Pulmonary mycoplasmoses of small ruminants*

P.-C. LEFÈVRE **, G.E. JONES *** and M.O. OJO ****

Summary: The pathogenicity of mycoplasmas isolated from the lungs of sheep and goats is very variable. This is described in Chapter I.

A single species (Mycoplasma type F38) is responsible for a specific disease entity, caprine contagious pleuropneumonia, which occurs in East and North Africa.

Three other species are known to act as primary pathogens: Mycoplasma mycoides subsp. capri, which is pneumotropic (though not exclusively so), Mycoplasma mycoides subsp. mycoides (large colony or LC type) and M. capricolum; the pneumotropisms of the latter, particularly for M. capricolum, are secondary.

Others, such as M. ovipneumoniae, are not pathogenic unless the resistance of the animal to disease has been lowered, or like M. arginini merely invade tissues without actual pathogenicity.

Pulmonary mycoplasmoses (primary and secondary) probably occur throughout the world, except for type F38, which has so far remained confined to certain parts of the African and Asian continents. It is necessary to be aware of the ambiguity of the term 'contagious caprine pleuropneumonia', which embraces two distinct infections (F38 sp. and M. mycoides subspp. capri and mycoides).

The clinical aspects of pulmonary mycoplasmosis occurring in small ruminants are reviewed in Chapter II, with reference to contagious caprine pleuropneumonia and other pulmonary mycoplasma infections (Mycoplasma mycoides subsp. capri, Mycoplasma mycoides subsp. mycoides and Mycoplasma ovipneumoniae).

The various microscopical, microbiological and serological methods currently available for the diagnosis of mycoplasmoses, in particular those involving the respiratory tract of small ruminants, are outlined and compared in Chapter III. Particular emphasis is given to the practical aspects of mycoplasma isolation, cultivation and identification, and details of suitable media are provided in an Appendix.

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* This Review, edited by P.-C. Lefèvre, has been prepared in five chapters by three authors as follows: Chapter I: P.-C. Lefèvre; Chapter II: M.O. Ojo; Chapter III: G.E. Jones; Chapters IV and V: P.-C. Lefèvre.

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The epidemiology of mycoplasmoses presented in Chapter IV depends on many factors, namely: pathogenicity and tissue tropism of the mycoplasmas; animal species and age; the poor survival of mycoplasmas outside the host, but with the possibility of healthy carriers among animals which have recovered; influence of environmental conditions and type of husbandry.

Treatment, which is based on antibiotic therapy (tetracyclines and macrolide antibiotics), is described in Chapter V. To be effective, it is essential that the dosage and duration of treatment are adequate.

At present, no vaccines are available (except for a vaccine used in Oman on a small scale against contagious caprine pleuropneumonia caused by strain F38), and prophylaxis depends essentially on the strict application of general disease prevention measures.


INTRODUCTION

Although the initial clinical descriptions of diseases caused by mycoplasmas in goats date back to 1816 for contagious agalactia and 1873 (or 1881) for contagious pleuropneumonia, the study of the mycoplasmoses of small ruminants has been neglected for many years. As far as the respiratory mycoplasmoses are concerned, there are two reasons for this lack of interest:

1. Bacterial superinfection is very common, particularly by *Pasteurella haemolytica* and *Pasteurella multocida* (types A and D), and this complicates the clinical and pathological picture, obscuring the role of mycoplasmas.

2. Difficulties have been encountered in the laboratory in isolating and propagating mycoplasmas, which are much more demanding than most other bacteria as far as the composition of culture media is concerned.

Major progress in knowledge of mycoplasmas has been made during the past thirty years. Despite this progress, many aspects of pathogenesis and epidemiology remain obscure.

I. GENERAL ASPECTS

Among the problems which have arisen from the study of pulmonary mycoplasmoses, the most important is the precise assessment of the pathogenicity of the various species of *Mycoplasma* which occur in the lungs of sheep and goats with respiratory disease (33, 51 and Table I). At present these organisms can be divided into three groups, as follows:

Major mycoplasmas possessing primary pathogenicity

Members of this group are:

- The agent of contagious caprine pleuropneumonia (CCPP), the type species of which is strain F38. This agent, which has been isolated from numerous outbreaks
**TABLE I**

*Mycoplasmoses of small ruminants*

<table>
<thead>
<tr>
<th>Syndrome or principal site</th>
<th>Species</th>
<th>Reference strains</th>
<th>Comments</th>
</tr>
</thead>
</table>
| Contagious agalactia syndrome | *M. agalactiae*  
*M. capricolum*  
*M. mycoides subsp. mycoides (LC)* | Pg 2  
Cal. kid  
Y-goat | These three mycoplasmoses are indistinguishable by symptoms and lesions (keratitis, conjunctivitis, mastitis). *M. capricolum* and subspecies *mycoides* can also be pneumotropic. |
| Contagious pleuropneumonias | *Mycoplasma* sp.  
*M. mycoides subsp. capri* | F38  
Pg 3 | This is the specific agent of caprine contagious pleuropneumonia. Strains of *capri* are certainly pathogenic, but seem to be quite rare. |
| Various pneumopathies | *M. arginini*  
*M. ovipneumoniae*  
*Mycoplasma* sp.  
*Ureaplasma* sp. | G.230  
Y-98  
A.1343 | The respiratory tract is the commonest location, but *arginini* can be found in various tissues, *ovipneumoniae* in ocular lesions and *Ureaplasma* in the genital tract. |
| Contagious mastitis | *M. putrefaciens* | KS.1 | This mastitis is not accompanied by arthritis or keratitis*. |
| Ocular infections | *M. conjunctivae*  
*A. oculi* | HRC.581  
19 L | These two are associated with keratoconjunctivitis, but it is not known if they are the primary pathogens. |
| Genital tract | *Mycoplasma* sp. | 2 D | No information about pathogenicity. |
| Saprophyte | *A. laidlawii* | Pg 8 | Part of the microbial flora of mucous membranes. |

According to P. Perreau (89).

* Da Massa *et al.* (1987) give a description of mastitis and arthritis in goats caused by *M. putrefaciens* *(Vet. Rec., 120, 409-413).*
of CCPP in Kenya (61, 68, 69), Sudan (41), more recently in Tunisia (88), Oman (53), Chad (Lefèvre, in preparation) and Turkey (53), has not yet been given a specific name, and taxonomists are uncertain as to its exact status.

Comparative studies in recent years have shown that strain F38 can be considered either as an entirely distinct species, or as a biotype of *M. capricolum*.

b) *Mycoplasma mycoides* subsp. *capri*, a historical agent of CCPP, but which produces histological lesions differing from those of CCPP produced by strain F38. In addition, this organism is not exclusively pneumotropic, for it has been isolated from mastitis and arthritis.

c) Agents of the contagious agalactia syndrome:
- *Mycoplasma agalactiae*
- *Mycoplasma mycoides* subsp. *mycoides* (large colony form)
- *Mycoplasma capricolum*.

The agalactia syndrome consists of a clinical triad of keratitis, arthritis and mastitis, about which there is no dispute. However, the last two species can have a pronounced pneumotropism.

**Associative mycoplasmas**

These are mycoplasmas which are isolated from diseased lungs either alone or in the company of other bacteria. Unaided, they seem to be incapable of producing the symptoms and lesions observed.

The type of respiratory disease considered here is of multifactorial aetiology, and it is difficult to determine the precise roles of the various factors, such as stress, virus, mycoplasma and/or bacteria. Representatives of this group are:
- *Mycoplasma ovipneumoniae*.
- *Mycoplasma mycoides* subsp. *mycoides* (small colony type), which, in conjunction with stressors, can produce lobar pneumonia in regions where contagious bovine pleuropneumonia still occurs (85).
- *Mycoplasma arginini*, very frequently isolated from lung lesions, but the subject of much controversy concerning its pathogenicity.

Finally, *Mycoplasma bovis* and *M. agalactiae*, very occasionally recovered from lungs, but of doubtful importance.

**Inapparent and atypical mycoplasmoses**

The species included here seem to be members of the normal microbial flora of mucous membranes, and do not seem to be directly pathogenic. They count as laboratory ‘finds’ and their importance in disease processes is probably small and perhaps even non-existent. They comprise members of the genera *Acholeplasma* and *Ureaplasma*.

**HISTORY**

In 1979 Perreau reviewed the caprine mycoplasmoses (87) and traced the principal steps in our knowledge of these diseases. Without going into detail (see Table II), two points deserve attention:
1. Most of the strains have been isolated since 1950, which explains why their taxonomy is still in dispute. However, it is reasonable to predict that the latest techniques, such as DNA homology and the electrophoretic profile of their proteins, will lead to a classification.

2. Most of the mycoplasmoses described so far have been in goats. Sheep are much less affected by these micro-organisms, particularly those possessing primary pathogenicity. It is evident that this difference between sheep and goats has important implications for epidemiology.

Only *M. ovipneumoniae* is isolated frequently in both sheep and goats.

**Table II**

Principal stages in the study of mycoplasmoses of small ruminants

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1816</td>
<td>Description of contagious agalactia by Metaxa in Italy</td>
</tr>
<tr>
<td>1873</td>
<td>Report of contagious pleuropneumonia of goats in N. Africa</td>
</tr>
<tr>
<td>1881</td>
<td>Hutcheon described contagious caprine pleuropneumonia in South Africa</td>
</tr>
<tr>
<td>1923</td>
<td>Bridré and Donatien cultured the agent of contagious agalactia, to which Wroblesky (1931) gave the name <em>Anulomyces agalaxiae</em></td>
</tr>
<tr>
<td>1937</td>
<td>Debonera studied the ‘Sparta goat disease’ in Greece, which he considered to be a form of contagious agalactia</td>
</tr>
<tr>
<td>1949</td>
<td>Isolation of <em>M. mycoides</em> var. <em>capri</em> from an outbreak of caprine pleuropneumonia in Turkey</td>
</tr>
<tr>
<td>1951</td>
<td>Longley isolated the agent of contagious caprine pleuropneumonia and called it <em>Borrelomyces peripneumoniae caprae</em></td>
</tr>
<tr>
<td>1955</td>
<td>Cordy described septicaemia with polyarthritis in kids. The agent, <em>M. capricolum</em>, was not identified until 1974</td>
</tr>
<tr>
<td>1955</td>
<td>Hanko and Otterlin reported from Sweden an infectious enteritis of sheep and goats, accompanied by necrotic mastitis</td>
</tr>
<tr>
<td>1956</td>
<td>Laws described an infectious peritonitis in Australia</td>
</tr>
<tr>
<td>1967</td>
<td>El Nasri obtained strains indistinguishable from the agent of CBPP from goats in Sudan</td>
</tr>
<tr>
<td>1976</td>
<td>MacOwan and Minette isolated strain F38 from outbreaks of caprine pleuropneumonia</td>
</tr>
</tbody>
</table>

According to P. Perreau (87)

**ECONOMIC IMPORTANCE**

It is difficult or even impossible to estimate the losses caused by pulmonary mycoplasmoses, because in general they are associated with other infectious agents, particularly viruses. However, in the case of primary mycoplasmoses numerous examples show how costly the disease can be in a flock.

Thus Perreau (86) found that 30% of kids died from septicaemia during an outbreak of infection with *M. mycoides* subsp. *mycoides* (large colony type). In another outbreak of *M. capricolum* infection, 103 of 105 kids died suddenly, with the disease appearing among the adult goats shortly afterwards.
Gupta et al. (37) recorded a mortality rate of 75% among kids affected with pleuropneumonia attributed to M. mycoides subsp. capri, M. mycoides (LC), M. capricolum and M. ovipneumoniae.

Similarly, MacOwan (63, 67) estimated that the mortality rate of outbreaks of CCPP caused by strain F38 was about 60-70%, and could occasionally reach 100%. These rates tally with those of Ojo (79) of 50-60% for goat pneumonia in Nigeria.

Antibiotic therapy is now definitely capable of reducing mortality considerably, but the actual losses (cost of treatment, production losses, growth retardation) are all the more difficult to assess.

Nevertheless, the seriousness of primary or secondary pulmonary mycoplasmoses has increased considerably in countries where the rearing of dairy goats has become more intensive. As in the case of other mycoplasmoses of animals, conditions created by large-scale, intensive husbandry greatly encourage the spread of pulmonary mycoplasmoses of small ruminants.

**GEOGRAPHICAL DISTRIBUTION**

Infections due to M. capricolum and M. mycoides subsp. mycoides (LC) occur worldwide. They are recorded more frequently in Europe and the USA, but are also observed in Africa and Australia.

M. mycoides subsp. capri seems to be rarer, but has nevertheless been isolated in America, Europe (Sweden), Asia and Africa.

M. ovipneumoniae and M. arginini are found on the five continents, the former apparently being harboured by the upper respiratory tract of sheep (3).

There are two explanations for the worldwide distribution of these four species:

- Firstly, it is highly probable that they have become widely distributed during the past twenty years with the development of international trade in breeding animals.

- Secondly, improvements in diagnostic techniques have undoubtedly helped to reveal agents of infection long present in many countries but which have been overlooked because of a lack of appropriate investigative resources.

The particular case of caprine contagious pleuropneumonia should be given special mention. Table III, derived from the FAO/WHO/OIE Animal Health Yearbook for 1986, lists the countries which have notified outbreaks of this disease.

However, the term covers two separate diseases: contagious caprine pleuropneumonia due to strain F38, and contagious pleuropneumonia caused by M. mycoides subsp. mycoides (LC) or subsp. capri.

As shown by MacMartin et al. (61), only the first of these two is the ‘classical’ form of CCPP. It is largely confined to East and North Africa, whereas the disease occurring in America and Europe involving the M. mycoides subsp. is quite distinct, with different lesions (66).

*To avoid confusion two distinct names should be given to these infections.*


**TABLE III**

*Geographical distribution of caprine contagious pleuropneumonias*

<table>
<thead>
<tr>
<th>Incidence</th>
<th>Middle East and Arabian Peninsula</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Angola</td>
<td>+</td>
<td>Bahrain</td>
</tr>
<tr>
<td>Benin</td>
<td>+</td>
<td>Iran</td>
</tr>
<tr>
<td>Cameroon</td>
<td>+++</td>
<td>Iraq</td>
</tr>
<tr>
<td>Chad</td>
<td>++</td>
<td>Jordan</td>
</tr>
<tr>
<td>Djibouti</td>
<td>?</td>
<td>Kuwait</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>+</td>
<td>Lebanon</td>
</tr>
<tr>
<td>Guinea Bissau</td>
<td>+</td>
<td>Oman</td>
</tr>
<tr>
<td>Kenya</td>
<td>+</td>
<td>Qatar</td>
</tr>
<tr>
<td>Libya</td>
<td>+</td>
<td>Saudi Arabia</td>
</tr>
<tr>
<td>Mali</td>
<td>?</td>
<td>Turkey</td>
</tr>
<tr>
<td>Mauritania</td>
<td>+</td>
<td>United Arab</td>
</tr>
<tr>
<td>Niger</td>
<td>?</td>
<td>Emirates</td>
</tr>
<tr>
<td>Nigeria</td>
<td>?</td>
<td>Yemen (Arab Republic)</td>
</tr>
<tr>
<td>Senegal</td>
<td>+</td>
<td>Yemen (P.D.R.)</td>
</tr>
<tr>
<td>Somalia</td>
<td>++ ( )</td>
<td></td>
</tr>
<tr>
<td>Sudan</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>Tunisia</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Uganda</td>
<td>?</td>
<td>Bangladesh</td>
</tr>
<tr>
<td>Zaire</td>
<td>?</td>
<td>India</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nepal</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pakistan</td>
</tr>
<tr>
<td>Americas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brazil</td>
<td>+ ? ( )</td>
<td>Greece</td>
</tr>
<tr>
<td>Dominica</td>
<td>+</td>
<td>Malta</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Source: FAO/WHO/OIE Animal Health Yearbook, 1986*

*Key: ( + ) exceptional occurrence + ? serological evidence only, no clinical disease*  
+ low sporadic occurrence ? suspected but not confirmed  
+++ enzootic () confined to certain regions  
+++ high occurrence |( ubiquitous  

**II. CLINICAL ASPECTS**

**CONTAGIOUS CAPRINE PLEUROPNEUMONIA (CCPP)**

This is one of the most important pneumonic infections of goats. Sheep are not affected under natural conditions. According to MacMartin *et al.* (61), the disease has been reported in more than thirty-one countries. These workers argue that 'classical' CCPP is caused only by strains of 'F38' mycoplasma, because the disease caused by the organism is readily transferred by contact to susceptible goats, while sheep and cattle are not affected; also, after subcutaneous inoculation, goats do not develop local oedematous reactions. The organism has since been isolated from natural cases of CCPP in the Sudan (41), Tunisia (88), Oman and from Turkish goats (53). F38 mycoplasmas are probably a new group of caprine mycoplasmas (25). The notion
that only F38-like mycoplasmas cause ‘classical’ CCPP is now accepted by mycoplasmologists.

In natural infection, the organism is acquired by inhalation of droplets from infected goats in close contact with susceptible ones (42, 70). The incubation period is between six and ten days in animals experimentally infected by the intratracheal-endobronchial route (43, 67). Hutcheon (1881, cited by MacMartin et al., 61) showed that in-contact susceptible goats fell ill after ten days. However, the incubation period is sometimes longer than ten days. MacOwan and Minette (70) also showed that fever developed after contact with febrile experimental CCPP cases in 26 ± 15 days. The average peak of temperature occurring within 48-72 hours of the onset of fever is 41.4°C, and the febrile condition remains over 40°C for four to nine days.

During this period coughing and painful respiration develop. The affected animals tend to lie down or lag behind the rest of the flock. The animals are disinclined to move, they maintain a posture with the forelimbs well separated and the head held low. They continue to feed for some time, but eventually there is laboured breathing, painful grunting and depression. In terminal cases the mouth is often kept open, with the lips covered with froth. Respiratory movements are accompanied by loud gurgling or grunting sounds. The tongue protrudes and the animals bleat distressingly. In acute cases, particularly in animals with primary infection, the illness lasts for about two days, while in other cases it may last several days.

In the absence of any control measures, morbidity in affected flocks is 100% and mortality is up to 70%. The organism is not reported to affect other body systems apart from the respiratory tract of goats.

OTHER PULMONARY MYCOPLASMA INFECTIONS

Pulmonary infections of small ruminants are often associated with *M. mycoides* subsp. *capri*, *M. mycoides* subsp. *mycoides*, *M. ovipneumoniae*, *M. arginini*, *M. agalactiae* and *M. bovis*.

*M. mycoides* subsp. *capri* has been isolated from natural pleuropneumonia of goats (22, 23, 77). Sheep are not naturally infected but the animals can be infected experimentally.

*M. mycoides* subsp. *mycoides* (the agent of contagious bovine pleuropneumonia) has been isolated from cases of pleuropneumonia in goats (15, 77, 85). The organism is also pathogenic for sheep, but has only rarely been reported in this species (81). Natural infection with the *M. mycoides* group in goats does not produce ‘classical’ CCPP.

The incubation period is stated by several workers as being between two and twenty-eight days. Longley (60) put the incubation period, on the basis of experimental transmission, at ten to fourteen days, and Solana and Rivera (107) at between three and eight days. Ojo (78) and MacOwan (63), using the endobronchial route, showed that acute pneumatic symptoms occurred in two to six days. It would appear that the incubation period varies with the route of experimental infection. Under natural conditions the incubation period is likely to be longer because of the influence of defence mechanisms of the body.
After the incubation period, symptoms of pleuropneumonia develop rapidly. The body temperature rises sharply to 41-42°C and may remain high for some days before death 2-14 days later. The affected animals show some symptoms similar to those of CCPP. In acute cases they show respiratory distress with the abdominal type of breathing. There may be coughing, during which the animal stands with forelimbs spread out, and is unwilling to move. The neck may be extended as if gasping for air. The affected animal continues to eat, though it may not put on weight. When the animal is forced to move, it may collapse and die due to respiratory failure. Mortality varies with the strain of the organism; the Smith strain of *M. mycoides* subsp. *capri* often produces 100% mortality. With other strains, the mortality may be 30% or less. Morbidity is generally 100%.

The *M. mycoides* group, notably *M. mycoides* subsp. *mycoides*, causes septicemia, and organisms can be isolated from lung, liver, blood, spleen, kidney, joint fluid and milk of fatal cases.

Acute fibrinous pleuropneumonia can develop in experimentally infected sheep. The clinical signs are similar to those shown by infected goats.

*M. ovipneumoniae* is probably a world-wide inhabitant of the respiratory tract of sheep and has been isolated from apparently healthy as well as diseased sheep (71). It has been isolated from sheep in Australia (111), Great Britain (28), Sudan (41), Nigeria (74), New Zealand (4) and many other countries.

Clinically, *M. ovipneumoniae* produces chronic respiratory infection which progresses slowly. The infection is often complicated by *P. haemolytica*. Interstitial pneumonia is characteristic of the infection. When complicated by *P. haemolytica*, proliferative lung changes may be produced (31).

Affected animals usually fail to gain weight, and there may be occasional coughing. The clinical picture does not permit differentiation from other mycoplasma infections.

*M. ovipneumoniae* has been isolated from the respiratory tract of goats (16, 40, 74). Subacute suppurative bronchiolitis and alveolitis were produced in young goats inoculated endobronchially with a culture of *M. ovipneumoniae* by Goltz et al. (35). Coughing, respiratory distress and radiographic evidence of lung consolidation were seen in one experimental goat.

*M. arginini* has been isolated from the respiratory tract of sheep (27) and goats (74, 77). The role of this organism as a primary pathogen is doubtful.

*M. bovis* has been isolated from the respiratory tract of goats (80), but it is incapable of producing pneumonia in goats. *M. agalactiae* has been isolated from pneumonic lungs of goats (74), but its role in pneumonia is not clear.

**DIFFERENTIAL DIAGNOSIS**

The symptoms of acute *P. haemolytica* infections in goats may resemble those of acute pleuropneumonia due to the *M. mycoides* group. Differential diagnosis in live animals can be assisted by taking a sample of fluid from the thorax. The fluid is examined under phase-contrast microscopy, mycoplasmas being identifiable by their characteristic morphology. Since *M. mycoides* subsp. *mycoides* and *P. haemolytica*...
produce septicaemia, blood cultures may yield these bacteria on appropriate culture media. *M. mycoides* subsp. *mycoides* infections, as seen in North America or Europe, may result in polyarthritis as well as pneumonia. Polyarthritis has not been described in African goats (56).

### III. DIAGNOSIS

The aetiological diagnosis of respiratory disease in small ruminants suspected as involving a mycoplasma can only be based on microbiological and, perhaps, serological evidence. Clinical diagnosis of the respiratory mycoplasmoses is confounded by immense variations which may be observed in symptom expression, morbidity and mortality, depending on the genetic constitution, age and management of the animals at risk, the novelty of the organism to them, the strain of the organism involved, stress factors (e.g. dehorning, castration) and the occurrence of intercurrent diseases such as PPR, heartwater, coccidiosis and type B *Clostridium perfringens* enterotoxaemia. Necropsy and histopathology, though capable of contributing to a diagnosis, are not definitive either, due to the limited range of responses of which the lung is capable. Despite these considerations, some generalisations can be made about the clinical and pathological manifestations for each mycoplasma species capable of causing respiratory disease in small ruminants:

- *M. mycoides* subsp. *mycoides* (LC): polyarthritis and mastitis are regularly observed in outbreaks (19, 101, 104). Lungs show interlobular oedema (96, 98).
- *M. mycoides* subsp. *capri*: exact importance and prevalence now uncertain. Perhaps a cause of sporadic outbreaks of pneumonia involving occasional deaths and chronic disease in survivors.
- Strain F38-like group: generally now considered to be the major or sole cause of typical, acute CCPP (61). Causes intralobular oedema in lungs (55). No other organs are affected. Sheep are entirely refractory to the organism.
- *M. capricolum*: sporadic fatal pneumonia in young kids; pleurisy not a feature (18). Arthritis and mastitis are the more common manifestations (90).
- *M. ovipneumoniae* (and *P. haemolytica* or *P. multocida*): commonly causes a chronic pneumonia with occasional deaths (32, 52, 110). Seen as anterior lung lobe lesions in the abattoir (3). An acute, often fatal pneumonia apparently involving only *M. ovipneumoniae* also occurs naturally in goats and has been reproduced experimentally in this animal (35).

Two other mycoplasma species, namely *M. arginini* and *A. laidlawii*, are commonly isolated from the respiratory tract of goats and sheep: *M. agalactiae*, *A. oculi* and *M. conjunctivae* are less frequently isolated from this site (52). None of these appears to be capable of producing or exacerbating pneumonic lesions. Several other mycoplasma species have been recorded from the lungs of goats and sheep, but most (if not all) were probably aberrant occurrences, and non-pathogenic for these hosts.

### MICROSCOPY OF EXUDATES AND IMPRESSION SMEARS MADE FROM LUNG LESIONS

The two *M. mycoides* subspecies and F38-like organisms all show branching filamentous morphology *in vivo*. They can be seen in lesion exudates or suspensions
by dark-ground microscopy; alternatively, they can be rendered visible in impression smears from cut lesions by the staining method of May-Grünwald-Giemsa. *M. capricolum* and *M. ovipneumoniae* both produce coccobacillary or very short filamentous forms in broth cultures. *In vivo*, *M. ovipneumoniae* is coccobacillary in form and difficult to distinguish in lesion smears from cell debris and other microorganisms. Though the *in vivo* appearance of *M. capricolum* has not yet been described, it is probably similar to that of *M. ovipneumoniae*.

**GEL PRECIPITIN TESTS TO DETECT ANTIGEN IN SAMPLES**

These tests are used in the diagnosis of CBPP (72), and have been found to be effective for *M. mycoides* subsp. *mycoides* (LC) infection in sheep (81). The tests are based on the precipitation in agar of soluble antigens of subsp. *mycoides*, in particular the galactan produced by the organism, using specific hyperimmune serum. The first forms of the precipitin test for CBPP were the agar gel diffusion precipitin test of White (116) and the interface precipitin test of Turner (114). More recent modifications, though not necessarily improvements (34), are the radial immunodiffusion test of Windsor (117) and the counter-current immunoelectrophoresis test of Philpott and Onoviran (94).

The applicability of precipitin tests to *M. mycoides* subsp. *capri*, F38-like organisms and *M. capricolum* does not appear to have been evaluated. None of these organisms has been shown to produce galactan, though subsp. *capri* elaborates glucan, a similar polysaccharide. The possibility that diffusible antigens are released *in vivo* by any or all of the three mycoplasmas, and that these are detectable with specific hyperimmune serum in a precipitin test, warrants investigation.

**ISOLATION OF MYCOPLASMAS**

**Collection of samples**

Samples from live animals comprise nasal and ear canal swabs; no information is available on the culturing of blood from naturally-infected sheep and goats.

Nasal and ear canal swabs cannot be used to identify individual pneumatic animals. As with pasteurellae, nasal carriage of mycoplasmas can, and often does, occur in the absence of pneumonia. Conversely, mycoplasmas may be undetectable, at least on a single sampling, in the nasal sinuses of an animal with respiratory mycoplasmosis, depending on the species of mycoplasma involved and the stage of the disease. Positive ear canal swabs merely indicate carrier status (17). Furthermore, both types of swab frequently reveal mixed mycoplasmal infections; identification of the species relevant to the disease under investigation cannot be made on titres of isolation. Despite these drawbacks, sampling of a herd or flock can provide valuable information. Nasal swabs can indicate the likely cause of an outbreak of pneumonia, and the morbidity of infection; and ear canal swabs can identify seronegative, symptomless carriers of pathogenic mycoplasmas. Immediately after collection, all swabs should be placed in transport or mycoplasmal medium.
The best necropsy samples are lung lesions, particularly from the interface between consolidated and unconsolidated areas, and pleuritic fluid. Bronchial swabs are equally effective for *M. ovipneumoniae*, but less so for the mycoplasmas associated with CCPP or CCPP-like disease. Other samples, in particular bronchial and mediastinal lymph nodes, tracheal epithelium, tonsillar tissue, blood and the other viscera, may yield mycoplasmas, particularly in cases of infection with the *M. mycoides* species, but these are rarely examined diagnostically.

If microbiological examination cannot be performed immediately, samples or indeed whole lungs can be stored deep-frozen at -20°C for considerable periods (months) with little apparent loss in viability of the pathogens. Transport of samples should always aim at keeping them as cool as possible, since pathogen viability diminishes rapidly with increasing temperature.

Lung lesions can be dispatched to other laboratories in freeze-dried form.

**Treatment of samples**

Swabs are suspended in 2-3 ml of culture medium. Tissue samples are best chopped with scissors then shaken vigorously, or pulverised in medium (1 g of tissue to 9 ml of medium). Tissues should not be ground, since this releases mycoplasmacidal lysolecithins. The suspension is usually prepared with a mycoplasma medium, but if samples are fresh and parallel bacteriological examination is desired, a high-quality bacteriological medium such as nutrient broth may be used to provide a suspension suitable for both forms of examination.

Pleuritic fluid, or a suspension of tissue or swab, is diluted through at least three tenfold steps (to a nominal 10^{-4}) and preferably more in each mycoplasma medium selected. Dilutions from one medium series should also be plated on the selected solid media. Serial dilution of samples has four benefits: it reduces growth suppression of mycoplasmas by specific (antibodies) and non-specific (complement, lysozymes, lysolecithins, etc.) body products, and by antibiotics which may have been administered to the animal before death; it reduces the potential problem of bacterial contamination; it overcomes the potential problem of ‘swamping’ of fastidious mycoplasmas by more exuberant species; and it indicates the infection titres of the mycoplasma(s) concerned.

**Media**

No single mycoplasma medium is suitable for all mycoplasma species for reasons which are still not clear. The media described below (see Appendix) are not the only ones suitable for screening respiratory tract tissue of goats and sheep for mycoplasmas, and many more medium formulations are provided by Stalheim (108) and Razin and Tully (97).

**Media for isolating F38-like organisms**

The fact that the major cause of CCPP (61) eluded isolation and recognition until 1976 (68) indicates the difficulties in culturing F38-like organisms. Wild strains are particularly difficult to grow in primary isolation. The meat-liver medium used by MacOwan and Minette (68), termed "viande foie goat" (VFG), was an adaptation of the meat-liver medium described by Al-Aubaidi and Fabricant (1), incorporating, *inter alia*, goat tissue broth and goat serum. This medium presents
difficulties for diagnostic laboratories of limited resources, both in the production of goat meat-liver and in obtaining goat serum free of antibodies to F38-like organisms.

The WJ medium of Jones and Wood (53) avoids these problems by using commercially-available broths and serum. WJ medium was found to be superior to VFG, SP4, GS, OB/OA, DE, TPM, modified Hayflick and modified Friis media (see Appendix) (91, 97) in the cultivation of strain F38 and, in a more limited comparison (involving only VFG, OB and modified Hayflick medium), of wild strains of F38-like organisms from field cases of CCPP (53). Two other media which use commercially-available components, and have been used with success in culturing strain F38 and F38-like organisms, are AC and TPM (49); their formulations are also given in the Appendix.

Media for isolating other glycolytic mycoplasmas

Growth of M. ovipneumoniae (common in both sheep and goats) on WJ medium is poor; preferred media are OB/OA or modified Friis. M. mycoides (both subspecies), M. capricolum and acholeplasmas readily grow on most media.

Media for the differential growth of arginine-hydrolyzing mycoplasmas

Approximately one third of all recognised mycoplasmas, though only three caprine/ovine species or strains (M. arginini, M. capricolum and strain 2D), can hydrolyze arginine. Reduction of any of the media referred to above to pH 6.5-6.7, and supplementation with arginine (generally L-arginine hydrochloride to a final concentration of 1% w/v), indicates the presence of arginine-hydrolyzing mycoplasmas in a culture, and specifically enhances their growth, partly through the suppressive effect of high arginine concentrations on several glycolytic mycoplasma species (58, 115).

Cultivation strategy for diagnostic mycoplasmology

For complete diagnostic coverage of the typical mycoplasmas that may be found in the respiratory tract of goats and sheep, it is suggested that at least three broth and two agar media are used. One broth medium and its agar analogue should be specifically selected for the cultivation of F38-like organisms (e.g. WJ, AC or TPM). A second broth medium and its agar analogue should be selected for the growth of M. ovipneumoniae (e.g. modified Friis or OB/OA). This broth could be used with tetrazolium chloride instead of phenol red as indicator, but better diagnostic coverage would be provided by use of a third broth medium incorporating tetrazolium chloride, such as modified Hayflick. Finally, a broth medium (e.g. OB or modified Friis) with arginine supplement should be included for the growth of arginine-hydrolyzing mycoplasmas. Media for the specific isolation of ureaplasmas are unnecessary: ureaplasmas are rarely found, and are most certainly of no importance, in lung diseases of sheep and goats.

The use of several different media in routine diagnosis has four major benefits: the efficiency of mycoplasma isolation is greatly increased; each medium enhances the growth of certain mycoplasma species at the expense of others, thus assisting cloning and purification; recognition of mixed mycoplasma infections is considerably facilitated; and the most important step in biochemical characterization, namely identification of the organism(s) as being glycolytic, arginine hydrolytic, both or neither, is achieved.
Media production, storage and quality control

Certain medium components, particularly serum and yeast extract, require monitoring for growth-promoting capacity on a batch basis before incorporation in routine diagnostic media. The deionized water used in media production should also be carefully monitored on a regular basis. Malfunctioning deionization plants are thought to be a frequent cause of poor mycoplasma isolation rates. Harvested aliquots of low-passage field isolates of the relevant mycoplasmas are generally used for monitoring medium components for growth-promoting capacity.

Broth media may be made up in large quantities and stored for up to three months at −20°C. Penicillin or its analogues, if used, should not be added until final dispensing, as these antibiotics rapidly lose activity in contact with proteins. Broth media are normally dispensed into bijoux (1.8 ml or 2.7 ml) or screwcapped tubes (4.5 ml) and stored, for up to three weeks, at 4°C. Agar media are best made with agarose (0.9% w/v), Noble agar (1.5% w/v), or Purified agar (0.6% w/v). Plates, which are poured to a depth of about 8 mm, should be used as fresh as possible and stored for no more than two or three weeks at 4°C before use.

Cultivation

Cultures are incubated at 37°C. Plates are best incubated in a humidified atmosphere of 5% CO₂, 95% air or N₂, or in a candle jar with a moisture source.

Broth cultures are examined daily for evidence of growth, namely colour change (yellow or acid change produced by glycolytic mycoplasmas, red or alkaline change by arginine hydrolyzers) and the appearance of floccular material. This may be no more than a ‘swirl’ from the bottom of the culture container when agitated, though the more heavily-growing species (M. mycoides and M. capricolum) produce some turbidity. Gross turbidity is indicative of bacterial contamination; cultures showing this are passed through membrane filters of 0.45 µm average pore-diameter (apd) before subculture. Broth cultures are subcultured by inoculation of fresh broth media with one tenth of their volume, or by streaking solid media with a loop.

Plate cultures are generally examined every two or three days (mycoplasmas remain viable longer on agar than in broth) using a stereobinocular microscope with 5 to 50× magnification and transmitted and incident light sources. Subculture of colonies, which may be up to 2-3 mm in diameter, is by transfer of excised agar blocks bearing isolated colonies to either agar or broth. Transfer may also be effected by drawing an agar plug bearing one colony into a Pasteur pipette, and discharging this into fresh broth medium.

Cloning and purification of isolates is performed by repeated transfer of single colonies representing each morphological type seen on agar. The morphology of colonies varies with the medium used, the mycoplasma species, its passage level and age of culture. Colonies of M. ovipneumoniae are generally centreless, with a lacy appearance. Wild strains of F38-like organisms in early passage also produce a high proportion of small, centreless colonies, with bizarre-shaped centred colonies scattered through them. The other classical mycoplasma species found in goats and sheep demonstrate the conventional ‘fried egg’ colony morphology, though all can produce bizarre-looking colonies in early passage levels.
A procedure that aids purification is the filtration of broth cultures through 0.45 µm apd filters before subculture; this ensures that aggregates of cells, which may be of different species, do not get passaged.

Cultures suspected of being bacteria should be examined for reversion to bacterial form by passage at least three times on solid mycoplasma medium from which bacterial inhibitors have been omitted. In practice, this is rarely necessary if a good antibiotic is incorporated in the medium (see Appendix).

Primary isolation broth media which have shown no indication of growth by seven days are generally subcultured blind at this time. Negative plates may be restreaked on themselves with an L-shaped glass rod. Growth on plates without concomitant colour change in broths using phenol red as indicator indicates that a broth medium containing an alternative indicator (e.g. modified Hayflick with tetrazolium chloride) should be additionally used in subculture.

Cultures of each sample, including one blind subculture, should be retained for a minimum of three weeks before being discarded. Titrations in broths, if performed in full (10⁻⁸ to 10⁻¹⁰), are also read at three weeks, being expressed as colour-changing units (ccu) per transfer volume. Growth on plates is expressed as colony-forming units (cfu) per ml. Ccu titres are usually one to two log dilutions higher than cfu titres, especially on primary isolation of wild strains, due to the superior growth of mycoplasmas in broths compared with solid media.

**IDENTIFICATION OF MYCOPLASMAS**

Wild strains should be passaged, and preferably cloned, several times before identification is attempted, to ensure full adaptation to artificial media and reliability of results.

The methodologies of biochemical and serological identification tests for mycoplasmas have recently been collated by Razin and Tully (97), but specific references to test methods are also given in the text below.

**Biochemical tests**

Many tests for biochemical reactions have been applied to mycoplasmas (4) and recommendations relating to the characterization of new species incorporate many of these tests (109). However, no test, used either singly or collectively, unequivocally identifies an isolate as a species; this can only be done, at present, on serological grounds. Furthermore, intraspecific variation in some biochemical reactions is often considerable, rendering their application valueless. However, a limited number of biochemical reaction tests do perform a useful function in a diagnostic laboratory as a preliminary screening system. They serve to narrow down the likely identity of an isolate, thus saving time and reagents in the final, serological phase of identification, and they provide supportive data when serological results are equivocal.

The tests most commonly used are glucose breakdown, arginine hydrolysis, reduction of tetrazolium chloride (aerobically and anaerobically), 'film and spots' formation, phosphatase activity, serum digestion and digitonin sensitivity. The first four tests (excluding anaerobic tetrazolium chloride reduction, which can be
accomplished by overlaying the modified Hayflick medium with sterile liquid paraffin) are applied in any case, if the full media screen suggested for caprine/ovine mycoplasmas is employed. 'Film and spots', in which there is wrinkling of the agar surface and the development of black spots within the medium in the vicinity of colonies, is demonstrable with strains of mycoplasm positive for this characteristic on most, if not all, media which contain a sufficiency of serum. Thus only three tests require additional labour input. Of these, serum digestion is particularly useful in identifying the four most pathogenic species but not in distinguishing between them. Sensitivity to digitonin distinguishes members of the family Mycoplasmataceae from those of the family Acholeplasmataceae (30): the test is generally performed at the same time as growth inhibition tests (see below). Reactions to these seven tests of the eleven most important mycoplasm species found in goats and sheep (including M. putrefaciens, not hitherto recorded from the respiratory tract) are summarized in Table IV.

Serological methods of identification

The traditional, and still current, basis of mycoplasma identification is by serology. Biochemical reaction tests provide only supportive, not definitive, evidence of identity, and more sophisticated techniques such as DNA hybridisation or restriction endonuclease analysis have still to be developed and evaluated in veterinary mycoplasmology. Serological tests generally demonstrated clear-cut distinctions between the previously described mycoplasma species. Latterly, however, the isolation and recognition of increasing numbers of species and strains has, in its wake, revealed the existence of considerable serological cross-reactivity between some mycoplasmas. Species definition by classical serological methods has therefore become, in some cases, a difficult and blurred area.

These observations are particularly true of the caprine/ovine mycoplasmas, and specifically the so-called 'M. mycoides group'. This appears to comprise two clusters, one containing subsp. mycoides (both large and small colony forms) and subsp. capri, and the other containing M. capricolum, F38-like organisms and members of the so-called bovine serogroup 7 (25). Cross-reactions with all serological tests are particularly marked within each cluster, but extend between clusters, and may also involve mycoplasmas outside the group, for example M. equigenitalium (from horses) and M. primatum (from primates). For these reasons, serological identification of an isolate suspected as being one of the 'M. mycoides group' should preferably be by at least two of the tests described below and should incorporate as controls hyperimmune sera against as many members of the group as possible.

Growth inhibition test (GIT) (13, 20)

This is probably the most commonly used serological diagnostic test in mycoplasmology laboratories. It is the simplest to perform, most specific and least sensitive of the four tests described here. It depends on the direct inhibition of growth on solid media by specific hyperimmune serum of high potency: the GIT therefore primarily detects surface antigens, in common with the metabolism inhibition and immunofluorescence tests.

There are several disadvantages to the GIT:

a) Only very potent antisera are effective, and these are used undiluted; the test thus makes extravagant use of valuable reagents. Even with such sera, zones of inhibition may be no more than 2 mm wide, and contain 'breakthrough' colonies.
TABLE IV

Biochemical reactions of the mycoplasmas of goats and sheep

<table>
<thead>
<tr>
<th>Test</th>
<th>Mmm(LC)</th>
<th>Mmc</th>
<th>Mc-c</th>
<th>F38</th>
<th>M. ovi</th>
<th>M. arg</th>
<th>M. agal</th>
<th>M. conj</th>
<th>M. putr</th>
<th>A. laid</th>
<th>A. oculi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose fermentation</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Arginine hydrolysis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Tetrazolium reduction</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>Aerobic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-/+</td>
<td>W</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>W</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Anaerobic</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>W/+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>‘Film and spots’ formation</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Phosphatase activity</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Serum digestion</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Digitonin sensitivity</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: Mmm(LC): *M. mycoides* subsp. *mycoides* (Large Colony type)  
Mmc: *M. mycoides* subsp. *capri*  
Mc-c: *M. capricolum*  
M. ovi: *M. ovipneumoniae*  
M. arg: *M. arginini*  
M. agal: *M. agalactiae*  
M. conj: *M. conjunctivae*  
M. putr: *M. putrefaciens*  
A. laid: *A. laidlawii*  
W: Weak reaction
b) The test can be applied only to isolates which have become well adapted to growth on solid media. A cloned isolate is preferred, but is not always essential.

c) The test can be too specific, such that growth inhibition of heterologous strains within the same species may be poor or non-existent. Thus false-negatives may frequently be encountered with monospecific antisera. This applies particularly to *M. ovipneumoniae* (which demonstrates a broad interstrain serological spectrum), *M. capricolum* (though data concerning this species are limited at present) and occasionally the *M. mycoides* subspecies. For effective diagnosis, a pool of serum against several strains of these organisms should be used: for *M. ovipneumoniae* the strains should be derived from both sheep and goats if both species are to be examined microbiologically.

d) Negative results in homologous tests have also been observed in older cultures, especially of *M. mycoides* (25). This was attributed to accumulation of extramembraneous material by the mycoplasma cells, which obscured membrane-bound antigens.

e) False-positive reactions are also encountered, particularly between members of the ‘*M. mycoides* group’, and between several species of *Acholeplasma*. The *M. mycoides* subspecies have also been found to cross-react in the GIT with *M. agalac­tiae* and *M. bovis*.

Despite these detractions, the GIT remains a valuable diagnostic test. For *M. ovipneumoniae* it is the best test, since the centreless colonies of this species cannot be subjected easily to the immunofluorescent test.

**Growth precipitation test (GPT)** (26, 57)

The GPT detects soluble cytoplasmic and extramembraneous antigens released by viable cultures and allowed to diffuse through solid mycoplasma growth medium towards various mycoplasma antisera. The interaction of antigens and specific antiserum produces one or more precipitin lines in the agar medium. The test is simple to perform, but lacks specificity and sensitivity; many diverse species of mycoplasma share cytoplasmic antigens in common. To quote Ernø and Peterslund (26), “A negative reaction indicates strongly that the two organisms in question (represented by culture and reacting antiserum) are not related at the species level. A positive reaction indicates a close relationship, but the strains are not necessarily of the same species”.

A combination of the GI and GP tests has been described (54). The precipitin reaction in this GIT/GPT is less readily identifiable than in the simple GPT, but under ideal circumstances the combined test can reduce the non-specificity of the GPT and increase the sensitivity of the GIT. The GIT/GPT is generally used when the identity of an isolate is suspected but not revealed by any of the other tests.

For both GPT and GIT/GPT (and the indirect fluorescent antibody test), it is essential that the anti-mycoplasma sera used be absorbed beforehand with lyophilized whole medium, to remove the anti-medium (generally medium serum) immunoglobulins that such antisera frequently contain.

**Indirect fluorescent antibody test (IFAT)** (99)

The direct fluorescent antibody test (DFAT) and the IFAT are probably the most effective of the various serological methods available for mycoplasma identification.
They are simple to perform yet more sensitive and sparing of antiserum than the GIT and GPT, and more versatile in application yet similar in specificity to the GIT and the metabolism inhibition test. Both DFAT and IFAT have been applied in several forms to the identification of mycoplasmas using fixed and unfixed colonies on agar, colony impressions, broth culture smears and tissue impressions or smears on slides, and cryostat sections of tissue. The form favoured by the author for the identification of isolates is the IFAT, using unfixed colonies on agar. This is slightly less specific than the DFAT, but easier to perform and possibly more sensitive.

Disadvantages of the IFAT are:

a) The cost of the epi-immunofluorescent UV microscope.

b) Difficulties and subjectivity in interpretation of results. Even with known pure cultures, only a proportion of colonies may demonstrate fluorescence, or fluorescence may be poor. It is essential to use agar cultures as young as possible. Autofluorescence by certain species (e.g. Acholeplasma laidlawii) may also be misleading.

c) The test cannot be applied to centreless colony species (e.g. M. ovipneumoniae).

Advantages of the test are:

a) Its high sensitivity and medium specificity (interstrain variations are rarely detectable) allow the use of single-strain antisera.

b) Serum potency is relatively unimportant.

c) Isolates do not require cloning. The test can therefore be applied to primary isolation plate cultures.

d) Identification can be achieved much more rapidly than with other serological tests.

e) Plate cultures need not be tested immediately, but may be stored, as soon as growth is observed, at 5°C for several days. This permits batch testing of several isolates at one time.

f) Isolates can be screened using pools of antisera (up to 20-30 monospecific sera per pool). Aberrant or unusual isolates can thus be rapidly screened and identified.

Metabolism inhibition (MIT) (95, 113) and tetrazolium reduction inhibition (TRIT) (106) tests

Metabolising glycolytic mycoplasmas change the pH of media to acid through breakdown of glucose to (mainly) lactic acid. Arginine-hydrolyzing mycoplasmas create an alkaline change by producing, finally, ammonia. These changes in medium pH are usually indicated by phenol red. The MIT is based, as its name indicates, on the inhibitory effect of specific serum on the growth, and therefore metabolism, of mycoplasmas as indicated by phenol red. The closely similar TRIT uses a different monitor of metabolism, namely the ability of some mycoplasmas (including M. agalactiae, M. capricolum and both M. mycoides subspecies) to reduce colourless tetrazolium chloride to red formazan. The TRIT is based on the same principle, and performed in virtually identical manner (in microtitration plates), to the MIT.
The MIT and TRIT are specific and generally sensitive. However, they are cumbersome to perform and unsatisfactory results are often obtained for various reasons. The time at which plates are read is critical, particularly in the MIT. Growth/metabolism may be over-exuberant, swamping any inhibitory effects of the antiserum, or insufficient, with variable colour change or formazan production in the wells. The plates used may contain inhibitory substances acquired during manufacture; these are eliminated by washing in ethanol then rinsing in deionized water. Plates must be fully sealed for good results.

Several attempts are often necessary to obtain satisfactory identification with the MIT or TRIT. Consequently, these tests are generally used, as with the GPT, only in the event of failure to obtain an identification with the GIT and IFAT.

EXAMINATION OF SERA FOR MYCOPLASMA ANTIBODIES

The three most frequently used serological tests in veterinary mycoplasmology have been the complement fixation (CFT), indirect haemagglutination (IHAT) and slide agglutination (SAT) tests. More recently described tests are the enzyme-linked immunosorbent assay (ELISA), the single reverse radial immunodiffusion test (SRRIT) and the similar single radial haemolysis test (SRHT). Other serological tests used in human mycoplasmology, including the GIT, MIT, IFAT and mycoplasmacidal test, have rarely been applied for epidemiological surveys in the veterinary field due to lack of sensitivity or difficulties in performance.

The diagnostic value of serology for identifying the cause of pneumonia outbreaks in goats and sheep remains largely unknown. The cross-reactivity of sera from animals experimentally or naturally infected with one member of the ‘M. mycoides group’ against antigens of other members of the group has not been sufficiently examined to permit conclusions on the ability or otherwise of serology to distinguish between infections with M. mycoides subsp. mycoides, subsp. capri, M. capricolum and F38-like organisms in the field. The situation is complicated by the fact that endemic infections, particularly with subsp. mycoides (100), subsp. capri (53) and M. ovipneumoniae (52), can produce a background of positive titres to these organisms in a proportion of apparently healthy animals. It is important, therefore, that serology be undertaken on a herd or flock basis, and that samples be paired whenever possible, being collected at the first visit to an outbreak and again three to eight weeks later.

The interpretation of serological findings should account for several diverse observations. One is that acute cases of CCPP rarely show positive titres to strain F38 up to the time of death (75). This is paralleled by a similar observation for subsp. mycoides antibodies in acute CBPP; it has been suggested that this is due to ‘eclipse’ of antibodies by circulating antigen. Another is that the times at which antibodies are first detectable, and peak, vary according to the mycoplasma involved. Goats in contact with others which had been experimentally infected with an F38-like strain were beginning to seroconvert (CFT titres of 1/16 to 1/128) after nine days and had titres of 1/512 to 1/4096 by day 22 (70). Muthomi and Rurangirwa (75) similarly showed that titres to an F38-like organism peaked at twenty-five to thirty days after experimental inoculation in both the CFT and IHAT (mean titres of 1/64 and 1/4096 respectively); this was followed by a rapid decline in titres to day 45. In contrast, sheep experimentally infected with M. ovipneumoniae and P. haemolytica were only just beginning to show rising titres to the mycoplasma by ELISA at two weeks after
inoculation; these titres reached a plateau eight weeks after inoculation (21). A third observation is that the development of positive titres does not necessarily imply the occurrence of disease. Goats in contact with others which had been experimentally infected with a strain similar to subsp. mycoides (LC) developed antibodies to the mycoplasma of 1/16 to 1/64 by the CFT, though none became clinically affected, or at necropsy had lung lesions or yielded the mycoplasma (64).

The CFT (10, 36, 75, 93, 105)

This is probably the most popular of the serological tests for veterinary use, though it has a major disadvantage in the high degree of technical skill required for its performance.

The CFT is more specific, though less sensitive, than the IHAT in the assay of antibodies both to subsp. mycoides (LC) (100) and to strain F38 (75). In both comparisons, specificity was judged by the presence of low or undetectable titres in normal animals. However, MacOwan and Minette (68) found a high degree of cross-reactivity in the CFT between strain F38 and hyperimmune sera to subsp. mycoides and capri. The antigen used was a whole cell preparation. In subsequent work MacOwan and Minette (70) used supernatant fluid from strain F38 cells which had been disrupted by repeated cycles of freezing and thawing. Other forms of antigen used in the CFT have been sonicated whole cells (strain F38; 75), supernatant fluid from a boiled suspension of cells (subsp. mycoides (LC); 100) and mycoplasma cells treated with 0.2% sodium dodecyl sulfate, then dialysed against phosphate-buffered saline (M. mycoides subsp. mycoides and M. capricolum; 59). The specificity of these preparations was not reported, but was probably low, because all included a high proportion of cytoplasmic antigens (24).

In applying the CFT to strain F38 antibodies in a sero-epidemiological survey of wild and domesticated livestock, Paling, MacOwan and Karstad (84) regarded titres of 1/32 or greater as positive.

The IHAT (11, 12, 75)

The IHAT is most commonly performed with erythrocytes which are either fresh and tanned, or treated with glutaraldehyde. The former is more sensitive, but suffers from the dual disadvantages of greater variability between tests and the requirement to sensitize cells with antigen each time the test is performed. Sensitized tanned erythrocytes are unstable and rapidly lose effectiveness. The use of glutaraldehyde-treated erythrocytes as antigen carrier reduces sensitivity, but makes the IHAT much more useful as a diagnostic test. Sensitized erythrocytes remain active for a year or more if kept at 5°C, and little further manipulation of this reagent is required before performance of the test. Chima and Onoviran (11), who used the test for M. mycoides subsp. mycoides (SC), also considered that glutaraldehyde treatment reduced cross-reactions by making the erythrocytes bind the more specific protein antigens, while tanned fresh erythrocytes detected antibodies against the polysaccharide fractions of M. mycoides.

The SAT

This test has been mainly used in veterinary mycoplasmology in the diagnosis of CBPP in cattle, and of chronic respiratory disease in poultry caused by M. gallisepticum. It depends on the clumping of whole mycoplasma cell antigen by specific
antibody, and can be performed in tubes, on slides or on a white tile using stained antigen. Its major virtues are simplicity and rapidity of performance. Its disadvantages are lack of sensitivity and specificity. Its applicability to the diagnosis of CCPP appears not to have been investigated.

The ELISA (8, 9, 21, 45, 76, 83)

This test was first applied in veterinary mycoplasmology to the diagnosis of enzootic pneumonia of swine caused by *M. hyopneumoniae* (9). It has since been used for several other animal mycoplasmoses, most notably *M. pulmonis* infection of laboratory rodents (45) and CBPP (83). The test depends on the adherence of proteins to plastic surfaces. Several forms of the test have been described; most now use microtitration plates. The simplest form comprises an initial coating of the plastic surface (e.g. microtitration plate, well or tube) with antigen, followed by addition of suitable dilutions of test and control sera, then anti-immunoglobulin (directed against the species and immunoglobulin class of interest) conjugated with an enzyme — e.g. alkaline phosphatase or horseradish peroxidase. The plates or tubes are thoroughly washed between each stage. Finally, substrate specific for the enzyme conjugate used is added. The substrate in degraded form is coloured, and the shade of colour (assayed photometrically) is related to the amount of enzyme remaining in the well or tube.

The ELISA is extremely sensitive and can cope with large numbers of samples when performed in microtitration plates. Its detractions are that it requires expensive apparatus to perform; results may take one to three days to obtain; it lacks specificity and can be capricious and unreliable. Improvements in performance and reliability of this relatively recently developed test, perhaps involving the use of monoclonal antibodies in an ‘antigen capture’ phase, can be expected.

The SRRIT (102) and SRHT (46, 48)

The SRRIT was described by Rurangirwa (102) as a means of detecting antibody to *M. mycoides* subsp. *mycoides* in CBPP. The single radial haemolysis test was used by Howard, Collins and Gourlay (48) for the detection of antibody to *M. bovis*; it has also proved satisfactory for several other mycoplasmas, including subsp. *mycoides* (46).

Both tests rely on the diffusion of antibodies present in the test sera (placed in wells cut into the agar) through agar containing antigen. In the SRRIT, soluble antigens are incorporated in the agar, and precipitin rings which form are recorded after overnight incubation. In the SRHT, the antigens (not necessarily soluble — whole cells may be used) are linked to ovine or bovine erythrocytes by a coupling agent such as chromic chloride: sensitized erythrocytes are suspended in the agar (indubiose was found to be best) at approximately 0.5% concentration, together with fresh guinea-pig serum. Results are recorded as zones of haemolysis after overnight incubation.

A major difference between the two tests is that the SRRIT detects non-complement-fixing antibodies, while the SRHT detects only complement-fixing antibody.

Both tests are reported to be sensitive, perhaps equivalent to the IHAT. Though their application to caprine/ovine mycoplasmas has not yet been described, their
**TABLE V**

*Suggested procedures for the isolation and identification of caprine/ovine mycoplasmas*

<table>
<thead>
<tr>
<th>SPECIMEN</th>
<th>Precipitin test for <em>M. mycoides</em> subsp. <em>mycoides</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incubation in 5% CO₂, 95% air or N₂, or candlejar</td>
</tr>
</tbody>
</table>

**Specimen**
- Phase contrast or dark ground microscopy of exudates; Giemsa stain of lung impression smear

**Broth media**
e.g.
- 1. VFG or WJB
- 2. modified Friis or OB
- 3. arginine broth
- 4. modified Hayflick with tetrazolium

At least 3 tenfold dilutions in each medium

**Cloning of isolated colonies**

**Biochemical tests**
- Glucose breakdown
- Arginine hydrolysis
- Tetrazolium reduction
- Digitonin sensitivity
- Production of 'film and spots'
- Phosphatase activity
- Proteolytic activity

**Serological tests**
- MIT or TRIT
- GIT
- IFAT
- GPT
- GIT/GPT

**Solid medium**
e.g.
- 1. VFG or WJA
- 2. modified Friis or OA

Solid medium without bacterial inhibitors to check for reversion to bacterial forms

**Modified from 'Methods in mycoplasmology', volume 1, Razin and Tully, 1983 (97).**
relative ease of performance (and therefore their suitability for a diagnostic laboratory) makes them worth examining in this field.

**RELATIVE MERITS OF THE DIFFERENT DIAGNOSTIC TECHNIQUES**

Diagnostic mycoplasmology is a time-consuming and often difficult discipline, the proper performance of which requires training and experience. Unfortunately, it is essential that any investigation of respiratory disease of goats and sheep should include, at least initially, acceptably comprehensive procedures for the cultivation and identification of mycoplasmas. Five species or strains of mycoplasma, and two species of pasteurella, may all be implicated in pneumonia of small ruminants, and only microbiological techniques can distinguish adequately between them. The serological techniques currently available are insufficiently specific, particularly for members of the 'M. mycoides group', to provide reliable evidence regarding the cause(s) of pneumonic outbreaks. Only when the organisms which occur in a locality or country have been identified can extensive use of serological techniques be made.

Serological tests offer a means of investigating the prevalence and morbidity of infections with mycoplasmas. They should be applied on a herd or flock basis; their use as a means of identifying infected individuals can be misleading, due to the common occurrence of false positive and negative reactions. The interpretation of serological results should always take into account the high degree of cross-reactivity between several of the mycoplasma species that can infect goats and sheep.

* * *

**APPENDIX**

* * *

**Chapter III - Diagnosis**

The formulations presented here give the variant of each medium as used by the author. Basic medium formulations have been subject to considerable variation by individual investigators, particularly with regard to serum (swine, horse and foetal or new-born calf are the most popular), broth bases (e.g. Bacto PPLO (Difco), Mycoplasma Broth Base (Pfizer and BBL), Heart Infusion, Brain-Heart Infusion, Hartleys Digest), agar (agarose, Noble agar, Purified agar, Ionagar) and bacterial/fungal inhibitors. Commonly used fungal inhibitors are thallous acetate (1/2,000-1/10,000) and nystatin (500 units/ml); either of these is used in conjunction with penicillin (250-1,000 units/ml) or ampicillin (0.1-1.0 mg/ml), which may be supplemented with either colimycin (100 µg/ml) or polymixin B (500 IU/ml); alternatively, a combination of methicillin and bacitracin (each at 0.15 mg/ml) may be used.

Included in some of the media given below is fresh yeast extract prepared as follows:

- 4 litres of deionized water are heated in a waterbath to 45°C. Fresh baker's yeast (500 g) is added to the water, and fragmented by hand. The suspension is then heated for 90 min to 80°C. Concentrated hydrochloric acid (20 ml) is added, and
the suspension is stirred at intervals while it is maintained at 80°C for a further 30 min. The suspension is centrifuged for 20 min at 2,000 rpm; the supernatant is passed through various clarifying and decreasing pore size filters until sterile-filtered through 0.22 µm apd (average pore diameter). The extract can be stored at -20°C for a maximum of four months before use.

Serum used in mycoplasma media is generally inactivated before use by heating at 56°C for 30 min to eliminate non-specific heat-labile inhibitory components.

Autoclaving of broths, agar, etc. is generally done at 121°C for 15 min.

**VFG Medium (63)**

The VFG base is prepared from 100 g muscle, 100 g goat liver and 120 g of fresh pig stomach. These are finely minced, and 1 litre of distilled water and 10 ml of concentrated hydrochloric acid are added while stirring. The suspension is incubated at 50°C for 24 hours, then heated to 80°C and filtered through clarifying filter paper. The filtrate is again heated to 80°C, then held at 4°C overnight. The cold solution is passed through filter paper, then adjusted to pH 7.6 with 10% (w/v) sodium hydroxide and warmed to 80°C. One per cent of buffer salt mixture (379 g anhydrous disodium phosphate and 90.8 g potassium dihydrogen phosphate) is added. The mixture is held at 4°C overnight, passed through filter paper, adjusted to pH 7.6 then sterile-filtered through Seitz filters or 0.22 µm apd membranes.

**Broth**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water</td>
<td>-</td>
</tr>
<tr>
<td>Noble agar*</td>
<td>-</td>
</tr>
<tr>
<td>Broth base</td>
<td>45 ml</td>
</tr>
<tr>
<td>Glucose (50% w/v)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Bacto-Yeast-Extract (Difco; 10% w/v)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Glycerol (neat)</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Goat serum (inactivated)</td>
<td>50 ml</td>
</tr>
<tr>
<td>Thallous acetate (5% w/v)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Penicillin (5 × 10^6 IU per 100 ml)</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Phenol red (0.1%)</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

**Agar**

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deionized water</td>
<td>20 ml</td>
</tr>
<tr>
<td>Noble agar*</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Broth base</td>
<td>45 ml</td>
</tr>
<tr>
<td>Glucose (50% w/v)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Bacto-Yeast-Extract (Difco; 10% w/v)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Glycerol (neat)</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Goat serum (inactivated)</td>
<td>30 ml</td>
</tr>
<tr>
<td>Thallous acetate (5% w/v)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Penicillin (5 × 10^6 IU per 100 ml)</td>
<td>0.2 ml</td>
</tr>
<tr>
<td>Phenol red (0.1%)</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

* Autoclaved with the deionized water before addition to the remaining components.

**WJB and WJA**

**Autoclaved portion**

<table>
<thead>
<tr>
<th>Component</th>
<th>WJB</th>
<th>WJA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto PPLO broth base</td>
<td>1.5 g</td>
<td>1.5 g</td>
</tr>
<tr>
<td>(without crystal violet; Difco)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacto-Tryptone (Difco)</td>
<td>1.5 g</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Bacto-Peptone (Difco)</td>
<td>0.3 g</td>
<td>0.3 g</td>
</tr>
<tr>
<td>Bacto-Yeast-Extract (Difco)</td>
<td>0.1 g</td>
<td>0.1 g</td>
</tr>
<tr>
<td>Deionized water</td>
<td>50 ml</td>
<td>55 ml</td>
</tr>
<tr>
<td>Agarose</td>
<td>-</td>
<td>0.9 g</td>
</tr>
</tbody>
</table>
Membrane filtered components

<table>
<thead>
<tr>
<th>Component</th>
<th>WJB</th>
<th>WJA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn calf serum (inactivated)</td>
<td>45 ml</td>
<td>40 ml</td>
</tr>
<tr>
<td>10 × Medium 199 without NaHCO₃, with glutamine (Gibco)</td>
<td>5.0 ml</td>
<td>5.0 ml</td>
</tr>
<tr>
<td>Fresh yeast extract</td>
<td>5.0 ml</td>
<td>5.0 ml</td>
</tr>
<tr>
<td>Calf thymus DNA (highly polymerised; 0.2% w/v)</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Glucose (50% w/v)</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>NADH (10% w/v)</td>
<td>0.1 ml</td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Ampicillin (100 mg/ml)</td>
<td>0.25 ml</td>
<td>0.25 ml</td>
</tr>
<tr>
<td>Thallous acetate (10% w/v)</td>
<td>0.25 ml</td>
<td>0.25 ml</td>
</tr>
<tr>
<td>Phenol red (0.4% w/v)</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
</tr>
</tbody>
</table>

Adjusted to pH 7.6-7.8 with 1 M NaOH.

AC (49)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain-Heart Infusion broth</td>
<td>14.8 g</td>
</tr>
<tr>
<td>Lactalbumin hydrolysate (Difco)</td>
<td>2 g</td>
</tr>
<tr>
<td>Bacto-Yeast-Extract (Difco)</td>
<td>2 g</td>
</tr>
<tr>
<td>NAD</td>
<td>0.01 g</td>
</tr>
<tr>
<td>Balanced salt solution</td>
<td>200 ml</td>
</tr>
<tr>
<td>Deionized water</td>
<td>200 ml</td>
</tr>
<tr>
<td>Horse or goat serum</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

TPM (49)

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto-Tryptose (Difco)</td>
<td>10 g</td>
</tr>
<tr>
<td>Bacto-Yeast-Extract (Difco)</td>
<td>3 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>1 g</td>
</tr>
<tr>
<td>Na₂PO₄ (anhydrous)</td>
<td>1.25 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>2.5 g</td>
</tr>
<tr>
<td>PPLO serum fraction</td>
<td>5 ml</td>
</tr>
<tr>
<td>Eagles vitamins (100 ×)</td>
<td>0.25 ml</td>
</tr>
<tr>
<td>Deionized water</td>
<td>400 ml</td>
</tr>
<tr>
<td>Horse or goat serum</td>
<td>100 ml</td>
</tr>
</tbody>
</table>

Modified Friis Medium (29)

Autoclaved portion

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 × Hanks balanced salt solution</td>
<td>3.0 ml</td>
</tr>
<tr>
<td>Deionized water</td>
<td>72 ml</td>
</tr>
<tr>
<td>Bacto-Brain-Heart Infusion (Difco)</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Mycoplasma Broth Base</td>
<td></td>
</tr>
<tr>
<td>(BBL Microbiology Systems, Cockeysville, MD, USA)</td>
<td>0.75 g</td>
</tr>
<tr>
<td>Lactalbumin hydrolysate</td>
<td>0.125 g</td>
</tr>
<tr>
<td>Yeast-Extract (Difco)</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Phenol red (0.1% w/v) solution</td>
<td>1.37 ml</td>
</tr>
</tbody>
</table>
Membrane filtered components

<table>
<thead>
<tr>
<th>Component</th>
<th>OB</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh yeast extract</td>
<td>3.65 ml</td>
<td></td>
</tr>
<tr>
<td>Glucose (50% w/v)</td>
<td>0.25 ml</td>
<td></td>
</tr>
<tr>
<td>Thallous acetate (2% w/v)</td>
<td>0.55 ml</td>
<td></td>
</tr>
<tr>
<td>Horse serum</td>
<td>10.0 ml</td>
<td></td>
</tr>
<tr>
<td>Swine serum (inactivated)</td>
<td>10.0 ml</td>
<td></td>
</tr>
</tbody>
</table>

**OB/OA Medium (28, modified)**

<table>
<thead>
<tr>
<th>Component</th>
<th>OB</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain-Heart Infusion (3.5% w/v)</td>
<td>20 ml</td>
<td>30 ml</td>
</tr>
<tr>
<td>Deionized water</td>
<td>54 ml</td>
<td>27 ml</td>
</tr>
<tr>
<td>Agarose</td>
<td>-</td>
<td>0.9 g</td>
</tr>
</tbody>
</table>

Membrane filtered components

<table>
<thead>
<tr>
<th>Component</th>
<th>OB</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swine serum (inactivated)</td>
<td>10 ml</td>
<td>30 ml</td>
</tr>
<tr>
<td>Fresh yeast extract</td>
<td>10 ml</td>
<td>10 ml</td>
</tr>
<tr>
<td>10 x Medium 199 without NaHCO₃, with glutamine (Gibco)</td>
<td>6.0 ml</td>
<td>3.0 ml</td>
</tr>
<tr>
<td>Ampicillin (100 mg/ml)</td>
<td>0.25 ml</td>
<td>0.25 ml</td>
</tr>
<tr>
<td>Thallous acetate (10% w/v)</td>
<td>0.25 ml</td>
<td>0.25 ml</td>
</tr>
<tr>
<td>Phenol red (0.4% w/v)</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
</tr>
</tbody>
</table>

Adjusted to pH 7.6-7.8 with 1 M NaOH.

**Modified Hayflick Medium (108)**

<table>
<thead>
<tr>
<th>Component</th>
<th>OB</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacto PPLO Broth without crystal violet (Difco)</td>
<td>2.1 g</td>
<td></td>
</tr>
<tr>
<td>Deionized water</td>
<td>70 ml</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>OB</th>
<th>OA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn calf serum (inactivated)</td>
<td>20 ml</td>
<td></td>
</tr>
<tr>
<td>Fresh yeast extract</td>
<td>10.0 ml</td>
<td></td>
</tr>
<tr>
<td>Calf thymus DNA (highly polymerised;0.2% w/v)</td>
<td>1.0 ml</td>
<td></td>
</tr>
<tr>
<td>Ampicillin (100 mg/ml)</td>
<td>0.25 ml</td>
<td></td>
</tr>
<tr>
<td>Thallous acetate (10% w/v)</td>
<td>0.25 ml</td>
<td></td>
</tr>
<tr>
<td>Triphenyl tetrazolium chloride (2% w/v)</td>
<td>1.0 ml</td>
<td></td>
</tr>
</tbody>
</table>

Adjusted to pH 7.6-7.8 with 1 M NaOH.

Broth for the promotion and recognition of arginine-hydrolyzing mycoplasmas

Modified Friis, OB or WJB may be used. A stock solution of L-arginine hydrochloride (20% w/v in deionized water) is included as 5% of the medium (final concentration of L-arginine HCl 1% w/v). The medium is adjusted to pH 6.5-6.7 with 1 M HCl.
IV. EPIDEMIOLOGY

DESCRIPTIVE EPIDEMIOLOGY

Contagious caprine pleuropneumonia (CCPP) caused by strain F38

From the first outbreak of CCPP in South Africa described by Hutcheon (cited by MacMartin et al., 61), the dramatic course of the disease has been clear: in nineteen herds totalling over 7,000 goats, the disease broke out a week after new animals had been introduced, and all the goats became affected within a few weeks. The mortality rate reached 68%, although in other herds infected subsequently it was only 40%. Hutcheon underlined the following two characteristics of the infection:

- the necessity for direct contact between animals;
- the possibility of a chronic carrier state of long duration, since the imported goats had been travelling for eight weeks, and were apparently healthy upon arrival.

More recently, in outbreaks recorded in Tunisia, Perreau (88) found that it took quite a short period, two to three weeks, for the disease to develop in a herd.

Contagious caprine pleuropneumonia caused by M. mycoides subsp. capri

The evolution of this infection is usually less dramatic than that caused by strain F38. It occurs one to three weeks after contact infection, for example after the regrouping of goats, and affects only a small proportion of the population.

In Mexico, Solana and Rivera (107) recorded a morbidity rate of 15-20% and a mortality rate of 10%.

Nevertheless, certain strains of subspecies capri seem to be very virulent, sometimes incurring a mortality rate of 100%, as noted by Ojo in Nigeria (77).

Pneumopathies caused by M. capricolum and M. mycoides subsp. mycoides (LC)

These organisms do not have a special affinity for lung tissue. The pneumonia which they provoke occurs mainly in young animals, usually following an outbreak of the contagious agalactia syndrome (mastitis, arthritis) among the adults.

According to Perreau (86), the following clinical forms are seen during an outbreak caused by subsp. mycoides:

- among adults: mastitis with severe general illness;
- among young animals: frequent symptomless septicaemia; acute polyarthritis (the commonest form); sometimes pneumonia or pleuropneumonia.

Other pulmonary infections

Their evolution is very variable, because it depends on predisposing factors, the environment and bacterial complications.
ANAlytical Epidemiology

Aetiology

Pathogenicity

Three groups of mycoplasmas are responsible for pulmonary mycoplasmoses:

a) Those possessing primary pathogenicity with exclusive or predominant pulmonary tropism.

- *Mycoplasma* strain F38: This seems to be the sole agent of ‘classical’ CCPP. MacOwan has shown that only strains of F38 fulfil Koch’s postulates, since this agent is present in every case of the disease and in no other diseases (67); it has been isolated in pure culture from every case examined; all the symptoms and lesions can be reproduced by inoculating the agent into a susceptible animal. He concluded that the name CCPP should be used only for infection by F38 (Table VI).

<table>
<thead>
<tr>
<th>TABLE VI</th>
<th>Comparison of strain F38 with the subspecies of Mycoplasma mycoides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain F38</td>
<td>Subspecies <em>capri</em> and <em>mycoides</em> (LC)</td>
</tr>
<tr>
<td>Route of inoculation</td>
<td>Endobronchial aerosol</td>
</tr>
<tr>
<td>Lung lesions</td>
<td>Intralobular, interstitial oedema</td>
</tr>
<tr>
<td>Species</td>
<td>Only goats</td>
</tr>
</tbody>
</table>

- *M. mycoides* subsp. *capri*: Described as the agent of ‘historic’ CCPP, this mycoplasma has a tropism which is essentially pulmonary, but it may also induce mastitis and arthritis.

b) Those possessing primary pathogenicity, but with only occasional pulmonary tropism. These are the agents of the contagious agalactia syndrome, *M. mycoides* subsp. *mycoides* (LC) and *M. capricolum* (Table VII).

<table>
<thead>
<tr>
<th>TABLE VII</th>
<th>Comparison of mycoplasmas responsible for contagious agalactia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species affected</td>
<td>Affinity for eyes, joints and mammary gland evolution</td>
</tr>
<tr>
<td><em>Mycoplasma agalactiae</em></td>
<td>Goats &amp; sheep</td>
</tr>
<tr>
<td><em>Mycoplasma capricolum</em></td>
<td>Goats &amp; sheep</td>
</tr>
<tr>
<td><em>M. mycoides</em> subsp. <em>mycoides</em> (LC)</td>
<td>Goats (rarely sheep)</td>
</tr>
</tbody>
</table>

According to P. Perreau (92)
Note that it is possible to grade these organisms according to their tropism, progressing from the contagious agalactia syndrome to contagious pleuropneumonia, as indicated in the figure below:

![Contagious organisms diagram]

- **Mycoplasma agalactiae**  
(Mycoplasma sp.) (type 2 D ?)
- **Mycoplasma capricolum**
- **Mycoplasma mycoides** subsp. **mycoides** (LC)
- **Mycoplasma mycoides** subsp. **capri**
- **Mycoplasma sp.** type F38

**c) Those possessing secondary pathogenicity:**

- **M. ovipneumoniae**: The pathogenicity of this mycoplasma is controversial, for it is often found in healthy lungs (50). Moreover, when isolated from pulmonary lesions it is accompanied by other bacteria, particularly *P. haemolytica* or *P. multocida* types A and D.

An abattoir survey in Norway of subacute pneumonia gave the following results (5, 6):

<table>
<thead>
<tr>
<th></th>
<th>M. ovipneumoniae</th>
<th>P. haemolytica</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonic lungs</td>
<td>98%</td>
<td>49%</td>
</tr>
<tr>
<td>Healthy lungs</td>
<td>28%</td>
<td>18%</td>
</tr>
</tbody>
</table>

The authors concluded that *M. ovipneumoniae* was only a potential pathogen which prepared the way for secondary bacterial infection.

- **M. arginini**: This seems to be devoid of pathogenicity.

**Resistance to environmental factors**

Mycoplasmas are fragile micro-organisms and are very sensitive to all disinfectants, detergents, heat (50-55°C) and acids (49).

They are destroyed by ultraviolet light and putrefactive changes, so that their life in the environment is of limited duration.

In culture they survive for seven to ten days at room temperature, three to four weeks at +4°C, and between six months and several years at -20°C.

Stored in the freeze-dried state at -20°C, they remain viable for at least ten years.
Modes of transmission

The low survivability of mycoplasmas outside the host means that close contact between an infected animal and a healthy animal is required for transmission to take place. While this contact must be close, it does not have to be prolonged: 24 hours is sufficient (88).

The airborne route is the commonest for pulmonary infections, with the upper respiratory tract serving as the portal of entry. Mycoplasmas become attached to the mucous membrane, and due to their inhibitory effect on phagocytosis, are able to multiply before eliciting an immune response (57).

Another significant route, particularly applicable to *M. capricolum* and *M. mycoides* subsp. *mycoides* (LC) is the oral route. Kids become infected by ingesting milk from dams with mastitis (18, 90).

Infection of an entire flock or premises occurs when an infected animal or a 'healthy' carrier is introduced. Of course, this is a threat only in those countries where goats are kept in large numbers in intensive or semi-intensive systems.

In countries where extensive husbandry is practised, pathogens spread when animals meet at watering points, or when they are driven to new grazing areas.

Factors related to the animal

Species of animal

The term 'pulmonary mycoplasmoses of small ruminants' may create confusion by implying that both sheep and goats are equally affected. This is not the case, for primary mycoplasmoses rarely occur in sheep. Under natural conditions, type F38 and the subspecies *capri* infect only goats. Similarly, subspecies *mycoides* (LC) is uncommonly found in the lungs of sheep.

By contrast, the frequency with which *M. capricolum* is found in the lungs of sheep is equal to, or greater than that observed in goats. Taoudi (112) recovered this mycoplasma from 20% of lung lesions in sheep, and from only 8% of goats.

Breed, sex and age

Breed and sex seem to play no part in the epidemiology of these infections, but age is an important factor. On the one hand, mortality is higher among young animals than among adults, and on the other hand, the clinical expression may vary with age, as shown by Perreau (86) in the case of subspecies *mycoides* (LC) infection.

The contagious agalactia syndrome is confined to adults, while young stock mainly develop septicaemia and pneumonia.

However, in the case of CCPP caused by type F38, young and adult animals are affected alike.

Carriage

The appearance of disease some distance away and sometimes after a long interval (several weeks or months) can be explained only by carriage of the mycoplasmas by apparently healthy animals. Usually they are goats or sheep which have recovered from infection without becoming bacteriologically sterile.
This fact was pointed out as early as 1881 by Hutcheon (cited by MacMartin et al., 61) in the case of CCPP in South Africa. Perreau (90) observed the same phenomenon during infection with *M. capricolum*, which re-appeared between eleven and thirteen months after symptoms had disappeared.

This carriage is not confined to the respiratory tract and udder. Cottew (17) and Hazell et al. (44) were able to isolate several species of mycoplasma (*M. mycoides* subspp. *mycoides* (LC) and *capri, M. capricolum, M. agalactiae*) from the external ear of goats and, in at least one case, this occurrence was related to an outbreak of pneumonia and arthritis.

Unfortunately, the duration of the carrier state after recovery is not known. The worst case would be for the mycoplasmas to be harboured until death, which would complicate considerably the prevention of these infections.

**Predisposing factors**

The environment as a whole plays an indisputable role in the appearance, evolution and severity of pulmonary mycoplasmoses, whether primary or secondary. The factors include:

**Type of husbandry**

The economic impact of mycoplasmoses is particularly serious under intensive husbandry. Overcrowding or even confinement favours contact and circulation of mycoplasmas.

The non-specific defence mechanisms of the upper respiratory tract are easily inhibited by changes in temperature or the relative humidity of the air. Good hygiene in animal buildings is an indispensable prophylactic measure.

In those countries where extensive husbandry is practised, the mixing of animal species (cattle, sheep and goats) may play a role in the persistence of infection, at least in the case of those mycoplasmas which do not have strict host specificity. In Africa, for example, the agent of contagious bovine pleuropneumonia has been isolated, at least once, from goats with pneumonia (85).

A correlation between the frequency of pneumonia caused by *M. ovipneumoniae* and the type of pasture was noted by Bakke and Nostvold (6).

**Climate and seasonal variations**

Not only is the occurrence of pneumopathies greater at certain times of the year (autumn and winter), but also their severity is dependent upon season, as observed by Melanidi (73) during an outbreak of contagious agalactia: the mortality rate was 94% in winter and 60% in summer.

**Intercurrent infections**

Among the factors which predispose lung tissue to invasion by mycoplasmas, viruses occupy an important place. Those encountered most often are parainfluenza virus type 3, and adenoviruses. In Africa the virus of the "peste des petits ruminants" and capripoxviruses have also been incriminated.
Any infectious or parasitic disease which lowers the non-specific defence mechanisms of the host can open the way to pulmonary infections.

CONCLUSIONS

The study of epidemiological mechanisms responsible for the occurrence and evolution of diseases has inevitably as its final goal the development of preventive measures.

As far as the pulmonary mycoplasmoses are concerned, the salient epidemiological features are as follows:

- the need for close contact, even if this is brief;
- the low resistance of mycoplasmas to environmental factors, contrasted with carriage for weeks or months by 'healthy' carriers;
- the important role of environmental and husbandry conditions in the occurrence and severity of the infections.

V. TREATMENT AND PROPHYLAXIS

TREATMENT

Because they have no bacterial wall, mycoplasmas are very resistant to those antibiotics whose action depends on inhibition of synthesis of such a wall: penicillin, cyclosine, polymyxin and bacitracin. Streptomycin is active against some species, but its use is not advisable because of the rapid development of streptomycin-resistant mutants.

As a general rule, the antibiotics which give the best results (2, 7, 39, 82) are:

a) the tetracycline group (tetracycline, oxytetracycline and chlortetracycline) at 5-10 mg/kg;

b) the macrolide group (or related compounds): tylosin, erythromycin or spiramycin at 25 mg/kg (an initial 50 mg/kg is recommended in the case of spiramycin).

It is essential to administer these antibiotics for at least five days, and to treat all exposed animals.

As far as tests for the efficacy of antibiotics in vitro are concerned, there is at present no standard procedure for mycoplasmas. According to Béranger (7), although the standard disk technique, based on measuring the zone of inhibition after the antibiotic has diffused, gives good results for certain antibiotics, it cannot be used as a general test. The micromethod for determining the minimum inhibitory concentration (MIC) in a liquid medium is preferred, because it is reproducible, reliable and in agreement with results obtained in animals. For example, erythromycin hydrochloride and tylosin tartrate are shown to be the most active, with a MIC of about 0.1 g/kg, followed by spiramycin (0.1-0.5 g/ml) and oxytetracycline (0.5-1.0 g/ml) (7).
It must also be borne in mind that failure of treatment and recurrence in the long term are not uncommon, particularly when the correct dosage and duration of treatment have not been respected. Coupled with the relatively high cost of antibiotics, the question of whether the treatment is appropriate has to be answered. The decision must be made by the veterinarian after a detailed study of the epidemiological situation on the premises.

PROPHYLAXIS

Immunoprophylaxis

The present state of knowledge regarding immunity during pulmonary mycoplasmosis has been reviewed by Howard and Taylor (47), who showed that:

1. A property of mycoplasmas is their ability to become attached to macrophages and neutrophils without eliciting phagocytosis. This may well explain how mycoplasmas can be carried by animals for long periods.

2. The generalised humoral response follows the conventional sequence of antibody formation (IgM, IgG and IgA) and may be responsible for the lesions observed.

3. The local immune response seems to provide better protection against subsequent infection than the generalised response of the body, whether humoral or cellular.

In fact, the main reason why few or no vaccines have been developed against the pneumopathies of small ruminants is probably the diversity of the causal agents.

Moreover, results obtained with vaccines against CBPP show that eradication or control of pulmonary mycoplasmoses by vaccination is difficult. Live, attenuated vaccines may have a residual pathogenicity for certain sensitive breeds, while inactivated vaccines are poorly immunogenic and protect for only a short time.

Trials are being conducted, however, with a vaccine against CCPP caused by strain F38. According to Rurangirwa et al. (103), this vaccine, inactivated by ultrasound and containing Freund’s complete adjuvant, provides complete protection for goats for at least six months. A live attenuated F38 vaccine was also found by MacOwan (65) to be safe to use and protective.

A vaccine against infection caused by *M. mycoides* subsp. *capri* has been developed in Nigeria, but it has not been possible to test its effectiveness in the field (79).

Hygienic precautions

There is no doubt that hygienic precautions have been the most effective method of control wherever they have been applied rigorously, namely in countries where the livestock industry is well structured and under continuous health supervision (87).

For disease-free premises:

Great care must be taken when introducing new animals onto the premises. Purchased animals must come only from flocks which have been free from disease for at least several months.
In areas where the movement of animals is unavoidable, every precaution must be taken to avoid contact between flocks, particularly at watering points.

*For infected premises:*

The conventional precautions must be observed scrupulously, including isolation of sick animals and slaughter of those unlikely to recover fully; thorough disinfection of the premises and of equipment; culling of recovered animals; no survivors to be sold (except for immediate slaughter).

* REFERENCES *


