Development, monitoring and evaluation of clinical mastitis protocols

John House*, Mark Humphris and Kiro Petrovski*****

* Faculty of Veterinary Science, University of Sydney, Camden, New South Wales

** The Milk Road, PO Box 877, Newry, Victoria

*** School of Animal and Veterinary Sciences, Adelaide University, Adelaide, South Australia

Introduction

Mastitis is the most common disease of dairy cattle and a frequent cause of premature cow attrition. Costs associated with mastitis include reduced milk yield, loss of milk quality premiums, culling, mortality, medications, labour, reduced reproductive performance, discarded milk, and transmission of disease to young stock (Pinzon-Sanchez and Ruegg, 2011). Effective disease prevention and management significantly contribute to sustainable and profitable dairy farming. While disease prevention strategies should be the primary focus, there is a shared responsibility to use therapies judiciously when disease occurs to optimise cow health and reduce development of antimicrobial resistance.

Mastitis is inflammation of the mammary gland. This is almost always secondary to intramammary infections. Hence, antimicrobial therapy is usually the mainstay of therapeutic interventions. The general principle of antimicrobial therapy is to achieve an antimicrobial drug concentration at the infection site, greater than the minimal inhibitory concentration, for sufficient time for elimination of infection. In practical terms, there are a number of challenges to evidenced-based therapeutic decisions: at the start of treatment, the causative pathogens, the concentration of antimicrobial drugs in the udder following intramammary or parenteral therapy, and the antimicrobial susceptibility are unknown. The objectives of this paper are to: 1) provide background information on common characteristics and cure rates of mastitis pathogens and different therapeutic options available; 2) present a decision tree example to facilitate the decision making process; 3) outline approaches to monitoring therapeutic outcomes and outline an approach to investigating treatment failure.

Background Information

Prudent antimicrobial use

Constraints on the use of antimicrobials in food producing animals include consideration of current and future efficacy, availability, cost, and potential for violative drug residues. With the increasing public and regulatory attention on food safety there is a global call for 'prudent or judicious' drug use to preserve the efficacy of antimicrobials and avoid introduction of antimicrobial resistant bacteria into the human food supply. Rational use of antimicrobials calls for selection of an antimicrobial with known pharmacokinetic and pharmacodynamic properties for use in a patient with a known disease process caused by a bacterial pathogen known to be susceptible to the administered dose of the chosen antimicrobial. The objective is to develop more effective treatment protocols for clinical mastitis to: (Hillerton and Kliem, 2002):

a) Eliminate infections and prevent recurrence of disease;
b) Reduce antimicrobial use for treatment of mastitis and so reduce the impact on resistance, and
c) Identify effective methods of control with limited or no use of antimicrobials.

Case definitions

The Countdown Technotes consider a cow to have clinical mastitis and require treatment when she has heat, swelling or pain in the udder and/or changes in her milk (wateriness or clots) that persists for more than 3 squirts. Flakes of milk that do not persist for more than three squirts reflect teat canal infections that warrant monitoring but no antimicrobial treatment (Preez, 1988). In developing mastitis treatment protocols, it is important to consider the different presentations of intramammary infections to ensure the most appropriate therapy. A mastitis severity scoring system exists to guide both diagnosis and therapy (Table 1). Clinical manifestations that may be observed are presented in Table 2.

Table 1 Mastitis Clinical Scoring System

Changes in	Non-infected	Subclinical	Mild clinical	Moderate clinical	Severe clinical
Cow	NAD	NAD	NAD	NAD	+
Udder	NAD	NAD	NAD	+	+
Milk	NAD	NAD	+	+	+
ICC	NAD	+	+	+	+
Culture / PCR	NAD	+	+	+	+

+ Changes detectable; NAD – no abnormalities detected; PCR – polymerase chain reaction; ICC – Individual somatic cell count.
Note: Culture and PCR status may change during the course of an infection either due to unculturable pathogen, intermittent shedding, limits of detection or clearance of the infection. In some 15-40% of cases no growth/PCR detection is obtainable.

Pathogens

In the most recent survey of mastitis pathogens from dairy cows in South Eastern Australia, the most frequent pathogens isolated were *Streptococcus uberis* (54.3%), *Staphylococcus aureus* (14.8%), *Escherichia coli* (11.7%), and *Streptococcus dysgalactiae* (8.9%) (Charman et al., 2012). The clinical features and anticipated cure rates of the common mastitis pathogens are presented in Table 3. A number of pathogens are known to be refractory to treatment. When there is a shift in the response to treatment, performing milk cultures to identify the infecting organism is recommended. In this situation, the possibility of *Mycoplasma spp*. should be considered and the laboratory notified so that the correct media is applied for bacterial isolation. Otherwise 'no growth' and diagnostic failure is the outcome.

Table 2 Clinical Manifestations of mastitis

Changes in	Description	
Milk	**Presence of clots/flakes**	
	Change in colour	Bluish, reddish, yellowish
	Change in consistency	Watery, serous, thickened, custard-like
	Change in smell	Yeast, purulent, foetid
Udder	**Swelling**	Hardness, pitting oedema
	Changes in colour	Redness, bluish
	Temperature	Warm or cold
	Tenderness	On palpation
Cow	**Kicking**	Fever
	Stepping	Toxaemia
	Not wanting to be milked	Recumbency
	Change in milking order	Dehydration
	Lethargic/Depression	Inappetence to anorexia
	Lame	Involvement of other organ systems

Table 3 Features of common mastitis pathogens

	Location	Anticipated Response
Environmental		
Streptococcus uberis (predominantly environmental)	Ducts +++/parenchyma+	82–91% clinical cure (Owens et al., 1997, McDougall, 1998, Wilson et al., 1999)
Streptococcus dysgalactiae	Ducts +++/parenchyma+	90–98% clinical cure (Owens et al., 1997, McDougall, 1998, Wilson et al., 1999)
Escherichia coli	Ducts + / cow+++	High rate of spontaneous cure (Royster and Wagner, 2015)
Klebsiella spp.	Ducts + /cow +++	Lower and slower rate of spontaneous resolution than E. coli, 37% vs 85%. (Hogan and Smith, 2012, Roberson et al., 2004)
Coagulase negative staphylococci	Ducts +++	85% Cure rate (McDougall et al., 2007a, McDougall et al., 2007b)
Contagious		
Streptococcus agalactiae	Ducts +++	Good, approaching 100%
Staphylococcus aureus	Ducts + / Parenchyma +++ Facultative intracellular / fibrosis / micro-abscess	Bacteriological cure 20 – 60%. Probability of cure influenced by parity, stage of lactation, historic ICC, number of infected quarters, antimicrobial susceptibility. (Sol et al., 1997)
Mycoplasma spp.	Parenchyma +++ / Cow +++ Facultative intracellular / bacteraemia	Poor / no cell wall / beta lactam antimicrobials ineffective
Miscellaneous		
Corynebacterium bovis	Teat	High prevalence generally reflects poor application of teat dip
Trueperella pyogenes	Parenchyma Fibrosis / Abscessation	Poor / Often associated with abscessed quarters
Nocardia spp.	Parenchyma Invasive / Fibrosis	Poor (May be associated with contamination at infusion)
Yeasts/Fungi	Parenchyma	Poor
Pseudomonas spp.	Cow +++	Poor (May be associated with contamination of water sources in parlour)

+ low likelihood, +++ high likelihood, ICC – individual somatic cell count

Therapeutic options

It is important to appreciate that host immunity is sometimes capable of eliminating the infecting organism leading to spontaneous cure. The rate of elimination of intramammary infection varies according to pathogen, drug, and cow factors. Three clinical trials have compared treatment outcomes to untreated controls (Royster and Wagner, 2015, Roberson et al., 2004). In these studies the average rate of cure was 49% (range 28–64%) in untreated controls versus 61% (range 48–78%) in the treatment groups. The average number of cows that would need to be treated to effect an additional cure can be calculated by dividing 100 by the percent reduction in persistent infection achieved with treatment. In this example 8.3 cows would need to be treated to effect one additional cure as compared to no treatment (100/12 = 8.3) (Royster and Wagner, 2015). Although some clinical studies have failed to demonstrate an improvement in clinical and bacterial cure with treatment, this scenario is more commonly associated with infections caused by coliforms (Guterbock et al., 1993).

The goal of antimicrobial therapy is to attain effective drug concentrations at the site of infection. The classification of mastitis into mild, moderate, and severe according to the nature of the secretion and condition of the mammary gland and cow fits with the concept of 3 potential therapeutic targets, or pharmacologic compartments:

1. The milk and epithelial lining of the ducts and alveoli of the mammary gland.
2. The interstitial tissues of the mammary gland.
3. The cow.

Intramammary antimicrobial therapy

Administering antimicrobials via the intramammary route can achieve concentrations as high as 100 to 1,000 fold those obtained via parenteral administration using less antimicrobial (Smith, 2010). The higher concentrations achieved are advantageous for infections of the milk compartment, such as streptococcal.

Table 4 Intramammary antimicrobial formulations available in Australia

Action	Drug	Distribution in gland#	Antimicrobial activity in milk*
Beta lactams	Amoxycillin + clavulanic acid	Good	Similar
	Ampicillin	Good	Similar
	Cefuroxime	?	Similar
	Cloxacillin	Limited	Similar
Protein synthesis inhibitors	Neomycin	Poor	Markedly Reduced
	Lincomycin	Good	?
	Novobiocin	Good	Reduced
	Oleandomycin	?	?
	Oxytetracycline	Limited	Reduced

**Antimicrobial activity derived from (Constable and Morin, 2003, Ziv, 1980b, Price et al., 1956)*

Potential disadvantages of intramammary antimicrobial delivery include an uneven distribution of the antimicrobial to the upper parts of the affected quarter, secondary to compression or blockage of milk ducts by inflammatory products. Intramammary infusion also carries a significant risk of iatrogenic infection associated with poor infusion technique. In a Victorian study 13% of cows developed a new infection with a different organism following intramammary therapy (Shephard et al., 2000). Tissues lining the teat duct are very susceptible to damage from rough cannula insertion, which may jeopardise antimicrobial function. Risk of damage is reduced by partial insertion of the cannula. Partial insertion has improved efficacy of dry-cow therapy compared to full insertion (Boddie and Nickerson, 1986).

There are seven different antimicrobial formulations on the market in Australia with a number of formulations sold by more than one company. The commercial products available utilise 10 different antimicrobial compounds (Table 4). There is a paucity of comparative data to indicate the relative efficacy of the different products in Australia and elsewhere. It is wrong to assume that products containing the same antimicrobials at the same concentration will have the same efficacy. Product formulation has a significant impact on the distribution of antimicrobials within the mammary gland and subsequently on product efficacy.

Parenteral Therapy

Parenteral therapy may have clinical advantages over intramammary infusion when multiple quarters within a cow are infected, significant swelling of the mammary quarter is present and diffusion of antimicrobials delivered by an intramammary route may be compromised, in the face of a Mycoplasma outbreak (to decrease spread at the time of treatment), when treating large numbers of cows in a ‘blitz therapy’ or when animal behaviour poses a safety risk to operators trying to infuse antimicrobials (McDougall et al., 2007a).

The characteristics of antimicrobials that include a reference to mastitis on their label or in the ‘Pest’ section of the Australian Pesticides and Veterinary Medicines Authority Pubcris database is presented in Table 5.

Antimicrobial susceptibility testing

There are numerous studies reporting the antimicrobial susceptibility of mastitis pathogens. Although antibiotic susceptibility undoubtedly plays a role in response to therapy, there is little evidence of a correlation between in vitro susceptibility testing and treatment outcomes in the cow (Royster and Wagner, 2015). The clinical predictive value of antimicrobial susceptibility is limited by incomplete phamacokinetic/pharmacodynamic data for commercial intramammary products and inadequate field studies validating susceptibility

Table 5 Parenteral antimicrobials registered for treatment of mastitis*

Antimicrobial	Dose	Chemical composition	Lipid solubility	MIC in milk	Milk to serum ratio[1]	Spectrum	Evidence where available	Comment
Procaine penicillin	12 mg/mL	Acid	Low	Unchanged	0.22	Gm + ve	Limited efficacy *Staph. aureus.* (Ziv and Storper, 1985)	
Penethamate hydriodide	10–15 mg/kg	Base	High	Unchanged	6.1	Gm + ve	[#]Improved clinical and SCC outcomes (St Rose et al., 2003, McDougall, 1998, Serieys et al., 2005, Ziv and Storper, 1985, McDougall et al., 2007a)	In a New Zealand trial, an overall bacterial cure rate of clinical mastitis of 76.4% was lower than that achieved with intramammary therapy with a combination of procaine penicillin and dihydrostreptomycin (84.9%) (McDougall, 1998)
Amoxycillin	15 mg/kg	Acid	Low	Unchanged	0.26	Gm+ve/ Gm-ve[§]	No data recovered	
Trimethoprim / Sulphadimidine	2.6–4 mg/kg T 13.3–20 mg/kg S	Base Acid	High	Increased	2.9–4.9 0.3	Gm+ve/ Gm–ve	No data recovered	Trimethoprim has a short half–life in adult cattle (T1/2 ~40 minutes) and is poorly absorbed from extravascular injection sites. At lower doses, similar to the recommended labelled doses in Australia, Trimethoprim has been reported to fail to reach therapeutic concentrations in the mammary glands in adult ruminants (Kaartinen et al., 1999, Guard et al., 1986).
Trimethoprim / Sulfadiazine	1.33–2 mg/kg T 13.3–20 mg/kg S	Base Acid	High	Increased	2.9–4.9 0.14–0.2	Gm+ve/ Gm-ve	Improved outcome coliform mastitis (Shpigel et al., 1998)	
Trimethoprim / Sulfadoxine	2.6–4 mg/kg T 13.3–20 mg/kg S	Base Acid	High	Increased	2.9–4.9 ?	Gm+ve/ Gm-ve	Not recommended (MacDiarmid, 1978)	
Neomycin	2–4 mg/kg BID maximum 3 days	Base	Low	Increased	0.5	Gm–ve	Not recommended (MacDiarmid, 1978)	
Tylosin	5–10 mg/kg	Base	High	Increased	4.5	Gm + ve	Similar efficacy to Penethamate (McDougall et al., 2007a)	
Erythromycin	2–4 mg/kg	Base	High	Increased	8.5	Gm + ve	Proposed to be effective against *Streptococcal* and *Staphylococcal spp.* (Satoh, 1974)	Tissue reaction and pain at the site of injection are often observed and may limit repeated drug administration
Oxytetracycline	4–10 mg/kg	Amphoteric	Moderate	Increased	0.6–1.4	Gm+ve/ Gm-ve	Limited data(Morin et al., 1998)	Distribution of oxytetracycline to milk following intramuscular injection of labelled doses (4mg/kg) is poor. Following intravenous injection oxytetracycline distributes to milk at similar concentrations to serum.

*Gm = ve–gram positive spectrum; Gm–ve–gram negative spectrum; T– trimethoprim, S–sulphonamide product, 1Milk pH approximately 6.6 [§]limited Gram–negative spectrum *Distribution data derived from (Ziv, 1980a) (Kaartinen et al., 1999)*

breakpoints (Constable and Morin, 2003). The minimal inhibitory concentration (MIC) breakpoints currently recommended for most antimicrobials are based on achievable serum and interstitial fluid concentrations in humans after oral or intravenous antimicrobial administration. The relevance of these breakpoints to milk concentrations in lactating dairy cows after intramammary or parenteral therapy is questionable (Constable and Morin, 2003). There are no antimicrobial MIC breakpoints established for any of the intramammary products available in Australia that have been established based on antimicrobial concentration achieved in the bovine mammary gland.

Extended therapy

Lactating cow therapies are designed to provide a short duration of therapeutic antimicrobial drug concentration and subsequently a short milk withholding period. Inadequate duration of therapy has been proposed as a potential cause of treatment failure (Milne et al., 2005). The aim of extended therapy is to prolong the duration of effective antimicrobial concentration in the udder. The risk of iatrogenic infection is an important consideration prior to implementing extended therapy.

A number of studies have been conducted evaluating the effect of increasing the duration of therapy on clinical outcomes for cows with chronic subclinical or recurrent clinical intramammary infections caused by *Staphylococcus aureus*, *Streptococcus uberis* and coliforms (Milne et al., 2005, Swinkels et al., 2013a). These trials have generally achieved higher cure rates than standard treatment regimens. However, the results have differed according to the causative pathogen and the magnitude of the improvement is sometimes limited (Swinkels et al., 2013b). Most of the research in this area has been conducted using intramammary formulations not available in Australia. On farm it is difficult to evaluate efficacy of mastitis treatments. Inflammation is often self-limiting after 4 to 6 days and is not always predictive of the presence of active intramammary infection or the need for additional therapies (Oliveira and Ruegg, 2014). Risk or outcome based guidelines for logical application of extended therapy have not been developed, thus recommendations for when to change or extend therapy are anecdotal (Oliveira and Ruegg, 2014). The indiscriminate use of extended mastitis treatment hoping for a better cure should be discouraged and is not economically logical or consistent with prudent antimicrobial use. No advantage was observed when all cases were enrolled prompting the recommendation that extended therapy not be used on an ad hoc basis but rather be targeted according to ICC, clinical mastitis history and bacterial culture results.

The currently available research suggests that approximately 5 to 10 cows would need to undergo extended therapy (as compared to standard therapy) to achieve one additional cure (Swinkels et al., 2013b). Extended therapy reflects an extra-label drug use requiring additional milk withholding. In the absence of with-holding data for this treatment regime, a common approach of practitioners is to double the label WHP. A further step to reduce risk of an antimicrobial violation is to test the milk for antimicrobials; the result needs to be interpreted with care as there are no registered individual antimicrobial cow tests, only tests registered for bulk milk.

Ancillary therapy for mastitis

Treatment success may be improved via administration of ancillary therapies including non-steroidal anti-inflammatories, oxytocin and fluid therapy. The application of ancillary therapies is based on the severity of mastitis. Of the ancillary therapies non-steroidal therapy has shown the most compelling evidence of benefit. Controlled studies have failed to demonstrate benefit with oxytocin. Fluid therapy is recommended for treatment of severe, toxic mastitis.

Treatment of subclinical mastitis

A somatic cell count in composite milk samples greater than 200,000 cells/mL (in the US, Europe and China) or 250,000 cells/ml (in Australia) is commonly used to indicate that one or more quarters are infected (Bradley and Green, 2005). The current individual somatic cell count threshold used in Australia is currently under review with the Australian Milk Quality Steering Group. The aim of treating subclinical mastitis during lactation, is to improve milk quality and rarely to increase milk production. The economic benefit may be equivocal due to the costs of treatment, milk discard, and low treatment efficacy (Sandgren et al., 2008). However, in herds with a high proportion of contagious pathogens, identification and treatment of subclinically infected cows that are likely to respond to therapy, may be advisable to reduce the risk of disease transmission (Royster and Wagner, 2015).

Available research indicates that treatment success is improved by extending the period of effective antimicrobial concentration. Patient selection criteria should take into account historic mastitis records and likely pathogen involvement. Higher cure rates are expected following extended treatment of younger animals, in early lactation (<100 days in milk (DIM)) with a recent infection (e.g. no history of chronic ICC) by a non-invasive pathogen. Despite extended treatment, lower cure rates should be expected when treating non-justifiable cases, for example old cows, chronic cases (e.g. with palpable changes in the quarter), repeat clinical cases, late lactation cows (>100 DIM) and cases that yield no growth on culture) (Swinkels et al., 2005, Bexiga et al., 2011).

Most of the registered therapeutic products are not labelled for treatment of subclinical mastitis, subsequently their use reflects extra label drug use. When considering the cost benefit, research indicates that approximately between 8–10 cows need to be treated to achieve one additional cure to the normal rate of self-cure. It has also been determined that ‘cured’ quarters are highly susceptible to re-infection (Salat et al., 2008).

Defining treatment success

The success of mastitis treatment can be measured by clinical resolution and microbial cure. Clinical resolution is the standard measure on farm. It is based on observation of the disappearance of clinical signs indicative of mastitis (Tables 1 and 2). However, it is not a definitive indication of pathogen elimination as the infection may regress to subclinical mastitis. Conversely, return to 'normal milk' and regression of signs of clinical mastitis of the udder is usually expected in 2–6 days. Premature estimation of clinical cure may result in falsely judging a case as a failure. Other parameters used to evaluate treatment success include cow side tests (e.g. the rapid mastitis test RMT), somatic cell count, recurrence of clinical mastitis and milk production (Hoe and Ruegg, 2005). Using RMT or ICC to define success is confounded by the lag between microbial cure and resolution of inflammation within the gland which may be incorrectly interpreted as treatment failure.

The time to resolution of inflammatory response is influenced by the inciting pathogen: for example in one study 42% of mastitic quarters returned to a 'trace' RMT score by 36 days of microbial cure with a range of 29% for Klebsiella spp to 78% for *E. coli*. (Roberson et al., 2004). When evaluating farm treatment protocols, it is necessary to have a pre-defined definition of success/failure to provide a consistent outcome assessment; for example, treatment success could be defined as the disappearance of the clinical signs of mastitis 1 day after the last treatments. In the absence of culture data it is difficult to distinguish treatment failure from a new infection. The time interval from treatment to re-emergence of clinical signs is utilised as a proxy for distinguishing treatment failure from new infection. The cut off is somewhat arbitrary with periods of time ranging from 7 to 14 days commonly applied (Roberson et al., 2004).

Development of clinical mastitis treatment protocols

Currently in Australia, mastitis treatment is usually initiated before the identity and susceptibility of the mastitis pathogen are known. Hence, the choice of antimicrobial for use in treatment is empirical, being based on factors such as previous culture results (i.e. herd profile), experience and treatment history. A description of the information required by a practitioner to develop a protocol will first be outlined before considering the development of treatment protocols with more rapid identification of the causative pathogen.

To develop treatment protocols, information regarding the mastitis pathogens isolated from the farm, previous treatment history and a sound knowledge of the farm's management are required (Table 6).

The concept of a mastitis treatment decision tree is to create a simple protocol that can be followed by staff to facilitate implementation of consistent and appropriate management interventions.

Unfortunately there is not a one size fits all for all occasions. For example, if a farm has significant problems with implementing aseptic intramammary treatment, it may be prudent to temporarily discontinue intramammary therapy and utilise parenteral therapy pending staff training.

Important concepts to be covered within the decision tree include whether to treat with antimicrobials, to dry the quarter or the cow off, or to cull the cow. The decision trees enables a process to classify the clinical severity.

An example of a generic treatment protocol, is presented in Figure 1. On farms with good records, attention to detail, and well trained staff, increasing complexity can be added to the protocol to include individual cow factors that may alter the treatment decision such as milk production, previous individual cell count history, stage of lactation, pregnancy status and any pathology of the udder.

Table 6 Information required in developing mastitis treatment protocols

Mastitis pathogens	Aim to attain 10 meaningful culture results from clinical mastitis cows per 100 cows milking per year
	Previously cultured mastitis pathogens
	Bulk tank and pooled hospital group PCRs (To screen for contagious pathogens *Streptococcus agalactiae* and *Mycoplasma bovis*)
Treatment history	Mastitis treatment records matched with calving dates.
	Ability to refer to individual cow somatic cell count history
Management	Ability of the milking staff to determine differences in the clinical signs of mild, moderate and severe mastitis
	Motivation of the manager to alter treatments depending on the age of the cow, previous ICC history or days in milk
	Ability and reliability of farm staff to administer treatments hygienically
	Ability to keep good records and have them readily accessible by milk harvesters to alter treatment options depending on the clinical presentation
	Preferred route of administration (Some managers may want to only use injectable antibiotics for safety reasons)
	Cost of products, milk and labour
	Preferred timing of administration (once daily, twice daily)

PCR – polymerase chain reaction; ICC – Individual somatic cell count

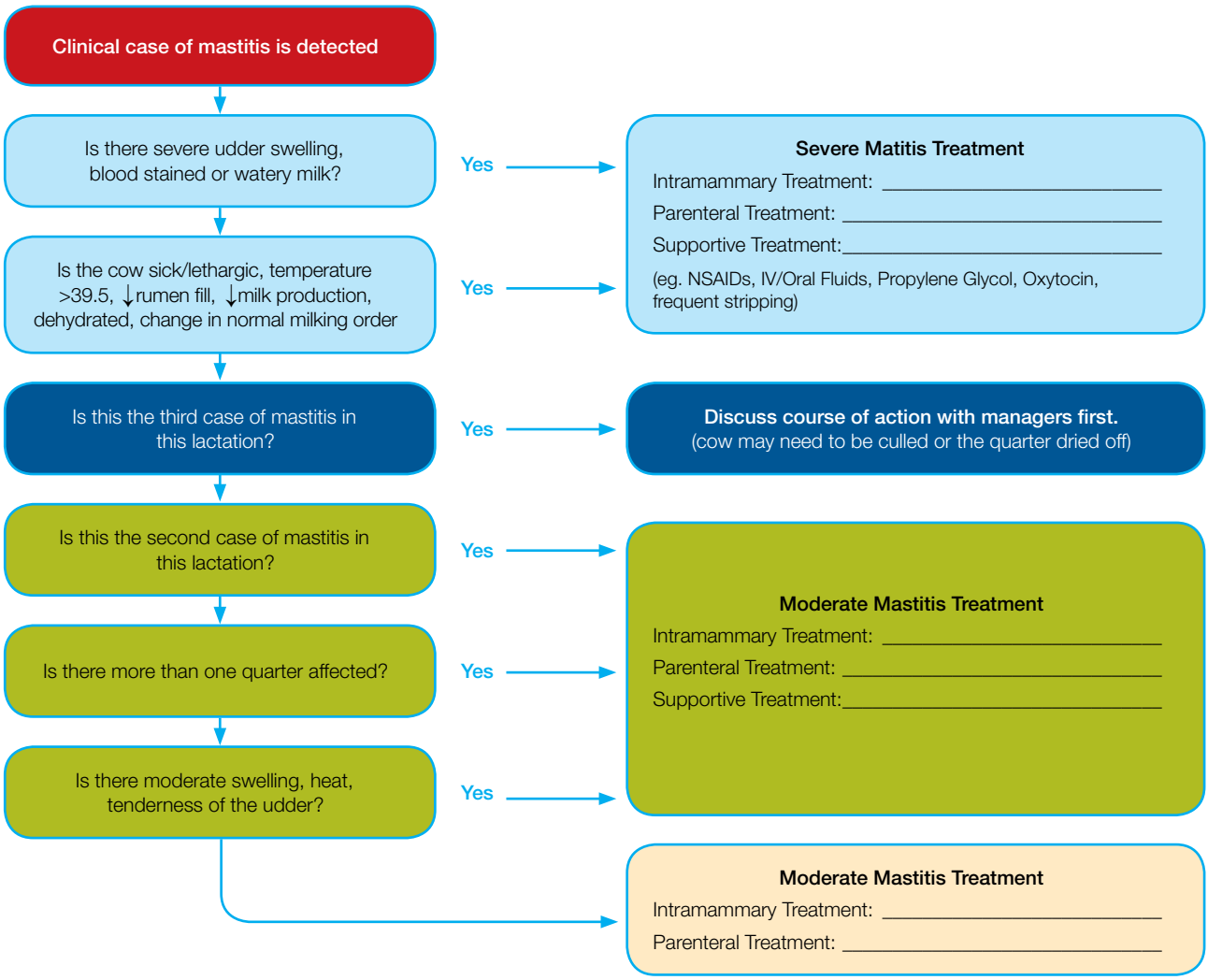

Figure 1 Generic mastitis treatment decision tree

Utilising rapid culture systems

The use of rapid culture systems to facilitate mastitis treatment decisions is an emerging global trend (Royster et al., 2014, Viora et al., 2014). Rapid culture systems provide useful information for guiding therapeutic decisions but are not always available. The more recent development of selective plating media that can distinguish the major pathogen groups can facilitate this process. It has been reported that treatment of mild or moderate clinical mastitis cases can be postponed for one day with minimal adverse effects, while producers wait for culture results (Wagner et al., 2007). The use of rapid culture systems to guide the strategic treatment of clinical mastitis in the United States has been found to reduce antimicrobial use by half without significant differences in days to clinical cure, bacteriological cure risk, new intramammary infection risk, or treatment failure risk within 21 d after the clinical mastitis event (Lago et al., 2011). The reduction in antimicrobial use reported from the US results from not treating cows from which there is no growth (typically 20–30 % of cases) or from which coliforms are recovered (~20 % in the United States and approximately 10% in Australia) (Charman et al., 2012).

Treatment and management considerations relevant to pathogen profile include:

1. Contagious versus Environmental
2. Contagious
   a. *Staph aureus* (difficult to treat effectively – age of cow, DIM, mastitis and SCC history and number of quarters affected should be considered prior to instigating therapy. A combination of intramammary and parenteral therapy may be beneficial)
   b. *Streptococcus agalactiae* (potential for rapid spread – isolation from a clinical case should prompt further investigation to define the herd's infection status)
   c. *Mycoplasma spp*. (no effective treatment, herd level risk associated with retaining clinically affected cows in the 'hospital' group. Isolation should prompt herd level management that includes risk management strategies to prevent disease transmission to other cows and young stock)
3. Environmental
   a. *Streptococcus uberis* (first case generally treated with standard intramammary treatment protocol, recurrent case may benefit from extended therapy)
   b. Other environmental streptococci (standard intramammary therapy likely to be successful)
   c. CNS (standard intramammary-therapy likely to be successful)

d. Coliform (mild cases likely to resolve without antimicrobial therapy), high colony counts generally associated with higher risk of persistence. Intramammary treatment with gram positive targeted therapies ineffective.

4. No growth – No need to treat with antimicrobials
5. Miscellaneous – May explain poor response to therapy for example *Pseudomonas spp, Bacillus spp, Serratia spp, Nocardia spp.* Identification of an 'unusual' colony type across multiple cows warrants further investigation and typing as it may lead to identification of an unusual source of environmental contamination, for example pseudomonas in parlour water supply.

Monitoring treatment outcomes

The Mastitis Focus Report

The Countdown Mastitis Focus Report (MFR) details a summary of mastitis events (both clinical and sub-clinical events) to assist advisers and dairy farmers to monitor mastitis in a particular herd. To allow benchmarking, a consistent methodology in analysing clinical mastitis events was developed. In the output below, calving mastitis is defined as clinical mastitis that requires treatment in the first 14 days after calving, 5% or less is achievable on Australian dairy farms. Clinical mastitis during lactation is defined as mastitis requiring treatment from 15 days after calving, reported on a monthly basis across the herd.

Treatment failure

The Mastitis Focus Report defines treatment failure (cases with extended therapy) as a cow with mastitis that receives more than 3 doses of a mastitis treatment within 10 days, see Figure 3. On some farms, the number of treatment failures recorded may be very high when the clinical mastitis treatment protocol is to administer more than 3 mastitis treatments as a routine practice. Subsequently, the MFR, like any test result, needs interpretation depending on individual farm management practices and how mastitis is recorded on farm dairy herd management software.

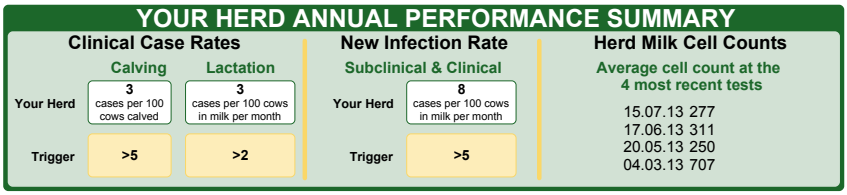

Figure 2 Mastitis Focus Report output

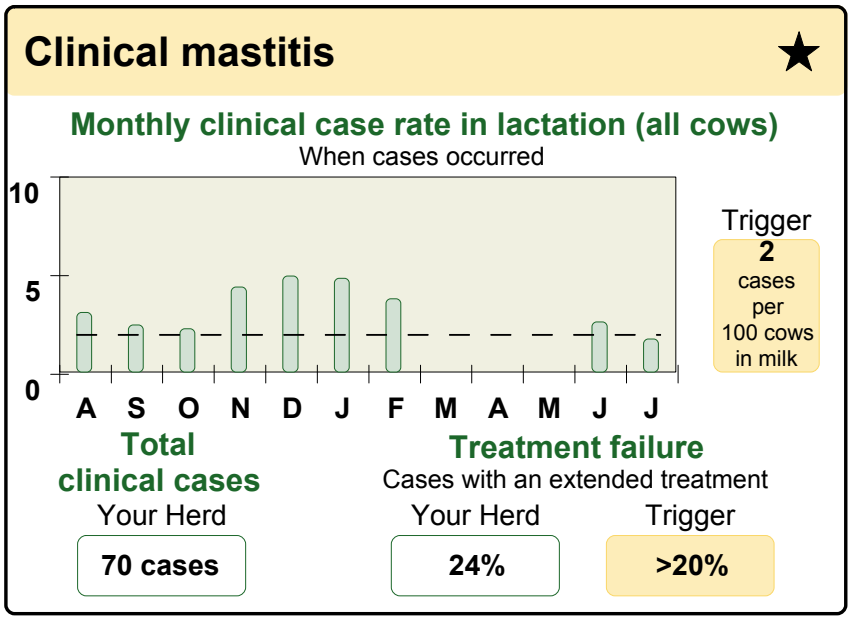

Figure 3 Mastitis Focus Report – Clinical Mastitis Management

New clinical case

The MFR records a new clinical case anytime a cow is treated more than 10 days after the last treatment. Depending on the data available, further in-depth analysis of clinical mastitis is possible. In Australian dairy farms, where routine cultures are rarely performed, or the affected quarter recorded, analysis is often done on a cow level (Rod Dyson, 2015, pers comm). Although an imperfect measure, a benchmark of 20% for repeat cases (the proportion of cows having a second ('new') clinical mastitis case within lactation) exists: when this figure is breached there is a need for investigation of apparent treatment failure.

Culling of mastitis cows

Mastitis is one of the top three reasons for culling dairy cows, alongside lameness and infertility (Esslemont and Kossaibati, 1997). Countdown guidelines recommend culling a cow if she has had three cases of clinical mastitis in the current lactation (Brightling et al, 1998). In data analysis completed by Livestock Improvement Corporation, NZ, the reported cure rates were 75% for first cases, 45% for second cases and 12% for cows treated a third time. Despite the significant cost to the producer of culling cows, it is an essential part of milk quality preservation and needs to be considered when developing mastitis treatment protocols. Evaluation of this management strategy is made easy when data is recorded with herd improvement records and can be accessed through the Mastitis Focus Report (Figure 4). Depending on the skill of the dairy manager and whether the causative pathogen is known, more specific detail regarding culling, can be identified in a treatment protocol. For example, an aged cow, having two quarters affected with clinical mastitis, that has had *Staph aureus* isolated should be culled after her second case of clinical mastitis. Culling should also be advised for

cows with prolonged history of high ICC, particularly continued high ICC after the use of antimicrobial dry cow therapy. Finally, culling should be also advised for cows that have chronic, palpable changes in the udder. An alternative to culling is permanently drying off the affected quarter.

Evaluating the efficacy of antimicrobial products

Evaluating efficacy of specific antimicrobial products is often very difficult due to a lack of culture information and accurate and complete clinical mastitis records. Excellent record keeping is important for meaningful comparative evaluation of therapeutic products. The robustness of a prospective investigation will be improved by establishing clear case definitions, randomisation of treatment allocation and clear definition of outcome assessments prior to conducting the on farm trial. Pathogen profile may lead to significant differences in treatment outcomes. Hence, defining the pathogen profile on the farm is useful for interpreting the relevance of trial results to other farming operations.

Approach to investigating clinical mastitis treatment failure

Despite an appropriate choice of antimicrobial, treatment of mastitis may be unsuccessful. A treatment failure rate greater than 20% (see previous section, 'monitoring treatment outcomes') or greater than 20% of cows having a second clinical mastitis case in the one lactation may be two reasons to instigate an investigation of mastitis treatment failure. It is not uncommon for dairy farmers to seek a 'stronger' intramammary preparation following treatment failure at an individual or herd level. This request should trigger a series of questions as it is a likely indicator of underlying mastitis management problems.

The practitioner needs to evaluate if the perceived treatment failure is due to herd management problems, misdiagnosis or ineffective therapy. A high treatment rate may result from treatment of sub-clinical mastitis (diagnosed from either herd test generated ICC or from a positive rapid mastitis result) or treatment of cows with teat canal infections.

Culling to control mastitis ★★★

	Your Herd	Trigger
Cows prone to clinical mastitis Cows in herd with 3 or more clinical cases in a lactation	5 cows	Any Cows
Cows infected in multiple lactations Cows still infected after 2 consecutive prior lactations despite intervening DCT	1 cows	Any Cows

Figure 4 Mastitis Focus Report output 'Culling to control mastitis'

Next, the practitioner determines the history of treated cows and the incidence of herd mastitis. In the face of high clinical mastitis rates, farmers may perceive new infections as treatment failures. A true high clinical mastitis rate (>2% per month during lactation) indicates the need for a complete Countdown Mastitis Investigation. Treatment success is reduced with delayed diagnosis. Herds that have high rates of involuntary culling due to reproductive failure and other non-mastitis related events may have higher rates of treatment failure reflecting retention of cows with chronic disease. Collecting and evaluating clinical mastitis records is required. This step is often difficult on farms with poor record keeping.

Completing a Milk-PCR of bulk tank and pooled hospital milk is important to identify the involvement of Mycoplasma: that may be causing poor response to treatment. If possible, culturing at least 20 clinical mastitis cows (some from cows that have had repeat clinical mastitis events at least 14 days after the last antibiotic treatment) is necessary in herds over 200 cows to gain a representative sample of the pathogens causing the mastitis (Brightling et al, 1998).

A milking-time visit is useful to assess clinical mastitis treatment procedures. The standard of hygiene, tube insertion technique, and general attention to detail will influence the risk of cross-contamination and introduction of bacteria during the treatment procedure. Contamination may also be associated with poor storage and handling of intramammary products. Clarification of data recording procedures at this time is also useful to verify the integrity of the available records.

In regards to drug-related treatment failure there is no information available in Australia that compares the efficacy of the different registered mastitis therapies. A summary of steps involved in investigating a perceived mastitis treatment failure is outlined in Figure 5.

Conclusion

Despite well established and successful control strategies to reduce mastitis on dairy farms, clinical mastitis during lactation is common. Clinical mastitis presents significant diagnostic, therapeutic and management challenges to both the prescribing veterinarian and the farm manager. With an increasing need and responsibility for prudent antimicrobial use, it is valuable to reflect on the indications and limitations of products for use in clinical mastitis. Veterinarians need to take an active role in improving treatment outcomes by improved prescribing, improving methods of product administration and monitoring outcomes. Farmer requests for better mastitis treatments should be viewed as an opportunity to assess managers' diagnosis, case selection for treatment, methods of treatments, culling policies and a trigger for more milk cultures to better understand herd udder health problems. New rapid and selective culture methods exist which will drive improved knowledge of the current spread of mastitis pathogens and judicious use of antimicrobials in the future.

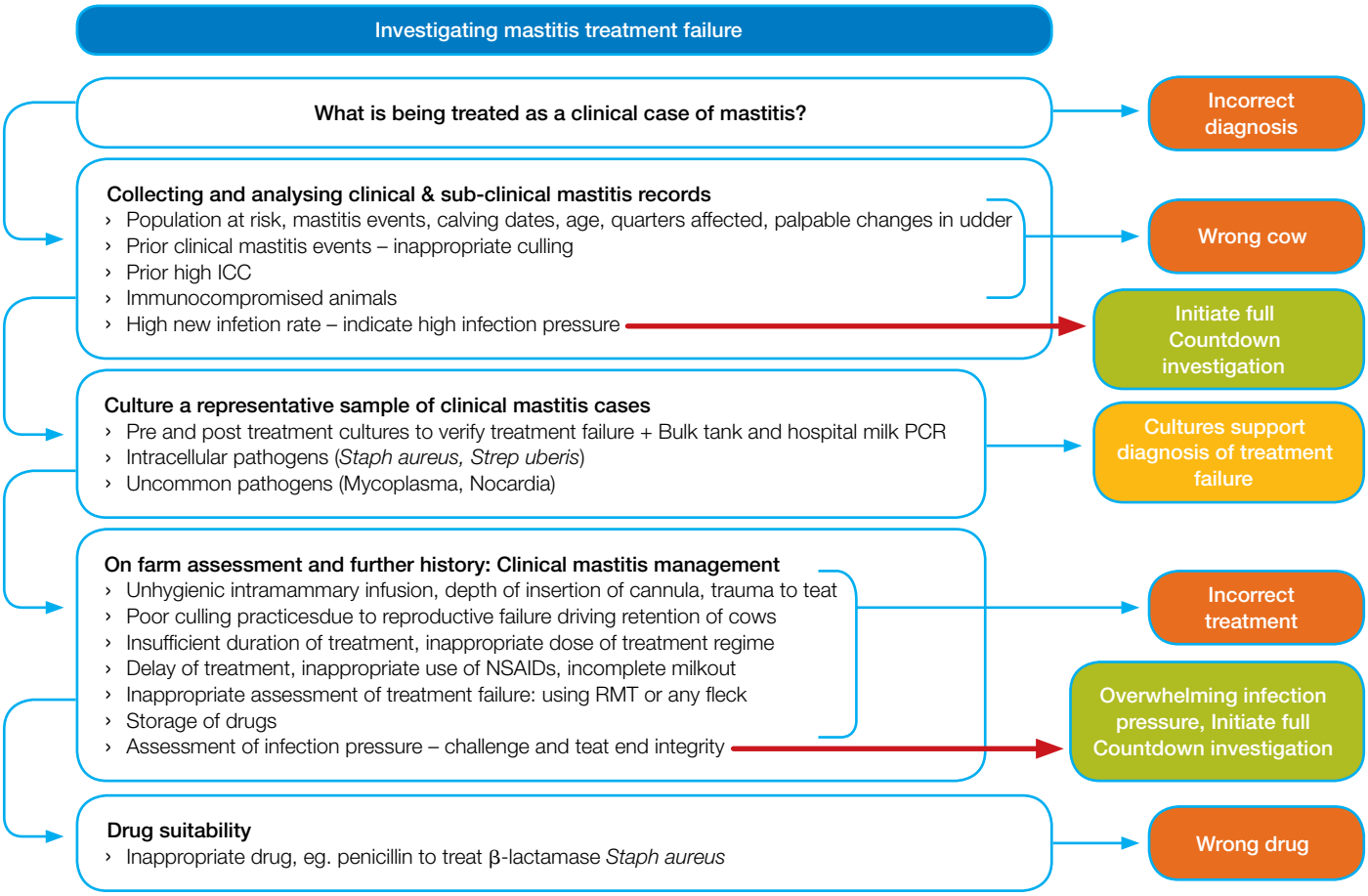

Figure 5 A summary of steps for investigating mastitis treatment failure

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