

Field lab: Reducing antibiotic usage in mastitis using on farm culture

Final report

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Summary

Take home messages

On farm culture and selective antibiotic treatment for clinical mastitis can be a potential tool for dairy farmers who are progressive and willing to reduce antimicrobial usage. Requirements are keen staff applying consistency in the procedures and a significant proportion of gram-negative mastitis pathogens. About a third of mastitis antibiotics may be saved. Cure rates are comparable but can potentially be affected by a treatment delay and limitations of test kits used, so close monitoring of outcomes is essential. Preventing mastitis should be a priority.

Context

Mastitis is the most common reason for antimicrobial usage in dairy cows. While significant progress has been made in reducing antibiotics at drying off by applying selective dry cow therapy, there is limited data monitoring the success of selective treatment of clinical mastitis based on quick identification of the causative bacteria, although it has been known for some time that some organisms, mainly Gram negative bacteria, show high spontaneous cure rates. This field lab investigates the concept using one particular test kit.

Trial design

On three farms, farmers were asked to treat mastitis of mild and moderate degree conventionally with antimicrobials in cows with an even number and apply on farm culture using MastDecide™ to treat gram-positive cases only in cows with an odd number. They were asked to submit the milk samples from the on farm culture to a reference laboratory to assess the agreement of the MastDecide test with reference bacteriology. Mastitis outcome parameters were compared between blanket treated and cultured cases.

Findings

There is moderate agreement with the reference laboratory, sensitivity against Gram positive organisms was 51 %, potentially due to a very sensitive laboratory test to compare. The correct treatment decisions were made in 62 % of the cultured cases.

Two of the farms had detailed records regarding mastitis outcome parameters, and there was no significant difference in any of the parameters analysed:

- Cell counts returned to below 200,000/ml after a 14 to 42 day window in about two thirds of mastitis cases, irrespective of blanket or culture led treatment
- Recurrence rates in the same lactation were very similar too, 39 % in the cultured and 38 % in the blanked treated group
- The median survival times after a case were 331 days for cultured and 426 days for treated cows, a difference which was not statistically significant.

Recommendations & next steps

On farm culture could be considered on progressive dairies, whether organic or non-organic. A first screening of pathogens, potentially in comparison with any test, should be done before embarking on selective treatment. It is recommended to look out for further test development and to monitor outcomes closely. Mastitis prevention should be prioritised.

Useful resources

Jong, E. De *et al.* (2023) 'Selective treatment of nonsevere clinical mastitis does not adversely affect cure , somatic cell count , milk yield , recurrence , or culling : A systematic review and meta-analysis', *Journal of Dairy Research*, 106(2), pp. 1267–1286. doi: 10.3168/jds.2022-22271.

Ruegg, P. L. (2018) 'Making Antibiotic Treatment Decisions for Clinical Mastitis', *Veterinary Clinics of North America - Food Animal Practice*. Elsevier Inc, 34(3), pp. 413–425. doi: 10.1016/j.cvfa.2018.06.002.

Schmenger, A. *et al.* (2020) 'Implementation of a targeted mastitis therapy concept using an on- - farm rapid test : antimicrobial consumption , cure rates and compliance', *Veterinary Record*, pp. 1–11. doi: 10.1136/vr.105674.

Farmer comment [<60 words] + name and occupation

Taking part in the field lab has enabled me to exchange knowledge and experience with colleagues which was a valuable experience. The idea of selective treatment is attractive, however, some outcomes on my farm were disappointing, as several Staph aureus cases were undetected and left untreated. While continuing to look out for all methods to reduce antimicrobial usage, I place my main efforts in improving the barns and reducing mastitis incidence, which has decreased by over 50 % since.

John Shiles, dairy farmer and vet

Main report

1 Field lab aims (up to 50 words)

Mastitis is the most common reason for antimicrobial use on dairy farms (de Campos *et al.*, 2021), and reducing antimicrobial usage is a common goal, supported by farmers, vets and consumers. By rapid typing of bacteria individual treatment decisions can be made, and the field lab aims to evaluate this concept.

2 Background (up to 250 words)

Conventional advice in the UK is to treat every case of mastitis with an antimicrobial. The main aims of this treatment are clinical cure (milk returns to normal consistency, udder inflammation signs disappear) and bacteriological/cytological cure (the infection is cleared, and somatic cell counts return to normal).

Recent research and practice has questioned this approach: Different bacteria show different cure rates, in particular Gram negative, coliform bacteria have a high cure rate without antibiotic treatment. An overview is given in Pinzón-Sánchez, Cabrera and Ruegg, (2011), with the percentage figures indicating bacteriological cure rates:

Bacterium	No Treatment	5 day treatment
Staph aureus	0 %	20 %
CNS	55 %	75 %
Strep uberis	25%	65 %
E coli	75 %	85 %
Klebsiella	35 %	45 %
No growth	90 %	90 %

The table data is as follows:

Bacterium	No Treatment	5 day treatment
Staph aureus	0 %	20 %
CNS	55 %	75 %
Strep uberis	25%	65 %
E coli	75 %	85 %
Klebsiella	35 %	45 %
No growth	90 %	90 %

Gram positive organisms (mainly Streptococci and Staphylococci) show a big difference in cure rates due to antibiotic treatment, and it would therefore be beneficial for farmers to rapidly identify the type of bacterium in order to inform a treatment decision. For this reason rapid test kits have been developed, which can be used on farm, however, an evaluation of most kits has not been carried out under UK conditions.

One of these kits, MastDecide™, has been evaluated against reference bacteriology, with a reported sensitivity (ability to pick up bacteria) of 84 % against Gram positive bacteria (Leimbach and Krömker, 2018). This test has been chosen for the field lab due to its simplicity and safety with regards to environmental contamination – once incubated the milk sample and reagents are totally sealed.

The farms use monthly milk recording which were accessible on two of the farms, so a two-fold evaluation was carried out:

- Evaluating the test against a reference laboratory using bacteriology followed by species identification using mass spectrometry
- Evaluation of cure rates and culling, comparing animals treated conventionally (blanket treatment) and animals treated or not treated according to culture result.

3 Methodology and data collection (up to 800 words)

After farm meetings and recruitment mailings three farmers who were dedicated to reduce their antimicrobial usage decided to take part in the field lab. One of the farms was organic, the other two non-organic. Farmers had group and on farm individual training sessions on taking sterile milk samples, incubating and reading/interpreting the test and recording and submitting the results. All farmers treated every case of mastitis with antibiotics before the trial, according to standard industry guidelines (AHDB).

Farmers were asked to continue treating all mastitis cases in even numbered cows with antibiotics according to their health plan as they did before the trial. In mastitis cases in odd numbered cows they were asked to take a sterile milk sample, apply the on farm test kit (MastDecide™) according to clear instructions and treat only Gram positive cases with antibiotics. All Gram negative and no growth cases do not receive an antibiotic. Any other supportive treatment (e.g. pain relief, Uddermint etc.) can be given as in the health plan to both groups, but farmers were asked to apply those in the same way to both groups and record all treatments.







Only mild and moderate cases of mastitis were included in the trial, severe cases with systemic signs (sick cow) were excluded, with farmers being advised to treat them immediately according to their health plan.

After incubating the MastDecide™ test farmers were asked to freeze the remainder of the milk samples and send them in batches to a reference laboratory (Quality Milk Management Service, Cedar Barn/Easton Hill, Wells) in order to compare the results of the test kit with standard culture/species identification. They were also asked to record details on date, quarter, grade, treatments, withdrawal period and days to clinical cure (normal appearance of milk) in order to assess any economic implications. The recording form is shown in Appendix 1.

Test Kit

The MastDecide™ test was used, which contains two tubes filled with liquid culture media and an indicator – growth will change the colour from pink to white. The test is to be run as follows:

MastDecide™ instructions:

<p>1. Prepare 2 test vessels per milk sample (test medium 1: white lid, test medium 2: yellow lid) -> See also step 3.-7. below.</p> 	<p>2. Carefully shake the milk sample (first milk of single udder quarters), which was taken under aseptic conditions.</p> 	<p>3. Use the disposable pipette to take 0,1 ml from the well mixed milk sample under aseptic conditions. The pipette needs to be completely filled.</p> 
<p>4. Carefully open the first test vessel (test medium 1: white lid). Only touch the outer surface of the vessel and its lid with your fingers.</p>	<p>5. Add the 0,1 ml of milk sample to the test medium 1 (white lid). Try to not touch the test vessel with the tip of the pipette.</p> 	<p>6. Close the lid of the test vessel carefully and shake it carefully several times. Do not put down the pipette in the meantime. Do not open the test vessel again afterwards. Incubation needs to be done at 37°C.</p> 
<p>7. Repeat steps 3.-6. with the test medium 2 (yellow lid).</p>	<p>8. Label the test vessels, if necessary.</p> 	

The sample tubes are placed in an incubator at 37°C and the test is read after at least 14 hours and a maximum of 24 hours.

MastDecide™ Interpretation and suggested action



Testmedium 1 (white lid)	Testmedium 2 (yellow lid)	Result	Action
White	White	Gram Positive	Treat
White	Pink	Gram Negative	Don't treat
Pink	Pink	No growth	Don't treat

Data analysis:

The data analysis begins with an estimation of the potential for antimicrobial reduction, followed by a calculation of the days of milk saved. The agreement between the MastDecide™ test against standard bacterial culture with species identification is assessed at four different levels: agreement on all cultured bacteria, agreement on major pathogens only, agreement on suggested treatment decisions and overall agreement. The exact criteria and the categorisation into major and minor pathogens are outlined in Appendices 2 and 3. The method used by the reference laboratory includes standard bacteriology cultures on blood, Edwards and McConkey agar, incubated for 72 hours, followed by accurate species identification using MALDI-TOF mass spectrometry. Results are given semi-quantitatively, stating either scant or heavy growth.

The second part of the analysis compares mastitis outcome parameters between cultured and blanket treated cows:

Somatic cell count 14-42 days post mastitis

The cell count in a window of 14-42 days post infection was used as a measure for cure. Data was collected using Interherd Plus (Pan Livestock Services) and milk records provided by National Milk Records (NMR). A cow was considered cured if her cell count was equal or below 200,000 cells/ml. Cure rates of blanket treated and cultured cows were compared.

Recurrence rate

Recurrence rates during the current lactation were established using Interherd Plus records and compared between blanket treated and cultured cows.

Survival analysis

Culling data was collected using milk records and the survival in the herd after a case is compared between the groups.

4 Results and discussions

Overall

A total number of 272 cases was originally enrolled between the three farms, but one farm contributed 78 % of the cases. The number of cultured and blanket treated cows were similar on two farms, suggesting good compliance with the selection protocol, with the exception of farm C, where a communication error led to wrong allocation of some cows. Total antimicrobial reduction due to culture was similar between the farms, around one third. On the two farms with accessible milk recording data, one organic and one non-organic, the impact of not treating a case results in 8 and 6 days of milk saved, respectively. This is usually the difference between the milk returning to normal appearance (clinical cure) and the withdrawal period of the medicines used, which is higher on organic farms. The non-organic farm routinely gave a pain relief drug with zero milk withdrawal to all mastitis cases. The organic farm gave pain relief in more severe cases, which will always trigger a minimum organic milk withdrawal period. A summary of the allocation, treatments, antibiotics saved and milk saved is given in Table 1:

Table 1: Summary of allocation and treatments

Farm	Total cases enrolled	blanket treated	total cultured	cultured untreated	% antibiotics saved of cultured	Days milk saved in untreated
A (non-organic)	211	118	93	32	34	6
B (organic)	30	14	16	8	50	8
C (non-organic)	31	8*	23*	7	30	Insuff. Data
Overall	272	140	132	47	36	N/A

*communication error, wrong allocation in some cows

Agreement of the MastDecide test kit with reference bacteriology.

Emphasis was placed on the sensitivity of the MastDecide kit to detect Gram positive bacteria, as missing infections which would benefit from antimicrobial treatment is a potential concern.

Farm A:

The sensitivity of the MastDecide kit for detecting all Gram positive bacteria was 48 % (21 out of 44), for Staph aureus it was 40 % (4 out of 10), while for Streptococcus sp it was 100 % (6/6).

Farm B:

The sensitivity for Gram positive bacteria in total was 43 % (3/7), for Staph aureus it was 50 % (1/2), for Streptococci it was also 50 % (3 out of 6).

Farm C:

The sensitivity for all Gram positive bacteria was 64 % (9/14), no Staph aureus was found on this farm, for Streptococci the sensitivity was 80 % (4/5).

A summary of the agreement on the three trial farms is given in table 2.

Table 2: Summary of agreement between the MastDecide™ kit and the reference laboratory (criteria see Appendix 2, classification of pathogens see appendix 3)

	% true all bacteria	% true major pathogens	% true treatment decisions	% true overall	Sens. All Gram pos
Farm A	43 % (30/69)	42 % (29/69)	61 % (42/69)	75 % (52/69)	48 % (21/44)
Farm B	50 % (7/14)	57 % (8/14)	64 % (9/14)	64 % (9/14)	43 % (3/7)
Farm C	64 % (9/14)	64 % (9/14)	64 % (9/14)	93 % (13/14)	64 % (9/14)
Total	47 % (46/97)	47 % (46/97)	62 % (60/97)	76 % (74/97)	51 % (33/65)

Mastitis cure rates

Mastitis cure rates were assessed on the two farms with complete records in two ways:

1. Single cell count reading after a case in a 14-42 day window

Somatic cell counts returning to low levels (below 200,000/ml) may be used as a proxy for cure. As most mastitis cases will have a high cell count during the clinical phase, the cows are allowed a 14 day recovery period, and the monthly recording in the window after these 14 days is used to assess cure. Not all cows had a reading in this time window, but of those who had the following results were obtained (table 3):

Table 3: Low SCC (< 200,000/ml) in the recording 14-42 days after a case

	% SCC 14-42 days below 200,000/ml		
	Cultured	Blanket treated	
Farm A	71 % (41 out of 58)	72 % (56 out of 78)	
Farm B	33 % (3 out of 9)	36 % (4 out of 11)	
Farm C	No data		
TOTAL	66 % (44 out of 67)	67 % (60 out of 89)	p=0.82

There is an overall minimal difference in the rate of return to low cell count, which is non-significant.

2. Recurrence rate in the current lactation

Mastitis cases were assessed whether the same cow will have another recorded case of clinical mastitis in the current lactation. The result is given in table 4:

Table 4: Recurrence rates in the same lactation

	Recurrence rates		
	Cultured	Blanket treated	
Farm A	40 % (33 out of 82)	40 % (46 out of 114)	
Farm B	33 % (4 out of 12)	21 % (3 out of 14)	
Total	39 % (37 out of 94)	38 % (49 out of 128)	P=0.87

The recurrence rates in the same lactation were very similar between the groups with no significant difference.

Culling and survival

A Kaplan Meier Survival curve combined the two groups between the two farms (Fig. 1):

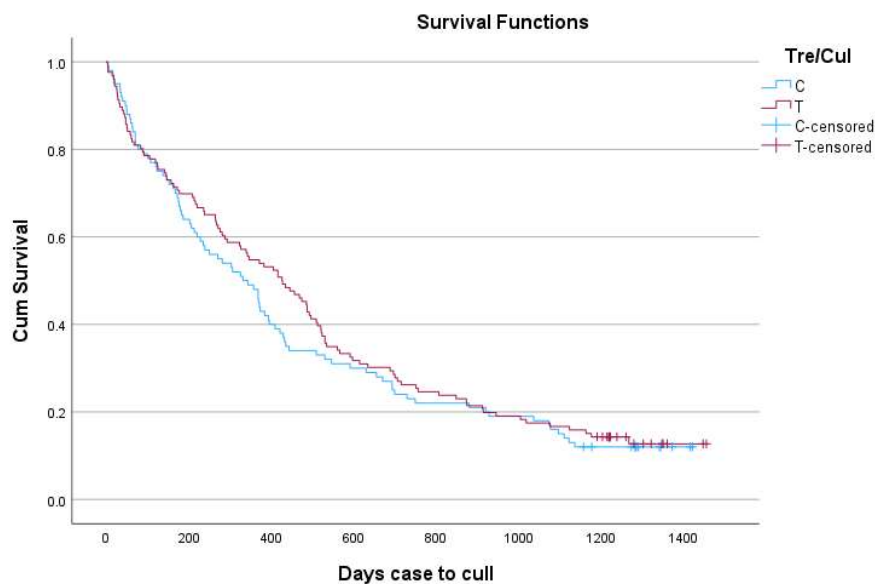


Fig. 1: Survival curves of treated and cultured cows

Comparing culling and survival in the herd between the groups, there was a numerical difference, median (50%) survival time for cows cultured was 331 days and for blanket treated cows 426 days, to test for significance a Cox proportional hazard analysis was carried out, which shows that the difference is non-significant ($p=0.486$)

Discussion

Several studies have shown the effectiveness of on farm culture and selective treatment of clinical mastitis. In the US, Lago *et al.*, (2011) and Vasquez *et al.*, (2017) showed reductions of antimicrobial usage in cultured cases of 49 and 68 %, respectively, with no significant differences in mastitis outcome parameters. In New Zealand (McDougall, Niethammer and Graham (2018) found a 25 % reduction in antibiotic usage with no increased re-treatment risk. Interestingly, in their study, the agreement between the on farm culture test, a commercially available four field culture plate, and the reference laboratory was only 56.9 %. The findings of the current study are similar.

The sensitivity figures of the MastDecide™ test to detect Gram positive bacteria against the reference laboratory were lower than in the study by (Leimbach and Krömker, 2018) – 51 % versus 83.6 %. Three reasons for this difference are possible

- The original study was carried out in a designated mastitis laboratory by trained staff, while the current study was done in an on farm situation, although all staff were highly skilled and received training and ongoing monitoring by a vet
- In the original study the reference laboratory only reported a result as positive if more than 300 bacteria (colony forming units) were found per ml of milk. In the current study smaller quantities are reported which may be below the threshold of a colour change in the MastDecide™ tube. So the question is whether the on farm kit is not sensitive enough or the reference test is “too sensitive”, reporting low numbers of bacteria of doubtful significance
- A considerable number of Staph aureus was found in the samples of this study, which may in combination with other differences in the species distribution explain the different sensitivities.

The question arises whether the MastDecide™ kit is “good enough”, and the mastitis outcome parameters (cure rate and recurrence rate) suggest that it is effective, however, with a questionmark on culling and survival – the latter may have turned out to be significant if a larger sample size was available. However, numerous other studies have supported the concept of on farm culture.

In Germany, Borchardt and Heuwieser (2022) found a 30 % reduction of antibiotic usage in a single herd, again with no significant effects on mastitis outcomes. Also in Germany Schmenger *et al.* (2020) evaluated the effect of a targeted mastitis treatment protocol which involves treating only cases which are caused by Gram positive bacteria, following an on farm test using MastDecide, but also exclude “non-worthy” cases from antimicrobial treatment, which are cases unlikely to achieve a bacteriological cure (chronic high cell counts and several cases of mastitis previous to the case). This led to a 73 % reduction of intramammary antibiotics and a 65 % reduction in injectable antibiotics, with no difference in mastitis outcome parameters. This is in line with Ruegg (2018), stating that only about 20-30% of mastitis cases benefit from antimicrobial usage.

A recent meta-analysis (Jong *et al.*, 2023), pooling and summarising available published studies, showed that selective treatment does not negatively affect bacteriological cure,

clinical cure, new intramammary infections, somatic cell count, milk yield, recurrence or culling.

The overall apparent sensitivity of the test to detect the bacteria targeted for treatment appears to be disappointing, however, the outcomes are encouraging, indicating either that the low sensitivity is a result of a very sensitive reference test or that it is less relevant for outcomes as it is commonly believed. In comparison, selective dry cow therapy is widely accepted as responsible antibiotic use, and (Kabera, Roy, Afifi, *et al.*, 2021) confirmed the validity of the concept in a meta-analysis. However, the selection criteria, somatic cell counts and clinical mastitis history, also have sensitivities of around 70 % only (Torres *et al.*, 2008), (Kabera, Roy, Keefe, *et al.*, 2021). This means out of 100 animals with subclinical mastitis 30 will get missed using those criteria, but the outcomes are still acceptable.

The return to normal somatic cell counts as well as the recurrence rates were almost identical between the groups, however, although not statistically significant, there appeared to be a numerically lower survival time of cows cultured, and this should be followed up in future studies. As reasons for culling were not collected, no definitive answer can be given whether on farm culture did affect survival rates on these farms.

5 Conclusions

Choice of test kit:

The current kit appeared to have a modest sensitivity against Gram positive organisms, and numerically appears to perform poor for Staph aureus. In Staph aureus problem herds, this test kit may be avoided. This underlines the need for standard bacteriology screening before embarking on an on farm culture programme. It is more suitable for farmers with environmental mastitis caused by E coli and environmental streptococci.

Farmers can use this test but are also advised to look out for new test developments and closely monitor mastitis outcome parameters which are likely to differ between farms. Successful farms are generally working in close continuous partnership with their vets.

Economics:

While on farm culture is an attractive way to reduce antimicrobial usage, the primary emphasis should be placed on the prevention of mastitis in the first place. This can lead to a significant reduction in antimicrobial usage as well as higher animal welfare by avoiding pain and also increases economic output. According to AHDB the average cost of a mastitis case is around £ 250-300, taking into account milk loss, treatments and a higher risk of culling. The cost saved by applying on farm culture varies according to the studies: (Down *et al.*, 2017) applied a computer simulation and concluded that only if there is a high (90 %) rate of Gram negative and no growth cases will the concept break even. (Schmenger *et al.*, 2020) state an average saving of 40 Euros per clinical case if a targeted concept is applied which also includes not treating “unworthy”, that is chronically infected cows with antimicrobials. But it is evident that the potential cost savings are minimal compared with the potential savings of reducing the incidence of mastitis in problem farms. However, cost is not the only motivator, probably a minor one considering the discussions at farmer’s meetings, and “doing

the right thing”, “future proofing”, working to meet buyers’ and consumers’ expectations are all higher on the agenda.

6 Tips and recommendations

- Farmers interested in the concept should speak to a vet with the same interest and set up a pre-screening programme and then agree whether it is suitable for the farm, which test kit to choose etc. Continue to work closely with your vet to monitor outcomes. At the same time measures to reduce mastitis incidence can be discussed and implemented
- A similar trial can be set up on any farm for farmers and vets trying out the concept
- Discussion groups are always helpful and may be run by vet practices to exchange experience, learn about new kits coming on the market etc.

7 Further reading

Borchardt, S. and Heuwieser, W. (2022) ‘Comparison of Immediate Blanket Treatment versus a Delayed Pathogen-Based Treatment Protocol for Clinical Mastitis Using an On-Farm Culture Test at a Commercial German Dairy Farm’, *Antibiotics*, 11(3), p. 368. doi: 10.3390/antibiotics11030368.

de Campos, J. L. *et al.* (2021) ‘Quantification of antimicrobial usage in adult cows and preweaned calves on 40 large Wisconsin dairy farms using dose-based and mass-based metrics’, *Journal of Dairy Science*. American Dairy Science Association, 104(4), pp. 4727–4745. doi: 10.3168/jds.2020-19315.

Cobirka, M., Tancin, V. and Slama, P. (2020) ‘Epidemiology and classification of mastitis’, *Animals*, 10(12), pp. 1–17. doi: 10.3390/ani10122212.

Down, P. M. *et al.* (2017) ‘Factors affecting the cost-effectiveness of on-farm culture prior to the treatment of clinical mastitis in dairy cows’, *Preventive Veterinary Medicine*. Elsevier B.V., 145, pp. 91–99. doi: 10.1016/j.prevetmed.2017.07.006.

Jong, E. De *et al.* (2023) ‘Selective treatment of nonsevere clinical mastitis does not adversely affect cure, somatic cell count, milk yield, recurrence, or culling: A systematic review and meta-analysis’, *Journal of Dairy Research*, 106(2), pp. 1267–1286. doi: 10.3168/jds.2022-22271.

Kabera, F., Roy, J. P., Keefe, G., *et al.* (2021) ‘Bayesian estimation of diagnostic accuracy of somatic cell counts history and on-farm milk culture using Petrifilm® to identify quarters or cows that should be treated with antimicrobials in selective treatment protocols at dry off’, *Preventive Veterinary Medicine*, 195(July). doi: 10.1016/j.prevetmed.2021.105452.

Kabera, F., Roy, J. P., Afifi, M., *et al.* (2021) ‘Comparing Blanket vs. Selective Dry Cow Treatment Approaches for Elimination and Prevention of Intramammary Infections During the Dry Period: A Systematic Review and Meta-Analysis’, *Frontiers in Veterinary Science*, 8(June), pp. 1–16. doi: 10.3389/fvets.2021.688450.

Lago, A. *et al.* (2011) ‘The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes’, *Journal of Dairy Science*. Elsevier, 94(9), pp. 4441–4456. doi: 10.3168/jds.2010-4046.

Leimbach, S. and Krömker, V. (2018) ‘Laboratory evaluation of a novel rapid tube test system for differentiation of mastitis-causing pathogen groups’, *Journal of Dairy Science*, pp. 1–9. doi: 10.3168/jds.2017-14198.

McDougall, S., Niethammer, J. and Graham, E. M. (2018) ‘Antimicrobial usage and risk of

retreatment for mild to moderate clinical mastitis cases on dairy farms following on-farm bacterial culture and selective therapy', *New Zealand Veterinary Journal*. Taylor & Francis, 66(2), pp. 98–107. doi: 10.1080/00480169.2017.1416692.

Pinzón-Sánchez, C., Cabrera, V. E. and Ruegg, P. L. (2011) 'Decision tree analysis of treatment strategies for mild and moderate cases of clinical mastitis occurring in early lactation', *Journal of Dairy Science*. Elsevier, 94(4), pp. 1873–1892. doi: 10.3168/jds.2010-3930.

Ruegg, P. L. (2018) 'Making Antibiotic Treatment Decisions for Clinical Mastitis', *Veterinary Clinics of North America - Food Animal Practice*. Elsevier Inc, 34(3), pp. 413–425. doi: 10.1016/j.cvfa.2018.06.002.

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Torres, A. H. *et al.* (2008) 'Using dairy herd improvement records and clinical mastitis history to identify subclinical mastitis infections at dry-off', *Journal of Dairy Research*, 75, pp. 240–247. doi: 10.1017/S0022029908003257.

Vasquez, A. K. *et al.* (2017) 'Clinical outcome comparison of immediate blanket treatment versus a delayed pathogen-based treatment protocol for clinical mastitis in a New York dairy herd', *Journal of Dairy Science*. American Dairy Science Association, 100(4), pp. 2992–3003. doi: 10.3168/jds.2016-11614.

Appendix 1: Mastitis recording sheet

Mastitis Culture and Treatment Recording Sheet

Name _____ Farm _____

Date detected	Cow	Even cow ID - treat	Odd cow ID - grow (culture)	Quarter(s) (circle)	Grade (circle) 1-milk change 2-milk and udder change	For Culture Cows only (circle)	Medicine(s)	Start date	Finish date	Total used (tubes or mls)	Date milk okay	Date milk in tank	Notes
		T	C	FL FR BL BR	1 2	G+ G- no gr							
		T	C	FL FR BL BR	1 2	G+ G- no gr							
		T	C	FL FR BL BR	1 2	G+ G- no gr							
		T	C	FL FR BL BR	1 2	G+ G- no gr							
		T	C	FL FR BL BR	1 2	G+ G- no gr							

Appendix 2: criteria for agreement between the test kit and standard reference laboratory:

- Overall bacteria: Any bacterium isolated in the culture
- Major pathogens: Most relevant mastitis causing bacteria (see table below)
- Treatment decision: based on the assumption that only Gram positive major pathogens are worthy of antibiotic treatment
- Overall outcome: “Correct” treatment decision plus cows “incorrectly” treated for Gram positive minor pathogens.

Appendix 3: Classification of all bacteria isolated by the reference lab, based on (Cobirka, Tancin and Slama, 2020), plus *Tuerperella pyogenes*:

Pathogen	Gram	Major/minor
<i>Acinetobacter guillouiae</i>	neg	minor
<i>Acinetobacter jonsonii</i>	neg	minor
<i>Aerococcus viridans</i>	pos	minor
<i>Bacillus licheniformis</i>	pos	minor
<i>Bacillus cereus</i>	pos	minor
<i>Bacillus circulans</i>	pos	minor
<i>Bacillus pumilus</i>	pos	minor
<i>Candida krusei</i>	(Yeast)	minor
<i>Candida tropicalis</i>	(Yeast)	minor
<i>Chryseobacterium</i> sp	neg	minor
<i>Citrobacter freundii</i>	neg	minor
<i>Corynebacterium amycolatum</i>	pos	minor
<i>Corynebacterium callunae</i>	pos	minor
<i>Elizabethkingia miricola</i>	neg	minor
<i>Enterococcus faecalis</i>	pos	major
<i>Enterococcus faecium</i>	pos	major
<i>Enterococcus malodoratus</i>	pos	major
<i>Enterococcus saccharolyticus</i>	pos	major
<i>Escherichia coli</i>	neg	major
<i>Glutamicibacter arilaitensis</i>	neg	minor
<i>Glutamicibacter mysorens</i>	neg	minor
<i>Klebsiella pneumoniae</i>	neg	major
<i>Kokuria salsicia</i>	pos	minor
<i>Laclercia adecarboxylata</i>	neg	minor
<i>Lactococcus gravieae</i>	pos	minor
<i>Lactococcus lactis</i>	pos	minor
<i>Lactococcus raffinolactis</i>	pos	minor
<i>Pseudomonas fluorescens</i>	neg	major
<i>Pseudomonas gessardii</i>	neg	major
<i>Pseudomonas libanensis</i>	neg	major
<i>Pseudomonas veronii</i>	neg	major
<i>Psychrobacter</i>	neg	minor
<i>Serratia liquefaciens</i>	neg	major
<i>Staph arlettae</i>	pos	minor

Staphylococcus aureus	pos	major
Staphylococcus chromogenes	pos	minor
Staphylococcus epidermidis	pos	minor
Staphylococcus equorum	pos	minor
Staphylococcus haemolyticus	pos	minor
Staphylococcus saprophyticus	pos	minor
Staphylococcus sciuri	pos	minor
Staphylococcus succinus	pos	minor
Staphylococcus warneri	pos	minor
Staphylococcus xylosus	pos	minor
Streptococcus dysgalactiae	pos	major
Streptococcus gallolyticus	pos	minor
Streptococcus parauberis	pos	minor
Streptococcus uberis	pos	major
Streptomyces sp	pos	minor
Trueperella pyogenes	pos	major

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