Contagious Caprine Pleuropneumonia

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Introduction
In August 1995, outbreaks of severe respiratory disease were reported in goat herds in the Western Lowlands of Eritrea close to the border with Sudan resulting in mortality and morbidity rates exceeding 60% and 90% respectively. Disease spread rapidly to all goat herds which came into contact with the affected livestock of refugees returning from Sudan following the end of the war between Eritrea and Ethiopia. The disease, which was confirmed as contagious caprine pleuropneumonia (CCPP) [1], soon became enzootic throughout the whole country and is now one of the most important causes of goat mortality. Eritrea has become the most recent of nearly 40 countries worldwide to become affected by CCPP, a disease which has been designated as list B by the Office International des Epizooties (OIE) because of its economic impact.

History
CCPP was first described in 1873 in Algeria by Thomas and known under the local name of "bou frida" because, in the majority of diseased goats, only one lung was affected [2]. Its contagiousness was not initially recognised because the disease was endemic in most areas under examination, so climatic conditions were thought to be responsible for disease outbreaks. However, a major outbreak in South Africa in 1881 following the introduction of goats from Turkey led the colonial veterinary surgeon, Duncan Hutcheon, to conclude that CCPP was highly infectious.

Research into the control of CCPP was initially hampered by confusion over the exact cause of the disease. Two mycoplasmas, *M. mycoides subsp. mycoides LC* and *M. mycoides subsp. capri*, were for some time implicated in the etiology of the disease because they caused a pleuropneumonia in small ruminants that resembled CCPP. It was not until 1976 that a highly fastidious mycoplasma, designated F38 but later named *M. capricolum subsp. capripneumoniae*, was isolated for the first time *in vitro* by MacOwan and Minette [3]. Once these workers had developed a suitable medium for the mycoplasma and carried out experimental infections, its role as the primary cause of classical CCPP was confirmed.

However, in spite of this confirmation, respiratory diseases, caused by *M. m. capri* and *M. m. mycoides LC*, are still referred to erroneously as CCPP particularly in the Middle East and India. A condition should only be termed as CCPP when the following criteria have been satisfied:

- *M. c. capripneumoniae* is isolated or there is strong serological evidence of the mycoplasma
- Lesions are restricted to lung and pleura and consist of a pleuropneumonia
- The condition is highly infectious with high levels of morbidity/mortality
- There is no enlargement of the interlobular septa of the lung

Causative Agent
The taxonomic status of F38 has long been unclear and it was only in 1993 that it became a subspecies of *M. capricolum*, and classified as *M. capricolum subsp. capripneumoniae* [4]. *M. c. capripneumoniae* belongs to the genus *Mycoplasma* and is one of about 200 species of the class *Mollicutes*. Five distinct groups of mollicutes were identified by phylogenetic analysis of the 16S rRNA sequences, one of which, the spiroplasma group, contains *M. c. capripneumoniae* which has been subdivided within the *M. mycoides* cluster. This cluster contains six important ruminant mycoplasmas including *M. m. mycoides SC*, the cause of contagious bovine pleuropneumonia, *M. m. mycoides LC* and *M. m. capri* which share immunological and biochemical properties. Their close relationship can lead to problems for diagnosis. Table 1 and Table 2 summarise the properties of *M. c. capripneumoniae*, some members of this cluster as well as other mycoplasmas capable of causing diseases in small ruminants.
Table 1. Mycoplasmas, Including M. capricolum subsp. capripneumoniae, Isolated from Small Ruminants with Respiratory Disease

<table>
<thead>
<tr>
<th>Mycoplasma</th>
<th>Host</th>
<th>Primary site of isolation (other)</th>
<th>Disease*</th>
<th>Pathogenicity</th>
<th>In vitro growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. c. capripneumoniae</td>
<td>Goat (Sheep)</td>
<td>Lungs</td>
<td>CCPP</td>
<td>High</td>
<td>Slow (5-7 d)</td>
</tr>
<tr>
<td>M. m. mycoides LC</td>
<td>Goat (Sheep, Cattle)</td>
<td>Resp. tract (udder, joints)</td>
<td>Plp, M, A, C</td>
<td>Moderate</td>
<td>Fast</td>
</tr>
<tr>
<td>M. m. capri</td>
<td>Goat (Sheep)</td>
<td>Respiratory tract (joints)</td>
<td>Plp, A, C</td>
<td>Moderate</td>
<td>Fast</td>
</tr>
<tr>
<td>M. c. capricolum</td>
<td>Goat, Sheep</td>
<td>Joints/Respiratory tract (udder)</td>
<td>Plp, M, A</td>
<td>High</td>
<td>Fast</td>
</tr>
<tr>
<td>M. ovipneumoniae</td>
<td>Sheep, Goat</td>
<td>Respiratory tract</td>
<td>Pneumonia</td>
<td>Low</td>
<td>Moderate</td>
</tr>
<tr>
<td>M. conjunctivae</td>
<td>Sheep and Goats</td>
<td>Eyes</td>
<td>KC</td>
<td>Moderate</td>
<td>Moderate</td>
</tr>
<tr>
<td>M. agalactiae</td>
<td>Sheep and Goats</td>
<td>Udder (joints, eyes)</td>
<td>M, A, KC, P</td>
<td>High</td>
<td>Fast</td>
</tr>
<tr>
<td>M. putrefaciens</td>
<td>Goats (Sheep)</td>
<td>Udder (joints)</td>
<td>M, A</td>
<td>Moderate</td>
<td>Fast</td>
</tr>
<tr>
<td>M. arginini</td>
<td>Ubiquitous</td>
<td>Respiratory tract</td>
<td>None</td>
<td>Low/non pathogenic</td>
<td>Fast</td>
</tr>
</tbody>
</table>

*Plp = pleuropneumonia; P = pneumonia; M = mastitis; A = arthritis; C = conjunctivitis; KC = keratoconjunctivitis; CCPP = contagious caprine pleuropneumonia.

Table 2. Major Biochemical Differences between Mycoplasmas of Small Ruminants

<table>
<thead>
<tr>
<th>Mycoplasma</th>
<th>Glucose fermentation</th>
<th>Arginine hydrolysis</th>
<th>Phosphatase activity</th>
<th>Film and spots</th>
<th>Casein digestion</th>
<th>Tetrazolium aerobic</th>
<th>Reduction anaerobic</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. c. capripneumoniae</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>varies</td>
<td>weak/+</td>
</tr>
<tr>
<td>M. mycoides LC</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. m. capri</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. c. capricolum</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. ovipneumoniae</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>varies</td>
<td>+</td>
</tr>
<tr>
<td>M. conjunctivae</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>M. agalactiae</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>M. putrefaciens</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>varies</td>
<td>+</td>
</tr>
<tr>
<td>M. arginini</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

Distribution

While the clinical disease has been reported in nearly 40 countries in Africa and Asia, M. c. capripneumoniae has only been isolated in 13 countries because few have the facilities for isolating and growing mycoplasmas [5] (Table 3). The only reports of suspected CCPP in Europe date back to the 1920s when an outbreak occurred in Greece following the seizure of goats from Turkey. Goncalves [6], however, reported a disease in goats in Portugal in 1980 which very closely resembled
classical CCPP but from which *M. m. mycoides LC* was isolated. In 1996, a suspected outbreak of pleuropneumonia clinically resembling CCPP was investigated in a goat herd in England containing some imported goats and which had suffered severe respiratory disease resulting in many deaths [5]. CCPP was quickly ruled out by newly developed polymerase chain reaction (PCR) tests and a combination of *M. ovipneumoniae* and *Pasteurella (Mannheimia) haemolytica* were eventually identified as the causes of disease. There have been no reports of the isolation of *M. c. capripneumoniae* on the American continent although other members of cluster have been described there.

### Table 3. Distribution of CCPP

<table>
<thead>
<tr>
<th></th>
<th>Confirmed by isolation of mycoplasma</th>
<th>Clinical disease reported or suspected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Africa</strong></td>
<td>Chad, Eritrea, Ethiopia, Kenya, Niger, Sudan, Tunisia, Uganda</td>
<td>Algeria, Burkina Faso, Benin, Cameroon, Central African Republic, Djibouti, Egypt, Libya, Mali, Nigeria, Somali, Zaire</td>
</tr>
<tr>
<td><strong>Asia</strong></td>
<td>Nepal, Oman, United Arab Emirates, Turkey, Yemen</td>
<td>Afghanistan, Bangladesh, India, Iran, Iraq, Israel, Jordan, Kuwait, Lebanon, Pakistan, Saudi Arabia, Syria</td>
</tr>
</tbody>
</table>

**Clinical Signs and Pathology**

Goats of all ages and sex can be affected [7]. The acute disease is more noticeable in naive populations in newly affected areas. The incubation period generally lasts on average 10 days but may vary between two and 28 days. The first signs are a reluctance to walk and the onset of fever (typically 41°C and occasionally 42°C) although animals continue to feed and ruminate. Respiration accelerates and becomes painful with violent coughing episodes. Animals stand with limbs abducted and neck extended (Fig. 1). There is continuous salivation and noses become blocked with a mucopurulent discharge. In the terminal stages the goats are unable to move and death follows quickly. In subacute or chronic forms signs are milder with coughing usually noticeable only following exercise.

Figure 1. Goat with acute CCPP. - To view this image in full size go to the IVIS website at www.ivis.org . -

The best description of the pathology of the disease was made by Hutcheon [8] himself writing over a century ago: "There is no thickening of the interlobular tissue in the diseased lung of the goat, which forms such a striking feature in bovine pleuropneumonia; the section of the diseased lung in the goat has the appearance of a somewhat granular-looking liver" (Fig. 2). These features also clearly differentiate disease caused by *M. m. capri* and *M. m. mycoides LC*. In short, the pathological lesions are localised exclusively in the lung and pleura and consist of a pleuropneumonia, unilateral heparisation, adhesions, pleuritis and an accumulation of pleural fluid (Fig. 3). The pleural exudate can solidify to form a gelatinous covering on the lung (Fig. 4).

Figure 2. Granular lung of goat affected by CCPP. - To view this image in full size go to the IVIS website at www.ivis.org . -

Figure 3. Hepatised lung, pleural fluid and fibrin from goat affected by CCPP. - To view this image in full size go to the IVIS website at www.ivis.org . -
A study to correlate clinical signs and early lesions showed that affected goats, killed up to a week after contact with affected animals, were free of lung lesions or clinical signs; between two and three weeks after contact, lung lesions were generally small and superficial characterised by hyperemia and edema with clinical signs being restricted to an infrequent cough; fever was first seen after nearly 4 weeks which correlated with lung consolidation, the area of which increased as the fever progressed [9].

Histological examination of the lung tissues often reveals an acute serofibrinous to chronic fibrino-necrotic pleuropneumonia with infiltrates of serofibrinous fluid and inflammatory cells, mainly neutrophils, in the alveoli, bronchioles, interstitial septae and subpleural connective tissue. Intralobular edema is more prominent but interlobular edema has also been reported. Peribronchial and perbronchiolar lymphoid hyperplasia with mononuclear cell infiltration is also present [3,10,11].

Ultrastructural examination of the lungs of goats naturally affected with CCPP confirmed histopathological findings of congested septal capillaries with inflammatory cells invading thickened septal walls [12]. The alveolar lumen contained serous fluid mixed with neutrophils and lymphocytes, some of which were necrotic. The most significant findings were a widespread hyperplasia of type II pneumocytes that had lost most of their characteristic lamellar ultrastructure and large numbers of mycoplasma-like structures lying close to the microvilli and membranes of these cells. The authors proposed that the loss of these lamellae may reduce surfactant production, as well as key enzymes synthesis, leading to increased surface tension within the alveoli and contributing to the atelectasis often seen at post mortem examination of CCPP cases.

Conditions which may exacerbate CCPP include concurrent viral infections, in particular, orf (contagious ecthyma) and pestes des petits ruminants and possibly other mycoplasma infections such as *M. ovipneumoniae*, a cause of disease in its own right; adverse weather conditions and stress caused by transhumance may also compound on-going disease.

Sheep were believed to be refractory to disease although possibly acting as a reservoir of infection [13]. However, there have been reports of sheep with respiratory signs from which *M. c. capripneumoniae* was isolated [14] and some which were also seropositive [15].

**Immunology**

Little is known of the immunology of CCPP despite a number of reported experimental infections [16,17]. More recently March et al., [18] monitored the humoral response of goats infected with a multipassaged *M. c. capripneumoniae* strain 19/2 with several serological tests and PCR. While there was little evidence after infection of the infectious agent or clinical or pathological disease, apart from elevated temperatures and a transient cough in one goat, serological responses were detected by latex agglutination test and competitive ELISA. Immunoglobulin G (IgG) immunodominant bands of 23, 40 and 44 kDa were seen by immunoblotting in all experimentally infected animals as well as in some sera from a natural outbreak of CCPP in Eritrea which additionally showed bands of 62, 70 and 108 kDa.

**Transmission**

Outbreaks follow the introduction of an infected animal into a susceptible herd. The mycoplasma is transmitted over short distances through the expulsion of infected droplets during coughing. The disease is readily contagious and only brief periods of contact are necessary for successful transmission [7]. No evidence of indirect transmission has been shown as the mycoplasma is highly fragile in the environment. As with many mycoplasma diseases, in particular contagious bovine pleuropneumonia, the disease is introduced into a region by asymptomatic carrier animals.

**Molecular Epidemiology**

Unlike other members of the *M. mycoides* cluster, *M. c. capripneumoniae* shows a surprising degree of heterogeneity particularly in the sequence of its two rRNA operons which both contain the genes for 5S, 16S and 23S rRNA [19]. These polymorphisms, representing point mutations in either gene, can be used to subtype strains, some of which may have epidemiological and, possibly, evolutionary significance. Sequencing the 16S rRNA genes of African strains identified two
distinct lines, I and II, both of which were represented in Central, North and East Africa; isolates from the Middle East were of the type II only although they could be further divided [20,21]. Sequencing the amplified products of a different gene(s), the H2 locus, Lorenzon et al., [22] divided strains into 4 major groups in which lineage A occurred exclusively in East Africa, B mostly in North Africa and the Middle East, C in Central Africa, and D, represented only by a single strain, was restricted to the United Arab Emirates. Subtyping with amplified fragment length polymorphism (AFLP) strongly supported the 16S rRNA sequence analysis by identifying two main lineages [23], but all studies have been hampered by a lack of strains; clearly more strains are necessary to provide a better understanding of the epidemiology and evolution of CCPP.

On a more local level, 10 of 11 strains of *M. c. capripneumoniae* isolated from four different regions of Tanzania had very similar profiles with AFLP. These profiles were also indistinguishable from two Kenyan and one Ugandan strain indicating the close association between small ruminants in these three neighbouring countries [24].

**Biochemistry**

Substantial diversity has also been reported in the metabolism of strains of *M. c. capripneumoniae* [25]. Some strains including the type strain F38 oxidise organic acids but not glucose, while others, including the recently isolated Eritrean strains, metabolise glucose. Such biochemical diversity within a mycoplasma species is unique and may present diagnostic problems as glucose fermentation is a key characteristic in their preliminary identification. However, even with glucose-metabolising strains, the addition of pyruvate to the medium leads to significantly higher yields in vitro [1]. Thus it may be that organic acids are also important energy sources for glucose-oxidising strains. Table 4 illustrates the diversity of strains of *M. c. capripneumoniae* in their substrate requirements.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Strain</th>
<th>Oman</th>
<th>Eritrea</th>
<th>Kenya</th>
<th>Turkey</th>
<th>Kenya</th>
<th>Kenya</th>
<th>Oman</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactate</td>
<td>19/2</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>7/1a</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Glucose</td>
<td>4/2 LC</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glycerol</td>
<td>19/2</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
<tr>
<td>2-oxybutyrate</td>
<td>7/1a</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
</tr>
</tbody>
</table>

**Diagnosis**

In spite of greatly improved media formulations, the isolation of *M. c. capripneumoniae* remains one of the more difficult tasks for the mycoplasma diagnostic laboratory. A major problem is the transport of the samples over long distances to a specialist laboratory which often results in the inactivation of the fragile mycoplasma. The samples of choice are the pleural fluid which contains high numbers of mycoplasmas and sections of hepatised lung preferably at the interface of normal and diseased tissue. The samples must be sent quickly in a cool condition but will become of little value if journey time is longer than two days. Sending frozen samples is recommended but not always practical. During the recent investigation of CCPP in Eritrea, excellent isolation rates of *M. c. capripneumoniae* were achieved from lyophilised lung samples even though isolation was not carried out for several weeks after arrival [14]. Choice of medium is critical and best results were obtained during the same investigation with a commercial medium (Mycoplasma Experience, Reigate, UK) [14]. A diagnostic medium for CCPP has also been developed by this company in which *M. c. capripneumoniae* develop coloured colonies in semi-solid medium [26]. Other media which have been shown to support the growth of most strains of *M. c. capripneumoniae* include H25P [27] or FP medium supplemented with 2 g per liter of sodium pyruvate [28]. Overgrowth of this fastidious mycoplasma by other mycoplasmas is another major problem with isolation. In particular, *M. ovipneumoniae* will grow at a much faster rate but can be separated from *M. c. capripneumoniae* by early cloning after recognizing the former’s characteristic "centreless" colonies.
The development of PCR has greatly improved CCPP diagnosis as it is now possible to detect the mycoplasma quickly even in mixed cultures and directly from clinical material such as pleural fluid and lung. PCRs based on the 16S RNA genes have been reported which enable the detection of all members of the *M. mycoides* cluster followed by specific identification of *M. c. capripneumoniae* by restriction enzyme digestion [28]. PCR has also been reported to be successful for detecting *M. c. capripneumoniae* directly from pleural fluid dried on filter paper which can be sent, overseas if necessary, to a reference laboratory [22].

Serodiagnosis of CCPP, on the hand, is a relatively easy task thanks to a rapid, specific and relatively sensitive test developed initially in Kenya. The latex agglutination test (LAT) uses a carbohydrate extracted from *M. c. capripneumoniae* linked to latex particles which agglutinate in the presence of specific antibodies in the blood of affected goats [29]. The test, which takes minutes to complete, is more sensitive than the complement fixation test [1] and easier to perform than the competitive ELISA [30] which should be used for confirmation. A LAT has also been described for circulating antigen and could provide earlier detection in affected animals before antibodies have appeared [31].

Disease Prevention and Control

Protection against CCPP was shown to be possible more than a century ago when Hutcheon subcutaneously inoculated goats with lung extract from affected animals [2]. Furthermore goats vaccinated with an attenuated broth culture of F38 did not succumb to contact infection [32]. This clearly demonstrated control was possible. Since then a number of different preparations have been produced which are reported to produce solid immunity even after one year. These include a vaccine composed of sonicated antigens emulsified with incomplete Freund’s adjuvant [33] and another in which lyophilised F38 is inactivated with saponin immediately before immunization [34]. The latter vaccine has been in use in Kenya for the last few years but has been modified so that the mycoplasma is inactivated with saponin for at least 12 hours at 4°C [35]. Kids older than 10 weeks of age are vaccinated to avoid interference by maternal antibody.

In other countries where vaccination is not practised, other control measures are used. Antibiotics such as the tetracyclines, fluoroquinolones and the macrolide family are generally effective clinically if used earlier enough [36,37]. However, the complete elimination of the mycoplasma is rarely achieved and treated animals should always be considered as potential carriers.

Movement restrictions and slaughtering of infected and contact animals is recommended for countries or regions that are newly infected.

Conclusions

The risk of introducing CCPP to the USA is probably very small as it does not import small ruminants from Asia or Africa. There is, however, a risk of introducing CCPP to those European Union (EU) states bordering countries like Turkey where disease is endemic or from Eastern Europe where surveillance for CCPP rarely occurs. Italy may also be threatened from North Africa, particularly Tunisia which is only a short boat ride away. Once introduced into the EU, the disease could theoretically spread throughout the member states including the UK via herd movements. The widespread use of antibiotics would suppress the overt clinical signs reducing the direct economic consequences with the disease remaining undetectable for several months or years as was the case in Italy following the introduction of contagious bovine pleuropneumonia in 1990. However, the costs of eradication would be significant. The key to control of this disease therefore, given an outbreak in Europe, would be the rapid identification of the disease enabling the destruction of affected and contact animals.

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