CHAPTER 3.7.3.

CONTAGIOUS AGALACTIA

SUMMARY

Description and importance of the disease*: Contagious agalactia is a serious disease syndrome of sheep and goats that is characterised by mastitis, arthritis, keratoconjunctivitis and, occasionally, abortion.* Mycoplasma agalactiae (Ma) *is the main cause of the disease in sheep and goats, but* M. capricolum *subsp.* capricolum (Mcc), M. mycoides *subsp.* capri (Mmc) *and* M. putrefaciens *produce a clinically similar disease, more often in goats, which may be accompanied by pneumonia.* Ma *and* Mcc *have been isolated from wild small ruminants such as ibex and mountain goats. Antibodies to* Mmc *and* Mcc *have been detected in South American camelids* (*alpacas, llamas and vicunas*)*, but no mycoplasmas have yet been isolated.*

Identification of the agent: *Definitive diagnosis requires the isolation of the causative mycoplasmas from the affected animals, which are then identified by biochemical, serological and, increasingly, molecular tests such as the polymerase chain reaction. Samples of choice include milk, conjunctival and ear swabs, and joint fluid. The sampling of bulk milk tank provides a convenient way of monitoring whole herds/flocks for causative mycoplasmas. All four mycoplasmas grow relatively well in most mycoplasma media although* M. agalactiae *shows a preference for organic acids such as pyruvate as substrates.*

Serological tests: *Detection of antibodies in serum by enzyme-linked immunosorbent assay* (*ELISA*) *provides rapid diagnosis of disease, but may not be very sensitive in chronically affected herds and flocks. Indirect ELISAs have been used routinely in control programmes for screening herds for* Ma*. Confirmation of infection by isolation and identification or detection by polymerase chain reaction is usually necessary in areas believed to be free of contagious agalactia. Serological tests are not widely available for* M. putrefaciens*.*

Requirements for vaccines: *Commercial vaccines for* Ma*, inactivated with formalin, are widely used in southern Europe, but are not considered to be very efficacious. Under experimental conditions,* Ma *vaccines inactivated with saponin have been shown to be more protective than formalised preparations. Live vaccines for* Ma *are used in Turkey, where they are reported to be more protective than inactivated vaccines. A commercial vaccine containing* Ma, Mmc *and* Mcc *is available. Autogenous vaccines for* Mmc *and, occasionally, for* Mcc *are believed to be used in some countries. No vaccines exist* for M. putrefaciens*, as the disease it causes is not considered to be sufficiently serious or widespread.*

A. INTRODUCTION

Contagious agalactia is a disease of sheep and goats that is characterised by mastitis, arthritis and keratoconjunctivitis, and has been known for nearly 200 years. It occurs wherever pastoralism and small ruminant dairy production is located in Europe, particularly in the Mediterranean regions, Asia, North Africa, and sporadically in the United States of America (USA). It is mainly caused by *Mycoplasma agalactiae* (*Ma*) (reviewed by Bergonier *et al*., 1997). An upsurge of contagious agalactia, caused by *Ma*, has been seen in sheep in Spain and France recently with increased cases reported in and around the Pyrenees and new outbreaks being reported in Corsica (Chazel *et al*., 2010). Italy still report more than 50 outbreaks every year, mainly notified on two of its Islands[1]. Frequent and numerous outbreaks occur in Iran and Mongolia (Nicholas *et al*., 2008).

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[1]   Data from OIE World Animal Health Information System: http://www.oie.int/en/animal-health-in-the-world/the-world-animal-health-information-system/the-oie-data-system/

Mycoplasma capricolum subsp. *capricolum* (*Mcc*) and *M. mycoides* subsp. *capri* (formerly *M. mycoides* subsp. *mycoides* LC [LC = large colonies]) have also been isolated from sheep and goats with mastitis and arthritis in many countries including Italy, where the disease is spreading (Marogna *et al.*, 2015), South America (Nascimento *et al.,* 1986) and Australasia (Cottew, 1971).

The clinical signs of infections caused by *Mcc, Mmc* and *M. putrefaciens* (*Mp*) are sufficiently similar to be considered indistinguishable from contagious agalactia caused by *Ma*. In addition, *Mp* also causes mastitis and arthritis in goats, which is very similar to that caused by *Ma*, *Mmc* and *Mcc* (Rodriguez *et al.*, 1994). Furthermore, the consensus of the working group on contagious agalactia of the EC COST[2] Action 826 on ruminant mycoplasmoses, which met in Toulouse, France, in 1999, was that all four mycoplasmas should be considered as causal agents of contagious agalactia. In France, *Mmc*, *Mcc* and *Mp* constitute over 80% of the mycoplasma isolations from goats with *Ma* accounting for less than 2%. *Ma* and *Mcc* have been isolated from wild small ruminants such as ibex and mountain goats in the Pyrenees and Alps (Chazel *et al.*, 2010; Verbsick *et al.*, 2008). There are occasional reports of the isolation of *Ma* (Chazel *et al.,* 2010) and *Mcc* from apparently healthy cattle (Pinho *et al.,* 2009), although Catania *et al.* (2016) isolated *Ma* from eyes, ears and brain spinal fluid of bulls.

Clinically, the disease caused by *Ma* is recognised by elevated temperature, inappetence and alteration in the consistency of the milk in lactating ewes with decline and subsequent failure of milk production, often within 2–3 days, as a result of interstitial mastitis (Bergonier *et al.*, 1997); lameness and keratoconjunctivitis affects about 5–10% of infected animals. Fever is common in acute cases and may be accompanied by nervous signs, but these are rare in the more frequently observed subacute and chronic infections where the disease is endemic. Pregnant animals may abort. *Ma* may occasionally be found in lung lesions (Loria *et al.*, 1999), but pneumonia is not a consistent finding. Bacteraemia is common, particularly for *Mmc* and *Mcc* and could account for the isolation of the organism from sites where it is only present transiently.

Arthritis, pleurisy, pneumonia, mastitis and keratoconjunctivitis may all result from infection with *Mmc,* which has one of the widest geographical distribution of ruminant mycoplasmas, being found on all continents where small ruminants are kept and wherever contagious agalactia and caprine pleuropneumonia are reported (DaMassa *et al.*, 1983; Nicholas, 2002); however the lack of diagnostic facilities for mycoplasma diseases in many countries means that it is probably under reported. *Mmc* is mostly confined to goats but has occasionally been isolated from sheep with reproductive disease and cattle with arthritis or respiratory disease. Cases usually occur sporadically and the disease may persist and spread slowly within a herd; nevertheless, in free areas, the disease shows high morbidity and mortality of kids. In Sicily a reported outbreak showed more than 40% mortality (Agnello *et al.*, 2012). After parturition, the opportunity for spread in milking animals increases, and kids ingesting infected colostrum and milk become infected. The resulting septicaemia, with arthritis and pneumonia, causes high mortality in kids (Bergonier *et al.*, 1997; DaMassa *et al.*, 1983).

Mcc is widely distributed and highly pathogenic, particularly in North Africa, but the frequency of occurrence is low (Bergonier *et al.*, 1997). Goats are more commonly affected than sheep, and clinical signs of fever, septicaemia, mastitis, and severe arthritis may be followed rapidly by death (Bergonier *et al.*, 1997; Bolske *et al.*, 1988). Pneumonia may be seen at necropsy. The severe joint lesions seen in experimental infections with this organism are accompanied by intense periarticular subcutaneous oedema affecting tissues some distance from the joint (Bolske *et al.*, 1988). In 2015 the first human isolate of *Mcc* was reported from a man with septicaemia (Seersholm *et al.*, 2015).

Mp is common in milking goat herds in western France where it can be isolated from animals with and without clinical signs (Mercier *et al.*, 2001). It has also been associated with a large outbreak of mastitis and agalactia leading to severe arthritis in goats accompanied by abortion and death without pyrexia in California, USA (Bergonier *et al.*, 1997). *Mp* was the major finding in an outbreak of polyarthritis in kids in Spain (Rodriguez *et al.*, 1994).

Antibodies to *Mmc* and *Mcc*, but not *Ma*, have been detected in South American camelids, including llamas, alpacas and vicunas, but as yet no mycoplasmas have been isolated (Nicholas, 1998). These camelids are affected by a range of mycoplasma-like diseases, including polyarthritis and pneumonia, so it is likely that mycoplasmas including *Mmc* and *Mcc* may be found in the future.

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2   European Cooperation in the field of Scientific and Technical Research.

B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for the diagnosis of contagious agalactia and their purpose

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification[3]						
Culture and identification of the organism	++	+++	+++	+++	–	–
Conventional PCR	++	+++	–	+++	++	–
Real-time PCR	++	+++	–	+++	++	–
Detection of immune response						
CFT	+++*	+	+++	+++*	+++*	+++*
ELISA	+++**	+	+++	+++**	+++**	+++**
Immunoblotting	+	++	+	++	+	++

Key: +++ = recommended method, validated for the purpose shown; ++ = suitable method but may need further validation;
+ = may be used in some situations, but cost, reliability, or other factors severely limits its application;
– = not appropriate for this purpose; n/a = purpose not applicable.
*for *Mcc* and *Mmc* only; **for *Ma* only.
PCR = polymerase chain reaction; CFT = complement fixation test; ELISA = enzyme-linked immunosorbent assay.

1. Identification of the agents

1.1. Selection of samples

Preferred samples from living animals include: milk from mastitic females or from apparently healthy females; nasal swabs and secretions; where there is a high rate of mortality/morbidity in kids; joint fluid from arthritic cases; conjunctival swabs from cases of ocular disease; and blood for antibody detection from affected and non-affected animals (Nicholas & Baker, 1998). The sampling of bulk milk tank provides a convenient way of monitoring flocks and herds for causative mycoplasmas. A distinctive smell of putrefaction in the milk is often the first sign of the presence of *Mp* infection in a herd. The ear canal has also been shown to be a source of pathogenic mycoplasmas, although in practice the presence of non-pathogenic mycoplasmas at this site may make confirmation difficult (Nicholas & Baker, 1998). Mycoplasmas may be isolated from the blood during the acute stage of the disease when there is mycoplasmaemia. From dead animals, samples should include: udder and associated lymph nodes, joint fluid, lung tissue (at the interface between diseased and healthy tissue) and pleural/pericardial fluid. Samples should be dispatched quickly to a diagnostic laboratory in a moist and cool condition. All four causative mycoplasmas are relatively easy to isolate from internal organs, joints and milk and grow well in most mycoplasma media, producing medium to large colonies in 3–4 days.

1.2. *Mycoplasma* isolation

The usual techniques used in the isolation of mycoplasmas apply to all four causative organisms (Nicholas & Baker, 1998). Many media have been reported to grow the causative mycoplasmas. Improved growth rates of *Ma* have been seen in media containing organic acids such as pyruvate and isopropanol (Khan *et al.*, 2004). The formulation of PRM medium (Khan *et al.*, 2004) is as follows:

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3   A combination of agent identification methods applied on the same clinical sample is recommended.

Heat-inactivated porcine serum 100 ml/litre, special peptone 20 g/litre, yeast extract 5 g/litre, glycerol 5 g/litre, sodium chloride 5 g/litre, HEPES (N-2-hydroxyethylpiperazine, N-2-ethanesulphonic acid) 9 g/litre, fresh yeast extract 100 ml/litre, sodium pyruvate 5 g/litre, 12.5 ml of 0.2% phenol red and ampicillin (200,000 International Units/ml. Make up to 1 litre in distilled water and sterilise by filtration. Adjust the pH of the broth medium to 7.6. Prepare solid medium by adding 10 g of LabM agar No. 1 (Bury UK, or agar of equivalent quality) and dispense into sterile Petri dishes.

Thallium acetate (250 mg/litre), which is toxic and inhibitory to some mycoplasmas but not those causing contagious agalactia, may be a necessary component of the transport medium to reduce bacterial contamination from clinical samples, but should be omitted once the mycoplasmas begin to grow *in vitro*. A satisfactory alternative to thallium acetate may be colistine sulphate (37.5 mg/litre).

1.2.1. Test procedure

i)   Make tenfold dilutions (10^1–10^6) of the liquid sample (milk, synovial fluid, conjunctival and ear swabs) or tissue homogenate in appropriate broth medium.

ii) Spread a few drops of each sample on the agar medium and dispense a 10% (v/v) inoculum into broth medium.

iii) Streak swabs directly on to agar medium.

iv) Incubate inoculated broths (optimally with gentle shaking) and agar media at 37°C in humidified atmosphere with 5% carbon dioxide.

v)   Examine broths daily for signs of growth (indicated by a fine cloudiness or opalescence) or changes in pH indicated by a colour change and examine agar media under ×35 magnification for typical 'fried egg' colonies.

vi) If no mycoplasma growth is seen after 7 days, subculture a 10% (v/v) inoculum of broth into fresh broth and spread about 50 µl of this on to agar media.

vii) Repeat as for step v. If no mycoplasmas are seen after 21 days' incubation, consider the results to be negative.

viii) If bacterial contamination results (seen as excessive turbidity), filter sterilise by passing 1 ml of contaminated broth through a 0.45 µm filter into fresh broth medium.

Clinical samples frequently contain more than one mycoplasma species so clone purification of colonies is often considered necessary before performing biochemical and serological identification, in particular the growth and film inhibition tests (GIT and FIT, respectively). However, cloning is a lengthy procedure taking at least 2 weeks. The immunofluorescence test (Bradbury, 1998), dot immunobinding tests (Poumarat, 1998) and, more recently, polymerase chain reaction (PCR) tests (see Section B.1.5) do not require cloning as these tests can detect the pathogenic mycoplasmas in mixed cultures, saving a great deal of time.

1.3. Biochemical tests

The first test that should be performed on the cloned isolates is sensitivity to digitonin, which separates mycoplasmas from acholeplasmas; the latter are ubiquitous contaminants that can overgrow the mycoplasmas of interest. Growth in liquid medium containing glucose (1%), arginine (0.2%), and phenolphthalein diphosphate (0.01%), on solid medium containing horse serum or egg yolk for the demonstration of film and spots, and on casein agar or coagulated serum agar to test for proteolysis, are among the most useful tests for differentiating the four mycoplasmas (Poveda, 1998). These biochemical characteristics, however have been increasingly found to be variable for the individual mycoplasmas and have little diagnostic value. The most impressive biochemical characteristic that differentiates *Mp* from all other mycoplasmas is the odour of putrefaction it produces in broth culture. Other features that may be helpful include: film and spot production seen on the surface of the broth and solid media caused by *Ma* and to a lesser extent by *Mp*; and the proteolytic activity of *Mcc* and *Mmm*LC on casein and coagulated serum.

1.4. Serological identification

Identification of isolates using specific antisera is usually carried out with the GIT, FIT (Poveda & Nicholas, 1998) or the indirect fluorescent antibody (IFA) test (Bradbury, 1998). A dot immunobinding test that is carried out in microtitre plates offers many improvements over the other serological tests such as rapidity and higher throughputs (Poumarat, 1998) but requires subjective judgements of staining intensity. For *Ma*, film inhibition may often be more reliable as growth inhibition is not seen with

all isolates; it can also be used for serodiagnosis. Film production by the mycoplasma may be enhanced by the incorporation of 10% egg yolk suspension into the solid medium.

1.4.1. Test procedure

i) Inoculate at least two dilutions of 48-hour cloned broth cultures (10^{-1} and 10^{-2}) on to predried agar media by allowing 50 µl of the cultures to run down the tilted plates using the 'running drop' technique (Poveda & Nicholas, 1998). Remove any excess liquid with a pipette.

ii) Allow the plates to dry. It is possible to apply two or three well separated running drops to each 90 mm diameter plate.

iii) Apply predried filter paper discs containing 30 µl of specific antiserum to the culture; ensure good separation of discs (at least 30 mm).

iv) Incubate the plates as for mycoplasma culture and examine daily by eye against a light background.

1.4.2. Interpretation of the results

A zone of inhibition over 2 mm, measured from the paper disc to the edge of mycoplasma growth is considered to be significant. Partial inhibition can occur with weak antiserum or where there are mixed cultures. Stronger reactions can be obtained if about 60 µl of antisera is added to 6 mm diameter wells made in the agar with a cork borer or similar device (Poveda & Nicholas, 1998).

In the IFA test, specific antisera are applied to colonies on solid medium. Homologous antiserum remains attached after washing and is demonstrated by adding fluorescein-conjugated antiglobulin, washing, and viewing the colonies with an epifluorescence microscope (Bradbury, 1998). Controls should include known positive and known negative control organisms, and a negative control serum. However like the immunobinding tests subjective judgements are required to assess staining intensity.

Antisera for these serological tests have traditionally been prepared against the type strains of the various *Mycoplasma* species, and most field isolates have been readily identified using these antisera. As more strains have been examined, however, some have been found to react poorly with these antisera, while reacting well with antisera to other representative strains of the species. Intraspecies variation in antigenic composition has not been reported for *Mp,* but occurs to some degree with *Ma* and with *Mcc* strains. Thus, diagnostic laboratories may need to have several antisera to enable all strains of the species to be identified.

1.5. Nucleic acid recognition methods

1.5.1. Polymerase chain reaction assays

PCR assays are routinely used in many laboratories and are extremely sensitive. They can provide a rapid early warning system when carried out on clinical samples, enabling a full investigation to take place when results are positive. However negative results should not be considered definitive, as culture of bulk tank milk samples may be more sensitive (Tatay-Dualde *et al.*, 2015). Several PCRs specific for *Ma* have been developed and show similar levels of sensitivity, although they are based on different gene sequences (Dedieu *et al.*, 1995; Subrahamaniam *et al.*, 1998; Tola *et al.*, 1997a). They can be used directly on nasal, conjunctival, synovial and tissue samples; they have been used on milk samples where they have been reported to be more sensitive than culture (Tola *et al.*, 1997a), although occasionally undefined inhibitors may interfere with the test. PCRs can also be used, more reliably, on mycoplasmas growing in culture; a 24 hour enrichment of the mycoplasma in the appropriate medium greatly facilitates PCR detection even in the presence of bacterial contamination (Nicholas, 2002). The use of immunomagnetic capture-PCR methods may be more rapid than culture enrichment for detecting *Ma* from milk samples (Sanna *et al.*, 2014). A PCR based method that uses denaturing gradient gel electrophoresis (DGGE) and mycoplasma-specific primers is capable of identifying the majority of small ruminant mycoplasmas including all the causative agents of contagious agalactia by their migration pattern (McAuliffe *et al.*, 2005). Isothermal PCR methods may be applicable; and has been described for detecting *Ma* (Rekha *et al.*, 2015).

A positive PCR result, particularly in an area previously free of contagious agalactia, should be confirmed by isolation and identification of the mycoplasma using standard procedures.

Individual PCRs have been reported for *Mmc* (Bashiruddin, 1998) and *Mcc* (Monnerat *et al.,* 1999) and *Mp* (Nicholas *et al.,* 2008; Peyraud *et al.*, 2003) respectively. In addition a multiplex test has been described that can detect simultaneously *Ma*, *Mcc* and *Mmc* (Greco *et al.*, 2001). Cillara *et al.* (2015) describe a PCR and restriction fragment length polymorphism PCR method based on the *lpd*A gene to discriminate between *Mmc* and *Mcc*.

1.5.2. Real-time PCRs

Several rapid real-time PCRs have been reported for *Ma* that provide advantages of speed, sensitivity and sample handling (Lorusso *et al.*, 2007). More recently a multiplex real time test has been described which detects all four mycoplasmas simultaneously (Becker *et al.*, 2012).

1.5.3. Micro-array analysis

Micro-array analysis has been applied to the detection of mycoplasmas. Using probes derived from the 23 rRNA genes and *tuf* gene target regions, Schnee *et al.* (2012) have described the identification of 37 mycoplasma species including all four contagious agalactia pathogens. At the time of publication some cross reaction was seen between *Ma* and the closely related bovine pathogen, *M. bovis*. The advantages of the test over PCR include ease of operation, high information content and cost-effectiveness.

1.5.4. Matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF)

Matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF) has been used to identify bacteria (Randall *et al.*, 2015), including *Mycoplasma* species and the causative organisms of contagious agalactia (Pèreyre *et al.*, 2013). The organisms need to be cultured first, and sometimes only the predominant species is identified in mixed cultures.

1.5.5. Test procedure

The PCR detailed for *Mycoplasma agalactiae* has been evaluated across several laboratories (Bashiruddin *et al.,* 2005) whereas other molecular methods referred to in this chapter for *M. agalactiae* and other contagious agalactia-causing species have not been evaluated to the same extent and are therefore not detailed. The following primers based on the uvrC gene have been shown to be specific for *Ma* (Subrahamaniam *et al.*, 1998). PCRs may need to be optimised in each laboratory. Positive and negative control DNA should be run in each assay.

MAGAUVRC1-L   CTC-AAA-AAT-ACA-TCA-ACA-AGC

MAGAUVRC1-R   CTT-CAA-CTG-ATG-CAT-CAT-AA

i)   Extract DNA from *Mycoplasma* isolates or clinical material using the appropriate method (Bolske *et al.*, 1988).

ii)   Carry out PCR methods in 50 µl reaction mixtures containing: 1 µl of sample DNA, 20 pmol of each primer (see above), 1 mM each dNTP, 10 mM Tris/HCl, pH 8.3, 1.5 mM MgCl$_2$, 50 mM KCl and 1.25 mM U *Taq* DNA polymerase.

iii)   Subject the mixture to 35 amplification cycles in a thermal cycler with the following parameters: 30 seconds at 94°C, 30 seconds at 50°C annealing temperature and 1 minute at 72°C.

iv)   Analyse the PCR products by electrophoresis on a 0.7% agarose at 110 V for 2 hours and visualise by staining with ethidium bromide. A 1.7 kb fragment is indicative of the presence of *Ma.*

2.   Serological tests

2.1. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assays (ELISAs) using sonicated or Tween-20-treated antigens have been reported to be more sensitive than the CFT for the detection of antibody to *Ma* in serum (Bergonier *et al.*, 1997). Problems of nonspecificity have been overcome by the use of monoclonal or protein G conjugates in the ELISA (Lambert *et al.*, 1998). The use of these conjugates enables the testing of sera from a wide range of mammalian species, including camelids.

Two commercial ELISA kits for *Ma* are available, one using a fusion protein (sensitivity 54% and specificity 100%) and the other using whole cells as target antigens (sensitivity 84% and specificity 96% in sheep or 90% in goats) (Poumarat *et al.,* 2012). There also appeared to be differences in the ability of the tests to detect the responses to different strains equally. The choice of test depends on the objectives of the proposed study, i.e. a less sensitive test would be sufficient for prevalence study where the disease was endemic while a more sensitive test would be required for disease detection in a disease-free region.

ELISAs are not widely available for the other three causative mycoplasmas.

2.2. Complement fixation

A standard complement fixation test (CFT) for *Ma* has also been applied to other mycoplasmas involved in the contagious agalactia syndrome (Bergonier *et al.*, 1997). Antigens are prepared from washed organisms, standardised by opacity, and lysed, either ultrasonically or by using sodium lauryl sulphate followed by dialysis. Sera are inactivated at 60°C for 1 hour, and the test is carried out in microtitre plates with overnight fixation in the cold or at 37°C for 3 hours. The haemolytic system is added, and the test is read after complete lysis is shown by the antigen control. A positive result is complete fixation at a serum dilution of 1/40 or greater for the following mycoplasmas: *Ma, Mcc*, and *Mmc.* The CFT is regarded as a herd test and at least ten sera are tested from each herd, preferably from acute and convalescent cases.

Some sera from healthy flocks react in the CFT using *Ma* up to a serum dilution of 1/20, but rarely react with the other two antigens. However, in flocks infected with *Ma*, sera giving a homologous reaction at 1/80 may cross-react at up to 1/40, the positive threshold, with the other two antigens. It is often difficult to perform the CFT if the quality of the test sera is poor; where possible, the ELISA is preferred.

2.3. Immunoblotting test

Immunoblotting tests have also been described for *Ma* and are considered as confirmatory tests for outbreaks in Italy (Nicholas, 1998; Tola *et al.*, 1997b). Strong bands at approximately 80 and 55 kDa were seen with sera with antibodies to *Ma,* while sera from healthy flocks show no bands or very faint bands of different sizes. Diluting the sera to 1/50 improves the discrimination between positive and negative sera (Nicholas, 1998). On the other hand, Poumarat *et al.* (2012), using a 1/5 dilution of serum, considered a serum positive for *Ma* from a flock/herd in France if it contained 4 bands: 80, 48, 40 and 30 kDa suggesting there may be some geographic differences in humoral responses.

C. REQUIREMENTS FOR VACCINES

Vaccines for the prevention of contagious agalactia due to *Ma* are used widely in the Mediterranean countries of Europe and in western Asia. No single vaccine has been universally adopted, and no standard methods of preparation and evaluation have been applied.

1. Vaccines for *Mycoplasma agalactiae* infection

1.1. Inactivated vaccines for *Mycoplasma agalactiae* infection

In Europe where live vaccines for *Ma* are not acceptable, attention has focused on the use of killed organisms, mostly using formalin and an adjuvant such as aluminium hydroxide in an oil emulsion. The titres of the preparations, before inactivation, are very high (10^8–10^{10} colony-forming units per ml) and are derived from laboratory strains. Some products are available commercially including a trivalent preparation containing *Ma, Mcc* and *Mmc* but there are few data on their efficacy. A formalin-inactivated oil emulsion vaccine was shown to be immunogenic and protective in a small trial in lactating sheep and also prevented transmission of *Ma* (Greco *et al.*, 2002). However, in a small trial, no potency was evident in a similar commercial product following contact challenge (Agnone *et al.*, 2013).

It is possible that in some instances the apparent lack of protection given by vaccines could be the result of animals being infected with one of the other four mycoplasmas involved in the contagious agalactia syndrome (Gil *et al.*, 1999). A multivalent formalin inactivated vaccine incorporating all four causative mycoplasmas and adjuvanted with saponin and aluminium hydroxide appears beneficial in preliminary trials (Ramirez *et al.*, 2001).

More recently vaccines inactivated with phenol or with saponin have given superior protection against experimental infections compared with formalin, sodium hypochlorite or heat-inactivated vaccines (Tola *et al.*, 1999).

1.2. Live attenuated vaccines for *Mycoplasma agalactiae* infection

Live attenuated vaccines against *Ma* have been used in Turkey for many years and have been reported to provide better protection in ewes and their lambs than inactivated vaccines (Nicholas, 2002). However they can produce a transient infection with shedding of mycoplasma. Live vaccines should not be used in lactating animals and should be part of a regional plan in which all flocks from which animals are likely to come into contact be vaccinated at the same time.

2. Vaccines for *Mycoplasma mycoides* subsp. *capri* infection

There is little recent published information on the availability of vaccines for *Mmc* (Marogna *et al.*, 2015) although it is believed that inactivated vaccines are widely used in many Mediterranean countries and in Asia suggesting that their production and use is localised (Bergonier *et al.*, 1997). Saponised vaccines have been reported in India which provoke a strong antibody response and show some protection (Sunder *et al.*, 2002).

3. *Mycoplasma capricolum* subsp. *capricolum* and *M. putrefaciens*

Although infections with *Mcc* and *Mp* can be severe, their prevalence is relatively low and, as might be expected, little or no work appears to have been carried out on preventive vaccination for these infections.

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NB: There are OIE Reference Laboratories for contagious agalactia
(see Table in Part 4 of this *Terrestrial Manual* or consult the OIE web site for the most up-to-date list:
http://www.oie.int/en/scientific-expertise/reference-laboratories/list-of-laboratories/).
Please contact the OIE Reference Laboratories for any further information on
diagnostic tests, reagents and vaccines for contagious agalactia

NB: First adopted in 1990. Most recent updates adopted in 2018.