

4

Rapidly find, treat and record clinical cases in fresh cows

Clinical cases of mastitis are costly and severely disrupt the flow of milking. Cases that are missed can markedly increase the Bulk Milk Cell Count (BMCC) because they produce very high numbers of somatic cells in their milk.

The number of clinical cases detected within a herd is a function of the intensity of observation, and advisers therefore need to be aware of how different operators detect mastitis. People who forestrip are likely to identify many more cases than those relying solely on observing a swollen quarter.

Early detection and treatment of all quarters with clinical mastitis reduces the risk of severe and intractable cases developing, and reduces the likelihood of infection being passed to other cows.

The Countdown Downunder warning level of “five cases per 100 cows in the first month of lactation” is based on diagnosis of mastitis following observations of heat, swelling, pain, abnormal walking, poor milkout, or intense observation following discovery of clots on the milk filter. This is typical of dairies in Australia where there is minimal pre-milking teat handling by the operator. In contrast, farmers who routinely forestrip will commonly identify cows with abnormalities in the first squirt of milk, followed by milk that is visibly normal. These cows should not be counted towards the warning level or treated as clinical cases.

The cost of a clinical case of mastitis

The likely cost of each clinical case during early lactation is estimated to average \$146 (see table over page). This assumes a milk price of 25 cents per litre, a labour cost of \$20 per hour, and a reduction in milk yield of 3% (Gunn et al 1998). The risk factors for mortality, culling and vat contamination are estimates based on general experience.

Countdown Downunder considers a cow to have clinical mastitis and require treatment when there is observable abnormality of the milk (wateriness or clots) that persists for more than three squirts of milk.

[Technote 10 details how to rapidly detect and treat clinical cases in lactating cows.](#)

Calculating the cost of a clinical case in the first month of lactation

Cost of treatment		\$
Intramammary antibiotics	=	10
Vet visit and drugs @ \$80 for 1 in 15 cases	=	5
Extra time in the shed 10 min/milking for 6 milkings @ \$20/hr	=	20
Discarded milk		
7 days of 20 L/day @ 25 cents/L	=	35
Decreased yield for remainder of lactation		
For cases in early lactation (calving to 30 days) estimated 3% reduction in 300 day yield of 5,000 L is 170 L @ 25 cents	=	43
Risk of mortality		
1 in 200 cases, cow value \$800	=	4
Risk of culling		
7 in 100 cases, replacement cost \$400	=	28
Risk of contamination of vat		
2,000 L in 1 in 1,000 cases	=	1
Total	=	\$146

This cost would be higher for mastitis cases occurring in mid-lactation, as Gunn et al (1998) estimated that the 300-day yield of pasture-fed cows with clinical mastitis was:

- 3.4% lower than cows without mastitis if it occurred in early lactation (calving to 30 days);
- 7.7% lower if the mastitis in mid (peak) lactation (31 to 100 days post calving); and
- 2.0% lower for cases occurring in late lactation (101 or more days post calving).

Using these figures, clinical mastitis in a herd of 150 cows is estimated to cost about \$1,800 in the first 100 days of lactation. This is based on quarter infection rates observed in Gippsland of 0.072 (72 clinically affected quarters per 1,000 cows) in the first 30 days after calving and 0.007 (seven clinically affected quarters per 1,000 cows) between days 31 and 100 (Gunn et al 1999).

4.1 Look for swollen quarters and check for heat and pain in all freshly calved cows.

The signs and techniques used to detect clinical mastitis are the same throughout lactation.

All freshly calved cows should be visually inspected for swollen quarters during the first two weeks after calving. Cows at high risk of mastitis should continue to be closely examined as the lactation progresses. These include cows that:

- have not milked out;
- have had a clinical episode of mastitis within the last month; or
- have recently had high Individual Cow Cell Counts (ICCC).

Cows that have swollen or painful quarters may appear lame – and this may be the first indication of a mastitis problem.

People who put cups on and take cups off should be inspecting every cow for swollen quarters at every milking. When viewed from behind, the two hindquarters should be examined for size and symmetry. In cows that have just calved, it can be difficult to pick swollen quarters and the best policy is to compare the suspect quarter with other quarters. In thorough inspections, forequarters can be viewed by lifting the hindquarters.

Freshly calved cows with suspect quarters by gross observation should have their udders palpated and foremilk checked.

Suspect udders should be palpated when they are empty after milking. The teat is palpated with the finger tips by gently rolling it between the thumb and first two fingers, and glandular tissue is palpated superficially and deeply with the flat of the hand and fingers (Donovan et al 1992). The udder tissue of acute cases may be hot, swollen or painful. In acute or chronic clinical mastitis cases with less obvious changes, a thorough examination is required to assess the consistency of udder tissue. Chronic changes usually manifest as fibrosis, which can be felt as firmness that is local (from pea to fist size) or diffuse (giving the quarter a firmer feel than its opposite number and usually a more nodular surface). Long-standing infections can ultimately result in atrophy (shrinking) of the mammary tissue as it becomes non-functional.

Foremilk inspections are used to detect wateriness of the milk, a few clots or flecks, or more obvious abnormalities such as flakes, discolourations and bloodstains. Milking staff may see 'strings' of mastitis material hanging from teat-ends. These are viscous debris (inflammatory products) that are expressed during milking and may therefore be more obvious to the 'cups-off' operator.

Confidence – High

Heat, pain, and swelling are classic signs of tissue inflammation.

Research priority – Low

[Technote 5.2 describes foremilk stripping.](#)

Confidence – Moderate

Forestripping is the single most effective way to detect clinicals.

4.2 Check milk from all quarters of freshly calved cows every day while they are in the colostrum phase (first 8 milkings, or 10 milkings for induced cows).

Technote 5.2 discusses foremilk stripping of cows during their colostrum phase.

4.3 Consider collecting milk samples for culture to identify the bacteria involved.

The general principles of collecting milk samples for culture discussed in this section are applicable to diagnosis of both clinical and subclinical mastitis, and also as part of investigation of problems in herds.

Milk cultures are recommended whenever a herd problem emerges, namely when there are more clinical cases than is acceptable or when cell counts are rising. Virtually all mastitis is caused by bacterial infection. Milk cultures indicate the type of bacteria in the herd (e.g. *Staph aureus*, *Strep agalactiae* or *Strep uberis*) so that appropriate management strategies can be developed. A number of milk samples are required to give a representative picture of what is happening in the herd (see below).

Culture costs vary from approximately \$6 to \$20 per sample depending on the number submitted at the same time, transport costs, etc.

Cultures of milk samples from clinical cases

It is not possible to determine the organisms responsible for a case of mastitis without culturing a clean milk sample.

Cultures from cases of clinical mastitis can provide useful information on:

- Pathogen identification. This allows veterinarians and other advisers to use their knowledge of the epidemiology of the organisms to suggest possible sources of the infection and useful control measures for the herd.
- Antibiotic sensitivity testing of the isolated organisms. These tests are only considered as a guide to the likely treatment efficacy in live animals because bacterial kill rates on sterile plates in a laboratory do not necessarily translate to curative treatment in inflamed udder tissue.

It is a good insurance policy to encourage farmers to take samples from all quarters with clinical mastitis – although they won't necessarily be submitted for culture. These should be collected before treatment (because the presence of antibiotics in samples make it difficult to grow bacteria) and stored frozen. The samples can be submitted to a laboratory if:

- a cow fails to respond to treatment;
- there is concern about the type of bacteria causing the mastitis; or
- there are a higher number of mastitis cases than expected (e.g. more than three clinical cases in the past 50 calvings, more than five clinical cases per 100 cows in the first month of lactation, or more than two cases per 100 cows per month in subsequent months of lactation).

Sampling strategy

Aseptic technique must be used to collect milk samples from the type of cases causing concern, prior to administration of any treatment. For example, if the concern is an outbreak of clinical mastitis in freshly calved cows, the samples should be taken from these clinical cases. Bacteria isolated from high cell count cows in the herd at the same time may not necessarily be relevant to the clinical mastitis outbreak. Samples from cases that have recurred or failed to cure may also be unrepresentative of the overall problem.

Confidence – High

Culture is the definitive method of identifying mastitis pathogens in herds – its cost is minor when compared to potential gains from incorporating the information into mastitis control programs.

Research priority – Low

In practice, it is only currently possible to determine the bacteria causing mastitis by doing a milk culture.

Alternative methods for identifying bacteria used overseas and in research are described in the 'Bacterial identification' FAQ sheet.

The 'Vat milk tests' FAQ sheet describes bulk tank milk cultures.

- Advisers are encouraged to copy and distribute Fact Sheet A of the *Countdown Downunder Farm Guidelines for Mastitis Control* to clients.
- It is essential for advisers to ensure the milkers' sampling technique is satisfactory – a physical demonstration is often very helpful.
- On most farms it is the milking staff who collect milk samples from clinical cases.

The number of milk samples to be examined depends on the number of cases of mastitis occurring and the reason for the sampling. For most herd problems preferably 10 samples (and a minimum of five) are needed to get a reasonably reliable indication of the mastitis causing organisms in the herd. For large herds (more than 200 cows), it is preferable to have 20 samples. Between 10-40% of samples may return a result of 'no growth' (see below).

If a herd problem appears to recur some time later (certainly if more than 12 months later), it is worth collecting another set of samples because herd profiles can and do change.

Recurring individual cases of clinical mastitis may have been 'superinfected' with other bacteria such as *Nocardia* species or *Pseudomonas* introduced during the previous treatment infusion. This will only be detected if subsequent milk samples are cultured.

Sample collection

The main problems associated with milk culturing occur when samples are collected and transported. If correct procedures are not followed, milk samples can become contaminated with bacteria from water, mud or faeces, or from skin (milkers' hands or cows). These environmental bacteria can multiply in the milk sample and confuse the test result. Sterile sample collection and delivery of cool samples to a laboratory within 24 hours, or immediately freezing the samples after collection and then later submission, avoids these problems.

Fact Sheet A in the *Countdown Downunder Farm Guidelines for Mastitis Control* gives a detailed description of how to aseptically collect milk samples.

A milk sample should be considered contaminated if three or more colony types are isolated from a quarter. The organism causing mastitis cannot be identified in contaminated samples. Contamination is often a result of poor sample collection technique, a dirty environment or dirty animals. Teat injuries, wet teats or udders, and hands contaminated with milk or water are common causes of contaminated milk samples. Where possible, advisers should not arrange to take milk samples on wet days or too soon after wet weather.

Storage and handling of milk samples

Most bacteria that cause mastitis survive refrigeration for several days or freezing for several weeks. *Nocardia* species are an exception to this general rule, as storage of samples for only a few hours or freezing can reduce the likelihood of isolating these organisms. The survival of *Staph aureus*, *Strep agalactiae*, *Strep dysgalactiae* and *Strep uberis* was not impaired in milk samples that were stored in a commercial freezer at -20°C for up to 16 weeks (Schukken et al 1989). Other studies have found a variable effect on streptococci, especially *Strep dysgalactiae* (Luedecke et al 1972, Murdough et al 1996).

The survival of *Escherichia coli* and *Arcanobacterium pyogenes* can also decrease during freezing, with recovery rates for both pathogens decreasing by about 20% in samples frozen for four weeks (Schukken et al 1989).

In a survey of the causes of clinical mastitis in East Gippsland, (Alison Gunn personal communication) there was a significant relationship between the proportion of samples from which no growth was obtained and the number

of days of storage (mostly in domestic freezers). She recommended that farm operators should be encouraged to submit frozen milk samples for culture within a month of collection.

Freezing may increase the detection of coagulase negative staphylococci (Schukken et al 1989) and possibly *Staph aureus*. The proposed mechanism for this increase is the release of intracellular bacteria after the destruction of leucocytes during the freeze-thaw process. Samples found to have negative growth when cultured fresh may become positive after freezing.

Inappropriate storage and handling on-farm will significantly reduce the chance of obtaining a meaningful culture result. It is not unusual to see samples sitting in the dairy for hours without refrigeration or on the dashboard of the car on Friday afternoon on the way to the veterinary clinic for submission to the laboratory. It is essential for advisers to ensure the farm procedure for storing and handling samples is satisfactory – a physical demonstration is often very helpful.

Laboratory techniques

Techniques used in laboratories must be appropriate to achieve reliable isolation and identification of pathogens. This involves consideration of:

- Methods of sample preparation, including warming and mixing especially after freezing.
- Possible pre-incubation in growth media.
- The choice of culture media.
- The methods of inoculating plates to ensure suitable combinations of inoculum volume and surface area are used. Different combinations may be optimal for different circumstances. For example, larger loop sizes holding 25 μ L or 50 μ L would be appropriate for milk samples from clinical cases containing less than 200 bacteria/mL, as the standard 10 μ L loop is likely to result in a culture with two or less colonies.
- Incubation temperature and times.
- Procedures for follow-up of samples with 'no growth', including tests for inhibitory substances, and examinations for other organisms.
- Procedures and tests for identifying pathogens from the primary culture.
- Procedures for antibiotic sensitivity testing.

At present in Australia there appear to be significant differences between laboratories in techniques for bacterial isolation, characterisation and antibiotic resistance testing, and there is no standard recording protocol. In addition to major laboratories, the number of small, local laboratories is increasing and many of these are not using established quality assurance procedures. One objective of Countdown Downunder is to establish uniform laboratory testing and reporting procedures and to facilitate agreement by all laboratories to use them.

A bacteriology guide for bovine mastitis is published in the Australian Standard Diagnostic Techniques for Animal Diseases (Claxton and Ryan 1993). Although this requires some updating, it provides a good start. In 1999, the National Mastitis Council in the United States released a revised edition of its 'Laboratory Handbook on Bovine Mastitis'. The handbook details microbiological diagnostic procedures that differentiate mastitis pathogens (National Mastitis Council 1999). Details can be obtained at its website at www.nmconline.org.

Reasons for milk samples yielding 'no growth' after culture

Clinical cases of mastitis from which no growth is obtained are both common and frustrating. Many published surveys of clinical mastitis report 10-40% of samples with no pathogen isolated. Probably the most common reason for 'no growth' is a decline in the number of bacteria in the sample, by the time it reaches the laboratory, due to poor storage and handling. Other reasons include:

- By the time the milk sample is collected, the infection has been eliminated by host defence mechanisms. This is suggested particularly in the case of coliform infections. Zorah et al (1993) found that 51% of 'no growth' samples from clinical cases in Queensland were ELISA positive to *Escherichia coli* antigens.
- Bacteria are present in too low a concentration to be detected by the laboratory culture technique used. For example, the inoculum size used on culture plates may be inadequate.
- Antibiotic treatment of the quarter before sample collection has interfered with the ability to culture the infective organism. When submitting milk samples from cows that are not responding to treatment or are repeat cases, it should be noted on the laboratory submission form if they have received antibiotics within seven days of sampling.
- Contamination of the sample with disinfectant at the time of collection has interfered with the ability to culture the infective bacteria.
- The pathogen may not grow under normal culture conditions. For example, standard bacterial culture conditions are unsuitable for the detection of obligate anaerobes, mycoplasma and fungi.
- The clinical signs of mastitis are due to non-bacterial causes such as toxic substances.
- Isolated bacteria may not be reported because they are not considered to be major mastitis pathogens. For example, coagulase negative staphylococci are traditionally considered minor pathogens although they have been reported to cause clinical mastitis (Timms and Schultz 1987).

Antibiotic sensitivity tests show which drugs are not likely to be effective. Just because an antibiotic is effective at killing bacteria on agar plates in the laboratory does not mean it will have the same success in the cow.

Antibiotic susceptibility testing

The disc-diffusion antibiotic sensitivity test (Kirby-Bauer method) is most commonly used in veterinary laboratories. The disc-diffusion method involves inoculating an agar plate with a standard inoculum, adding discs containing standardised quantities of antibiotics, incubating for 18 hours and measuring the zones of inhibition. In disc-diffusion tests, isolates are reported as susceptible, intermediate or resistant to the antibiotics that were tested. Many of the discs in use were designed in human laboratories and some drugs listed on the antibiotic sensitivity report may not be registered for use in cattle.

The fact that an antibiotic is found to inhibit growth in the laboratory does not necessarily mean that it will be successful in curing infections from the udder. However, antibiotic sensitivity testing does give an indication of which drugs are NOT likely to be effective (Ziv 1997).

4.4 Select the antibiotic to be used – consult your veterinarian.

The goal of treatment is to cure the infection (bacteriological cure), return the affected mammary glands to normal milk production (clinical cure), and minimise pain and suffering of the cow. Ideally, the treatment period should be as short as possible and there must be no risk of antibiotic residues entering the milk vat.

Staphs or Streps cause more than 80% of clinical mastitis cases in Australia. Antibiotics are the basis of most treatment regimens and are administered by infusion into the affected quarter (intramammary route) or by intravenous, intramuscular or subcutaneous injection (parenteral or systemic routes).

Other support therapies such as oral or intravenous fluids and anti-inflammatories may be used in very severe cases. Frequent stripping out and use of oxytocin to aid milk let-down are important adjuncts. Farmers should always be encouraged to remove milk from mastitic quarters, despite the fact that antibiotics have been administered.

Most cases of clinical mastitis are treated without the benefit of bacteriological examination of the milk before treatment is commenced. The treatment selected is based on the severity of the mastitis, the history of the farm (including previous milk culture results and responses to treatment), and the field experience of the farmer and the prescribing veterinarian. In herd with clinical mastitis problems, milk samples should be submitted for culture to establish the farm profile of mastitis-causing organisms and develop appropriate treatment and control protocols.

Treatment should always be administered according to the directions given on the label and by the prescribing veterinarian. Recommended withholding periods must be observed for milk and meat.

Intramammary antibiotics

Intramammary treatment is practical and effective for cases where the inflammatory response does not occlude the teat canal or cistern.

Intramammary formulations should have the following qualities:

- The formulation should cause minimal irritation to the udder.
- The active ingredient must be effective against the bacteria.
- The active ingredient must distribute well through the mammary gland and persist in sufficient concentrations to effect a cure in localised areas of infection.
- The antibiotic should exhibit a low degree of binding to milk and udder proteins.
- The antibiotic should have a low degree of ionisation in the udder – in this form they are better retained in the udder.

In general, a smaller amount of active ingredient is required to achieve therapeutic concentrations when intramammary products are given compared to systemic doses. However, the inflammatory process in affected glands may impede distribution of antibiotics.

Confidence – High

Antibiotics are legally restricted drugs that must be prescribed by a veterinarian.

Research priority – Low

Although comparative efficacy data for many product formulations is not readily available, there are appropriate antibiotics for the common pathogens.

Only use products registered for administration to food producing animals. Drugs such as phenylbutazone and gentamicin MUST NOT be used.

Intramammary products available for use in lactating cows in Australia (October 1999)

Product name	Company	Treatment course in affected quarters	Active ingredients
Ampiclox LC	Jurox	One syringe every 12 hours for three treatments	Ampicillin 75 mg, Cloxacillin (sodium salt) 200 mg
Cepravin LC	Schering-Plough	One syringe every 12 hours in each of three successive milkings	Cefuroxamine sodium 250 mg
Lincocin Forte	Pharmacia & Upjohn	One syringe, repeat at intervals not less than 12 hours, do not administer more than three consecutive doses	Lincomycin hydrochloride 200 mg, Neomycin sulphate 200 mg
Mastalone Blue	Pfizer	One syringe, repeat daily for three days	Oxytetracycline 185 mg, Oleandomycin 100 mg, Neomycin 100 mg
Orbenin LC	Pfizer	One syringe every 48 hours for three syringes	Cloxacillin (benzathine salt) 200 mg
Special Formula 17900 Forte V	Pharmacia & Upjohn	One plaset per quarter per day repeated at 24-hour intervals for three days	Neomycin sulphate 150 mg, Novobiocin 100 mg, Dihydrostreptomycin 100 mg

Technote 4.10 shows the recommended withholding periods for meat and milk for these intramammary products.

A conflict exists between the duration of treatment (in many cases, longer treatment is associated with improved cure rates) and the desire to minimise the period over which milk must be withheld from the vat. All treatments have specified minimum treatment courses that should be adhered to.

Dry Cow Treatment preparations should never be used in lactating cows. Inadvertent use of Dry Cow Treatment would require milk to be discarded for extended periods of time.

Systemic antibiotics

Acute mastitis cases may benefit from both intramammary and systemic antibiotics. Peracute cases often require systemic antibiotics and anti-inflammatory preparations, and possibly intravenous fluids. The prognosis for peracute cases in cows with severe clinical signs (as indicated by body temperature, dehydration, etc) is poor regardless of treatment.

Systemic antibiotics have the advantage that drug distribution is not impeded by local inflammatory reactions in the udder. However, to be effective, systemic antibiotic treatments must be absorbed from the injection site and pass from the blood into the udder. Their major difficulty is penetration of the "blood-milk barrier".

Drugs move across the blood-milk barrier by passive diffusion of the non-ionised parts of the molecule according to the principle of osmosis. This barrier is penetrated by the non-ionized, lipid soluble, non-protein-bound drug fractions.

Weak acids (e.g. penicillin G) are almost completely ionised in blood and have poor tissue penetration. On the other hand, penethamate hydroiodide achieves concentrations in the milk that are 5-10 times higher than other penicillin salts due to its basic and lipophilic properties. This treatment results in high levels of penicillin in the udder because it is hydrolysed as it crosses into milk liberating active benzyl penicillin.

Milk-to-plasma ratios of some antibiotics used to treat mastitis (Anderson 1989)

Antibiotic	Milk-to-plasma ratio
Trimethoprim	3.7
Lincomycin or Erythromycin	3.0
Tylosin	2.0
Tetracycline	0.7
Streptomycin	0.5
Ampicillin	0.25
Sulphadiazine	0.21
Penicillin G	0.15

Allowing for antibiotic sensitivity patterns, antibiotics with high milk-to-plasma ratios are most suitable for systemic administration.

Recent clinical reports and studies suggest “that the combined systemic and intramammary antibiotic treatment may result in a slightly but significantly higher rate of bacteriological cure in the treatment of acute staphylococcal and streptococcal mastitis” (Ziv 1997).

Published cure rates of antibiotics

Very little information is available to assess the efficacy and cost-effectiveness of treatment of clinical mastitis during lactation, and to compare products in Australian conditions.

In a review of antibiotic treatment of clinical mastitis during lactation, Craven (1987) reported average cure rates for each antibiotic from scientific papers that stated the number of quarters treated and had rigorous bacteriological assessment. From this data (see table below), it was not possible to draw firm conclusions about the relative effectiveness of different products given the wide range of cure rates for similar antibiotics. There was a consistently greater bacteriological cure rate for treating *Strep agalactiae* infections than those due to *Staph aureus*, although cure rates are low for both organisms treated with neomycin.

Two recent clinical trials compared antibiotic products in clinical cases on commercial farms in Australia or New Zealand:

1. McDougall (1998) reported clinical cure rates of greater than 80% in 798 clinical quarters, predominantly due to *Strep uberis*, treated either with:
 - A course of high potency intramammary penicillin-dihydrostreptomycin – containing 1 g of procaine penicillin and 500 mg of dihydrostreptomycin. This product is not available in Australia.
 - Two subcutaneous injections of penethamate hydriodide in aqueous solution of 10,000,000 IU followed by 5,000,000 IU 24 hours later. (This dose and method of administration is not recommended by the manufacturer of penethamate hydriodide in Australia. On the Australian label, the manufacturer specifies daily intramuscular injection of 5,000,000 IU for cattle.)
2. Wraight (1998) compared cefuroxime (Ceprevin LC, Schering Plough) with cloxacillin (Orbenin LA, Pfizer Animal Health). Pathogenic bacteria were isolated from 61% of pre-treatment samples, including *Strep uberis* (32%), *Staph aureus* (18%) and *Escherichia coli* (7%). There was no significant difference between treatments with overall clinical cure rates of 82% in 416 cases and bacteriological cures rates of 70%.

Efficacy of treatment with different antibiotics (Craven 1987)

Antibiotic	Cure of <i>Staph aureus</i>			Cure of <i>Strep agalactiae</i>		
	Mean (%)	Range (%)	Reports (no.)	Mean (%)	Range (%)	Reports (no.)
Penicillin	32	0 – 87	12	84	50 – 100	11
Cloxacillin	41	21 – 84	14	92	40 – 100	8
Neomycin	27	25 – 36	2	27	27	1
Tetracycline	54	17 – 96	8			
Erythromycin	63	51 – 76	2			
Pen / <i>Strep</i>	39	21 – 78	5	91	91	1

Technote 5 gives a list of actions that should be considered when managing outbreaks of *Strep agalactiae* or *Staph aureus*.

Technote 1 gives a list of actions that should be considered when managing outbreaks of *Strep uberis* or *Escherichia coli*.

Specific mastitis treatments

The causative bacteria is usually not known at the time of treatment of individual cases so that the choice of treatment is based on the herd history, clinical judgement, and results of recent milk cultures.

Specific antibiotic treatment is indicated when cultures have been performed and the pathogen identity is suspected or confirmed. Some features of treatment of clinical cases caused by common pathogens are listed below:

Strep agalactiae

- *Strep agalactiae* is highly sensitive to most of the commonly used antibiotics, and a high cure rate (>90%) can be expected using the correct antibiotic.
- Treatment stops shedding of *Strep agalactiae* by cows with clinical mastitis.
- Treatment should be part of a total mastitis control program.

Staph aureus

- Bacteriological cure rate during lactation is low (about 30-60%) because *Staph aureus* causes micro-abscesses in the udder, survives inside cells, and some forms are resistant to commonly used antibiotics (e.g. strains with the enzyme beta-lactamase are resistant to penicillin).
- The best hope for successful treatment is in young cows with recent infections (of less than two weeks duration).
- Treatment of clinical mastitis may reduce *Staph* shedding, and result in milk returning to clinical normality.

Strep uberis

- Experience shows some cases readily respond to treatment and others are quite refractory to treatment. Recent research has found that field strains of *Strep uberis* are able to invade and live in epithelial cells, which may partially explain why infections are refractory to treatment (Keefe and Leslie 1997).

Escherichia coli

- Toxins produced by *Escherichia coli* cause the clinical signs of mastitis. In many cases, bacterial numbers are falling when clinical signs appear.
- Treatment aims to remove toxin by frequent stripping out and use of 30-60 IU oxytocin, and to minimise the effects of toxin by using anti-inflammatory agents and possibly intravenous fluids.
- Systemic antibiotics are given when the cow is extremely ill or when intramammary infusions are unlikely to diffuse through tissue because the udder is greatly swollen.

Supportive treatment

Injection with the milk ejection hormone oxytocin may help remove milk and debris from hard, sore quarters. Oxytocin is a Prescription Animal Remedy and can only be obtained through veterinarians.

There has been some discussion about treating mild clinical cases with oxytocin and frequent stripping rather than using antibiotics. In mild clinical cases of coliform mastitis, milk will usually return to normal within several milkings if stripped frequently with 100 IU oxytocin (Guterbock et al 1993). (This dose is higher than the Australian label recommendation of 30-60 IU.) In other circumstances, oxytocin tends to alleviate clinical signs rather than effecting a bacteriological cure. In a study of 40 herds, Hallberg et al (1994) found that it was economically beneficial to use intramammary antibiotics to treat clinical mastitis in lactating cows as this reduced the number of pathogens in the milk and increased the cure rate and number of quarters returning to normal milk.

Flunixin meglumine inhibits prostaglandin production and limits exudate at the site of inflammation. In contrast with corticosteroids, flunixin does not inhibit white blood cell mobilisation at the infection site. Passage of flunixin from blood to milk is poor, with levels in milk about 1% of those in blood. Nevertheless, it has a useful systemic effect and helps reverse the clinical signs of shock in toxic forms of coliform or staphylococcal mastitis.

Salicylates, such as aspirin, may help reduce fever and inflammation but have a low potency and relatively short half-life. Although they are not registered for use in cattle in Australia, some practitioners find them to be useful supportive treatment (Whittem 1997a). In contrast, phenylbutazone has a long half-life (36-72 hours in cattle depending on the dose) but its action may be cumulative and toxic. Phenylbutazone is NOT APPROPRIATE FOR USE IN CATTLE in Australia. Large doses of dexamethasone (1-3 mg/kg) have been used to treat septic shock in people with good results, but the treatment for cattle is costly and may impair the natural defence mechanisms within the udder.

Large volumes of isotonic intravenous fluid (25-40 L) can markedly improve the chances of survival of cows suffering from acute toxic mastitis. In the early stages of shock (for example, in cows that had a normal fluid status two hours earlier) small volumes of hypertonic saline have been used as an initial treatment to help restore the circulatory blood volume.

Technote 4.6 discusses off-label use of animal treatments.

Confidence – High

There is strong evidence that udder infusions can introduce pathogens unless strict attention is paid to sterile technique.

Research priority – Moderate

It may be beneficial to use systemic antibiotics rather than intramammary preparations in targeted herds.

- Advisers are encouraged to copy and distribute Fact Sheet B in the *Countdown Downunder Farm Guidelines for Mastitis Control* to clients.
- It is necessary to emphasise that udder cleanliness required for good milking hygiene is not equivalent to, or stringent enough for, sterile intramammary infusions.
- Demonstration of teat end preparation and intramammary infusion to staff who administer the treatments is worthwhile.

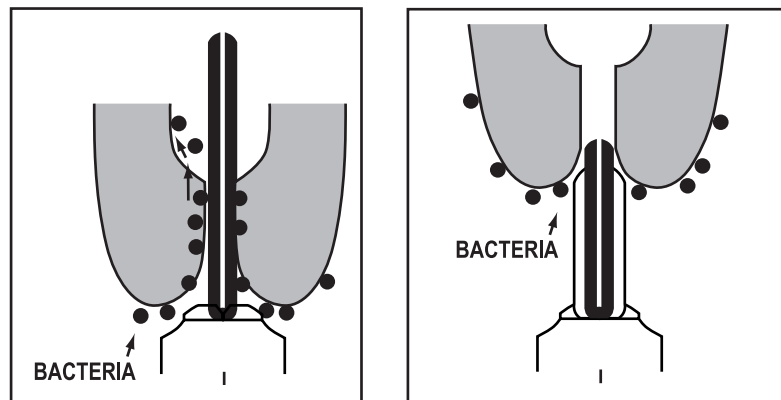
4.5 Administer the treatment as recommended.

Administration of intramammary preparations

The nozzle of intramammary treatments can introduce bacteria into teats if the teat end is not properly disinfected. Fact Sheet B in the *Countdown Downunder Farm Guidelines for Mastitis Control* gives a detailed description of the correct way to administer intramammary treatments.

Ideally, antibiotics are given by partial insertion of short nozzle tubes just inside the teat canal (1-2mm). This is unlikely to be achievable in cows that are not used to having their teats touched, and may therefore not be appropriate for many Australian dairy herds. If an operator is not confident that short nozzle tubes will be used correctly, long nozzle tubes should be used rather than risk damaging the teat canal epithelium.

Use short nozzle tubes (right) when possible and insert just inside teat canal



Administration of intramuscular antibiotics

Standards adopted by the Australian beef industry (CattleCare) to prevent carcase downgrades and chemical residue problems are:

- All injections are to be given into the muscles of the neck.
- Injections are to be given in no more than 10 mL doses at any one site. For example, when giving a 30 mL dose, inject 10 mL into each of three different sites.

This is especially important for dairy cattle that may be culled within 12 months of treatment.

4.6 Use the full course of antibiotics (as specified on the label).

The efficacy and treatment course for lactating cow formulations have been established through extensive research for registration of the products (www.dpie.gov.au).

Only affected quarters of clinical mastitis cases should be treated. As a significant proportion of cows with clinical mastitis have more than one affected quarter, all quarters should be checked at each milking during the treatment course to enable early detection and treatment of affected quarters.

Regardless of whether a clinically affected quarter shows rapid improvement, it is important to use the full course of antibiotic treatment specified by the product manufacturer to reduce the likelihood of infection recurring because of inadequate treatment, and to minimise the development of antibiotic resistant strains of bacteria.

The development of antibiotic resistance

There is limited information on the rate that bacteria are developing resistance to antibiotics commonly used to treat infections in food-producing animals.

The likelihood of antibiotic resistance developing broadly depends on the:

- prevalence of resistant bacteria in the animal population;
- frequency of antibiotic use in the animal population; and
- type of exposure to the antibiotics, e.g. short treatment courses of high doses of antibiotic confer less selective pressure than long-term exposure to low doses of antibiotic.

In addition to these factors, the rate of spread of antibiotic resistance within and between animal species will be influenced by the opportunity for contact between animals and the host specificity of bacterial strains. It is therefore likely to vary significantly with management systems, mix of enterprise types and geographic location.

One of the few published studies on the change in prevalence of resistant mastitis bacteria is in Finland, where Myllys et al (1998) reported an increase of 27% in the proportion of *Staph aureus* strains resistant to at least one antibiotic (mostly due to strains capable of producing beta-lactamase). There is currently no substantial data set that enables comparisons of this finding with what is happening in the Australian dairy cattle population.

An expert advisory committee (JETACAR), considering the future management of antibiotic use in food-producing animals, recommended that a mechanism for measuring the rate of development of resistance in Australia be established. A surveillance system to measure the incidence and prevalence of antibiotic-resistant bacteria and resistance genes in all areas of antibiotic use (including medical and veterinary applications) may be appropriate (JETACAR 1999).

Confidence – High

It is important to use the full course of antibiotic treatment specified by the product manufacturer to reduce the likelihood of infection recurring and to minimise the development of antibiotic resistant strains of bacteria.

Research priority – Low

The JETACAR report can be viewed at the website www.health.gov.au/pubs/jetacar.htm

Off-label use

Off-label use refers to an unregistered use of a product. This includes any deviation from the manufacturer's recommendations, such as using a:

- a different dose rate than stated on the label;
- a different route of administration;
- a different treatment interval; or
- a drug for a different purpose to that stated on the label.

Off-label use can only be authorised by a consulting veterinarian and only where state legislation permits. It is done at the vet's discretion, taking knowledge of safety and efficacy into account, and is usually restricted to situations where no suitable registered product is available or where scientific evidence supports off-label use.

In food-producing animals, veterinarians prescribing off-label use of drugs become liable for setting appropriate withholding periods. These should be given to the client in writing. Wherever possible, the proposed treatment should be explained to the owner and informed consent obtained before treatment is started.

4.7 Milk the quarter out fully at least every milking.

Section 4.4 describes supportive treatment for clinical mastitis cases.

4.8 Clearly mark treated cows.

All milking staff should be familiar with the system used on each farm to identify cows that have been given antibiotic treatment. The Food Quality Program (1999) gives examples of systems for temporary cow identification, all of which can be very effective.

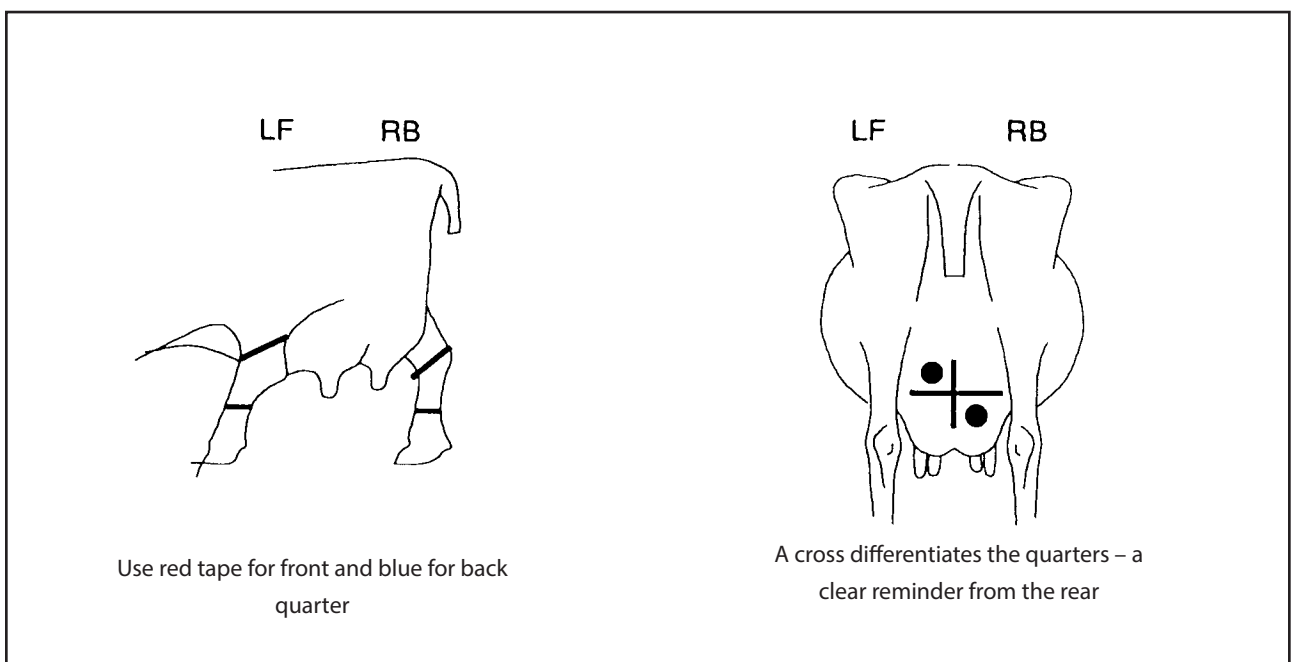
A separate identification system for marking cows that have received Dry Cow Treatment allows for easy recognition if cows rejoin the herd in error, and can help relief milkers or casual staff avoid mistakes.

Confidence – High

Clearly marking treated cows decreases the risk of antibiotic residue violations.

Research priority – Low

Two examples of temporary cow identification (Food Quality Program 1999)



Comparison of methods of temporary identification by the New Zealand Ministry of Agriculture (Food Quality Program 1999)

Method	Visibility	Durability	Ease of use
Velcro strip	Excellent	Good	Easy to apply and remove
Insulation tape	Excellent	Good	Easy to apply and cut off
Plastic hock strap	Excellent	Very good	Easy to apply and remove
Spray paint (non-scourable)	Variable	Good	Very simple
Spray paint (scourable)	Variable	Very poor	Not suitable
Tailpaint	Good	Excellent	Messy. Can paint over with new colour after treatment to avoid confusion
Paint stick/raddle	Good	Excellent	Simple; use like a crayon

4.9 Record all details.

Fact Sheet E of the Countdown Downunder Farm Guidelines for Mastitis Control shows essential information to be recorded for each clinical case (including cow identification, episode date, treatment details and withholding periods).

Advisers should encourage dairy farmers to keep permanent records of clinical mastitis cases so they can manage individual cows and assess herd-level mastitis control. For example, they can:

- make decisions about how to dry-off cows (if selective Dry Cow Treatment is being used);
- make decisions about which cows to cull;
- identify 'suspicious' cows (if clots are found on the filter or bulk milk cell counts rise);
- assess the number of mastitis cases and their response to treatment;
- calculate the cost of clinical mastitis in their herd;
- identify risk periods (e.g. stage of lactation) for clinical mastitis;
- determine the main mastitis pathogen(s) in the herd; and
- review the effectiveness of mastitis control and udder health on farms.

Herd improvement organisations have started providing services that can link clinical case information with details of the cow's age, production, individual cow cell counts (ICCC), previous clinical mastitis history, and Dry Cow Treatment history (see example).

Examples of measures used to assess clinical case management

Example measures	Whole herd	Group comparisons within the herd		Application
		Lactation number	Previous Dry Cow Treatment (yes/no)	
Level of clinical mastitis in the herd (% or rate)				Describe the level of clinical mastitis.
Clinical cases by days after calving or in the dry period (% or rate)				Identify risk periods.
Episodes per clinical case (no.)				Differentiate individual cow from herd problem.
New versus chronic infections (% or rate)*				Identify repeat cases for culling. Assess effectiveness of Dry Cow Treatment strategy.
Volume of milk discarded (L)				Identify priority control measures.
Duration of treatment (days)				Establish cost of treatment.

* Requires ICCC data.

4.10 Observe withholding times for milk and meat.

Withholding periods (WHP) refer to the minimum period of time that must elapse after the last administration of a drug before an animal or its products are sold for human consumption.

Pharmaceutical companies provide recommended withholding periods for their products. Antibiotic residues in milk or meat will not exceed the relevant Australian Maximum Residue Limit if treatments are used according to the label directions and milk or meat are withheld for the specified withholding periods.

Recommended withholding periods are based on trials that specify the:

- class of livestock, e.g. lactating cows;
- dose rate, e.g. milligrams of drug per kilogram liveweight of animal;
- dose interval, e.g. given once daily;
- duration of treatment course;
- route of administration, e.g. intramammary infusion or intramuscular injection;
- use of drugs within their expiry date;
- use of drugs stored in accordance with label directions; and
- pattern of use for which they are registered, e.g. individual animal treatments.

Technote 3.1 gives comparable information for Dry Cow Treatments.

Technote 4.4 describes the active ingredients and treatment course for these intramammary products.

Any deviation from the registration specifications described above may lead to changes in the withholding periods for a product. Such changes are unlikely to be linear (e.g. doubling the dose cannot be extrapolated to a simple doubling of the required withholding periods) (Whittem 1997b).

When giving systemic treatments for mastitis it is important to calculate the correct dose, as withholding periods for milk and meat change markedly when drugs are used at higher dose rates than specified on the label. Weights can be measured on scales or by using girth measurements and height sticks as a guide.

Recommended withholding periods for milk and meat after Lactating Cow Treatment (October 1999)

Product	Withholding period		
	After last infusion		After last feed of treated milk
	Milk	Cow meat (days)	Calf meat (days)
Ampiclox LC	72 hours	30*	30*
Cepravin LC	72 hours (6 milkings)	7	Must not be fed to bobby calves
Lincocin Forte	96 hours	30	30
Mastalone Blue	7 days	30*	30*
Orbenin LC	96 hours	30	30*
Special Formula 17900 Forte V	72 hours	30	30

Figures with an asterisk are not from material approved by the National Registration Authority, but provided by the pharmaceutical companies as consistent with NRA-approved withholding periods for other products. Contact the manufacturer or a veterinarian for further information.

High dose rates constitute an 'off-label' dosage and, for any prescription drug, can only be considered with written permission from a veterinarian. They are a common cause of antibiotic violations.

For registration purposes, the National Regulatory Authority requires withholding periods to be based on the product sold for consumption. Consequently, withholding periods for intramammary antibiotics for lactating cows refer to cows and calves sold for meat or vats of milk.

In Australia, failure to observe withholding periods after treatment is the most significant cause of residue non-compliance (Nicholls et al 1994). In dairy cattle, antibiotic violations are often associated with:

- inadvertent use of Dry Cow Treatment in lactating cows (note that Dry Cow Treatment is registered only for use immediately after a cow's last milking for a lactation);
- failing to identify treated cows;
- failing to record treatment dates;
- cows treated with Dry Cow Treatment at drying-off mistakenly rejoining the milking herd;
- 'off-label' drug use and
- cows treated with Dry Cow Treatment calving before expiry of the Minimum Dry Period.

Antibiotic residue tests

Traces of antibiotic in milk may cause allergic reactions in people and inhibit some starter cultures used in cheese production. National and international regulations stipulate the maximum levels of antibiotics that may be present in milk and these thresholds are often extremely low (Victorian Dairy Industry Authority 1999). Dairy companies perform regular screening tests to detect inhibitory substances in the vat milk that they collect. The dairy industry also conducts an independent survey of bulk raw milk for antibiotic (and other) residues, called the Australian Milk Residue Analysis. This service provides a credible monitoring system that helps the Australian Quarantine Inspection Service to sign off on European Union exports.

Any factory will conduct tests for farmers if there are concerns that antibiotics may have contaminated the vat. The tests include microbial inhibition tests such as the widely used Delvotest SP (DSM Food Specialists) and Disc assay (Difco Laboratories), or assays such as Lak Teck (Idetek Inc.), Penzyme (SmithKline Beecham Animal Health), CITE (IDEXX Corp.) or Charm II (Charm Sciences Inc.). The tests should be performed by operators experienced in using the kits to obtain valid results.

Milk should be withheld from the vat if there is any suspicion that they contain antibiotic residue. Samples can be submitted for antibiotic testing to help resolve problems.

These screening tests have been designed and validated for use on vat milk and are likely to give false positive test results if they are applied to individual milk samples. For example, non-specific inhibitory substances present in the milk of freshly calved cows or clinical mastitis cases are likely to give a positive Delvotest[®] SP test result (Cullor et al 1993). Inhibitory substances and antibiotic residue detected in an individual milk sample may not be excessive once it is diluted with clean milk in the vat. However, testing individual milk samples with factory screening tests provides a cheap and conservative approach to ensuring contaminated milk does not go into the vat.

Vat screening tests are relatively non-specific and vary considerably in their ability to detect all antibiotic families. A more sophisticated and expensive method for quantifying and identifying the type of antibiotic present is High Pressure Liquid Chromatography (HPLC). Although this is more suited for testing milk samples from individual cows, the cost (about \$100) is likely to be prohibitive in normal circumstances.

4.11 Discard milk from all quarters of cows that receive treatment.

Even when a single quarter has been treated with intramammary antibiotic, it is possible that some antibiotic will be absorbed into the bloodstream and pass into the milk of normal quarters. The risk of antibiotic contamination is too great to include milk from treated cows in the vat.

4.12 Make a particular effort to minimise spread of bacteria from infected cows to other cows.

Technote 8 describes good hygiene during milking.

4.13 Consult your veterinarian for advice about the following options if a clinical quarter fails to respond by the end of a full course of treatment (as listed on the label).

The reasons why a clinical quarter may fail to respond to treatment need to be considered when giving advice to clients. These may include:

- **Inappropriate choice of drug.**
Drugs which do not have the spectrum of activity required to combat infections in a particular herd will be ineffective.
The pharmacological properties of some drugs make them inappropriate for use in mastitis therapy. For example, although some drugs are effective in vitro they may be ineffective in vivo if they are unable to cross to the site of the infection.
- **Physical obstruction preventing drugs reaching the site of infection.**
Examples are accumulations of inflammatory cells and hyperplasia of alveolar epithelium.
Infections, such as Staph aureus, can lead to fibrosis and formation of micro-abscesses within the udder. Many antibiotics are unable to cross these barriers in sufficient concentration to reach the minimal inhibitory concentrations required at the site of infection.
- **Attributes of the bacteria.**
Staph aureus bacteria, sensitive in vitro to the antibiotic used, may gain refuge within the acid phagolysosomes of macrophages and polymorpho-nuclear neutrophils with the udder. Antibiotic penetration of cells may be poor and even if they gain access to the cell they may not distribute to the phagolysosomes.
Other organism-related reasons for treatment failure include infections that are resistant to useable antibiotics (e.g. Pseudomonas, mycoplasma, yeasts, etc.) and the emergence of L-forms ('naked' acapsular forms that resist beta-lactam antibiotics).

- If a milk sample was collected and frozen before the initial treatment, it can be cultured to determine the causal bacteria and their antibiotic resistance.
- Pathogens introduced at the time of treatment (due to poor technique when giving intramammary infusions) will only be identified by resampling the quarter.

Options when there is no response to treatment

Options that can be considered when a clinical quarter fails to respond to a full course of treatment are discussed below.

- Repeating the treatment but treating for an extended time with the antibiotic. Oxytocin should be used at milking to assist as much milk removal as possible in conjunction with repeated antibiotic treatments. Note that repeated treatments may extend the required withholding periods.
- Trying a different antibiotic treatment. This will be effective if the infection is more susceptible to the new antibiotic or if the physical properties of the antibiotic allow it to reach the infection site more effectively. The longer a case of clinical mastitis persists, the greater the degree of fibrosis and abscessation that may occur, and the less likely the quarter is to respond to antibacterial treatment. Some cases just do not respond to treatment.
- Drying-off the infected quarter if it is not hot and swollen. The cow should be in good general health apart from the infected quarter. A simple method of drying-off a quarter is to stop milking the quarter, as long as it is monitored to ensure that it does not develop into an acute case of mastitis. It is important that these quarters are permanently identified to prevent accidental attachment of cups to these teats at the time of milking. Dry Cow Treatment must not be used in a quarter when the other quarters are continuing to be milked. Dry Cow Treatments are not registered for use in lactating cows. Some antibiotic will be absorbed into the bloodstream and passed out in the milk from the normal quarters, so there is an unacceptable risk of antibiotic contamination of the vat. At the end of lactation it is not appropriate to use Dry Cow Treatment in a quarter that has been dried off during lactation because intramammary Dry Cow Treatments will not be absorbed in dry quarters. Advisers may consider using injectable antibiotics at the end of lactation in these cows. Stubborn cases of mastitis may be treated by preventing the quarter from producing further milk permanently while retaining the cow in the herd with three viable quarters. An alternative approach is to infuse an irritant chemical solution (5% copper sulphate, or a solution of chlorhexidine diacetate as per Boddie and Nickerson 1994) into the affected quarter to produce a chemical mastitis that causes it to permanently dry-off. From the animal welfare perspective, the short-term inflammation caused is preferable to the long-term inflammation and other potential problems associated with chronic mastitis. It is notable that most farmers do not report any significant drop in production of the other three quarters during the chemical cauterisation treatment. It is not acceptable, for animal welfare reasons, to remove a teat by use of an elastrator ring or other means (unless the teat is gangrenous as a result of the mastitis infection).

- Drying-off the affected cow.
Drying-off cows with quarters that fail to respond to treatment removes a source of infection to other cows in the milking herd. These cows may be treated with Dry Cow Treatment and the affected quarter closely monitored – if the quarter becomes hot and swollen, or the cow becomes systemically ill, treatment with lactating cow product in the infected quarter may need to be reinitiated.
- Culling chronically infected cows from the herd.
Culling chronically infected cows is an important component of any mastitis control program. The recommended withholding period for meat must be observed if the cow has been treated with antibiotics.

Using chemical solutions to permanently dry-off quarters:

- The cow should be in good general health.
- The quarter is milked out thoroughly with hand stripping and oxytocin injections.
- The quarter is infused with 20 mL of 5% copper sulphate solution and not milked for seven more days. After infusion, it exhibits a large degree of inflammation with associated heat, swelling and mild to moderate pain. The pain and heat generally subside within the initial week post treatment and the swelling over the next weeks.
- On the eighth day, it is hand-stripped; if there is any sign of milk, the procedure is repeated. If there is only serous fluids, the quarter is cleared of as much fluid as possible and not milked any further.

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