample was applied to the on-farm culture bi-plates and the time the sample was delivered to the microbiology laboratory and applied to the culture bi-plate may be responsible for the different results. One case detected and identified as gram positive using on the culture bi-plates in the microbiology laboratory was identified as 'mixed growth' in the on-farm culture bi-plates. This may have been the result of contamination during the on-farm culturing of the milk sample.

- A practical limitation of any on-farm milk culture program will be the availability of culture plates. Commercial microbiology laboratories deal in high volumes of material and are likely to always have adequate supplies in stock. A dairy with an on-farm milk culture program must be aware of inventory and re-order at an appropriate time. Culture plates have a limited shelf-life and may produce false results after expiration. In this single farm study, culture plates were custom made and were always used before the expiration date.

Culture results could be affected by the skill of the laboratory staff and variations in culture technique.

- Microbiologists are highly trained in the culture of bacteria, the testing of bacterial colonies grown on culture plates and the interpretation of test results. Most dairy producers are not. In this study, the on-farm culture results of 10 of 135 milk samples would have resulted in a different treatment decision compared to the treatment decisions based on the microbiology laboratory results. However, the on-farm culture system does allow producers to re-sample and re-culture the milk within a short time period. This difference in results demonstrates the need for ongoing checks of on-farm culture results against results from a microbiology laboratory to ensure optimum quality control of the on-farm culture program.

- There were some minor differences between the on-farm bi-plate culture results and the microbiology laboratory bi-plate culture results. The results of the on-farm cultures and photographs of the culture plates were reviewed by the author. There were no discrepancies in the interpretation of the bacterial growth on the culture plates. This suggests that the skill of the technician (producer versus trained laboratory technician) in applying the milk sample to the culture plate and in interpreting the culture results does not appear to be a major limiting factor in an on-farm milk culture program. However, a quality control program should be in place for all on-farm milk culture programs.

- The applicator used to apply the milk sample to the culture plate can have a bearing on the results. Applicators applying larger volumes of milk (cotton bud tipped applicators such as used on-farm) may produce an excessively thick layer of one type of bacteria which could grow over and obscure other types of bacteria. Applicators as used in the microbiology laboratory have sharp edges which can cut into the culture media during the application of the milk sample. A higher degree of skill or dexterity is required to use this type of applicator

- Milk samples in the microbiology laboratory were applied to the culture plates in a specific pattern designed to avoid overgrowth of bacterial colonies and create individual colonies. These individual colonies were selected for further identification testing. On the study farm, milk samples were applied in a single zig-zag or streak pattern which can result in some types of bacteria being hidden by more exuberant growth of other types of bacteria.

The target outcomes for the different culture systems are not the same. It is problematic to try to use only one system to achieve both outcomes.

- The on-farm culture systems outcome target is to drive the selection of an appropriate treatment protocol: to use or not to use antibiotics in the treatment of clinical mastitis cases. In almost all cases of acute clinical cases of mastitis, the growth of a gram positive organism justifies the use of an antibiotic based treatment protocol. This is a time sensitive outcome and under ideal circumstances culture results are required 24 hours after mastitis is detected. If not available, the cow could be presumed (correctly or incorrectly) to have a gram positive infection and receive antimicrobial treatment.

- The outcome target of a microbiology laboratory is the detailed identification of the specific bacteria in the milk sample. The variety of culture methods and secondary tests which can be performed in the microbiology laboratory allowed the identification of the bacterial species (*Streptococcus uberis*, *Escherichia coli*, etc.). The on-farm culture system is not suitable for determining if cows are infected with contagious bacteria (such as *Streptococcus agalactiae* or *Staphylococcus aureus*). The microbiology laboratory routinely produces this higher quality data and should be used whenever a producer is trying to identify cows carrying contagious bacteria.

There are several potential benefits from an on-farm milk culture program. Using the on-farm culture bi-plate data from this study and assuming the standard treatment protocol for a case of mastitis included antimicrobials (intramammary infusion and/or intramuscular injections), the potential number of cows receiving antimicrobials would be reduced from 135 to 38. When the total number of individual doses of antimicrobials (intramammary infusions or intramuscular injections) is considered, the number of treatment administrations is reduced from 405 (the expected number if no culture results were available) to 114 (based on 24 hour culture results). Aside from the obvious decrease in treatment costs, significant additional benefits exist: milk residue risk is reduced (greater risk of accidental contamination of the milk vat if a higher number of cows are being treated), antimicrobial stewardship is improved and potential food safety issues are decreased, tissue trauma due to intramuscular injections is reduced (less injection associated lameness, carcase value of cull cows is increased), and the risk of needle stick injuries to treatment and waste disposal staff is reduced.

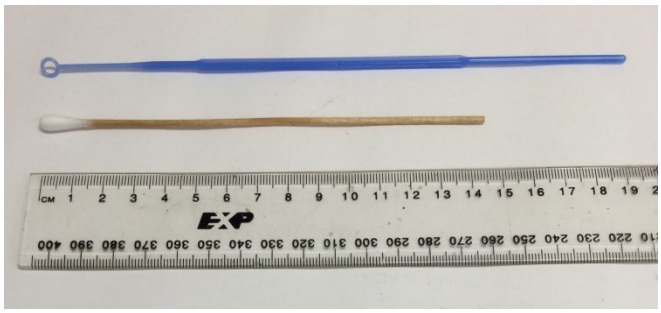
Although not part of this study, the antibiotic sensitivity pattern of any isolated disease-causing bacteria can only be determined using the standard laboratory methods commonly found in a microbiology laboratory. This information can help guide the selection of medications for the creation of and periodic updating of the farm's treatment protocols. The simple on-farm bi-plate culture system used in this

study is not suited to this task. Producers who desire this data should periodically submit milk samples to a microbiology laboratory.

Conclusion

On-farm milk culture programs are relatively simple to establish. Techniques can be mastered in a short period of time. Timely results from these on-farm programs offer significant benefit to dairy producers in the selection of treatment protocols for mild to moderate cases of clinical mastitis. Simple on-farm culture systems, such as used in this study, have very specific purpose and should not be used as a substitute for a microbiology laboratory in circumstances which require detailed identification of particular bacteria species or in certain control programs such as elimination of cows carrying *Streptococcus agalactiae*.

Figure 1. Applicators used to apply milk to the culture plates.



Top: Plastic culture loop. Loop holds 0.1 ml of milk.
Bottom: Cotton bud tipped applicator

Figure 2. Culture Bi-Plate Containing Selective Bacterial Growth Media

Left side: Selective media for Gram Positive bacterial growth
Right side: Selective media for Gram Negative bacterial growth

Figure 3a. Example of Growth of Gram Positive Bacteria on the Culture Bi-Plates

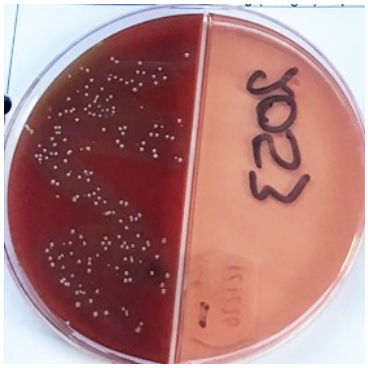


Figure 3b. Example of Growth of Gram Negative Bacteria on the Culture Bi-Plates.

Table 1. Comparison of Microbiology Laboratory and On-Farm Culture Results: Gram Negative, No Growth & Non-significant (mixed) Growth Cultures.

Microbiology Laboratory Culture Results	**On-Farm Bi-Plate Culture Results**
38 Gram negative (all *E. coli*)	33 Gram negative
	5 No growth
22 No bacterial growth	21 No growth
	1 Gram positive
30 Very light mixed growth cultures (no pathogens detected)	28 No growth
	1 Gram positive
	1 contaminated sample
2 Light mixed growth cultures (no pathogens detected)	2 No growth

Table 2. Comparison of Microbiology Laboratory and On-Farm Culture Results: Gram Positive Growth Cultures.

Microbiology Laboratory Culture Results	**On-Farm Bi-Plate Culture Results**
20 *Streptococcus uberis*	17 Gram positive
	2 Gram positive & Gram negative *
	1 No growth
3 *Streptococcus bovis* (required 48 hour culture period)	1 Gram positive
	2 No growth
1 *Streptococcus pneumonia*	1 Gram positive
2 *Enterococcus faecium* #	2 Gram positive
4 *Streptococcus* spp (not typeable)	2 Gram positive
	2 No growth
2 Coagulase negative *Staphylococcus*	2 Gram positive
11 *Streptococcus dysgalactiae*	7 Gram positive
	2 Gram positive & Gram negative*
	2 Gram negative

* - On the bi-plate, this result indicates there was growth on both the gram positive selective media and on the gram negative selective media.
# - A *Streptococcus*-like organism commonly found in manure.

Table 3. Comparison of Bi-Plate Cultures performed in the Microbiology Laboratory and On-Farm.

Bacterial Growth Category	Microbiology Laboratory Bi-Plates	On-Farm Bi-Plates
Gram negative only	35	29 - Gram negative 6 - No growth
No bacterial growth	28	25 - No growth 3 - Gram positive
Gram positive only	33	32 - Gram positive 1 - No growth
Mixed growth*	2	1 - Mixed growth* 1 - Gram positive only

* The culture resulted in growth of both gram positive and gram negative bacteria from the milk sample.

Table 4. Comparison of Microbiology Laboratory and On-Farm Culture Results: Antibiotic Treatment Selection - Gram negative, No growth & Non-significant (mixed) Growth Cultures.

Microbiology Laboratory Culture Results	**On-Farm Bi-Plate Culture Results**
60 Gram negative or No growth cultures	59 Gram negative or No growth cultures
	1 Gram positive
32 mixed growth cultures (no pathogens detected)	30 No growth
	1 Gram positive
	1 contaminated sample

Table 5. Comparison of Microbiology Laboratory and On-Farm Culture Results: Antibiotic Treatment Selection - Gram Positive Growth.

Microbiology Laboratory Culture Results	**On-Farm Bi-Plate Culture Results**
43 *Streptococcus* spp., *Enterococcus* spp. or *Staphylococcus* spp.	36 Gram positives
	7 Gram negative or No growth

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