

Contagious caprine pleuropneumonia and its current picture in Pakistan: a review

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ABSTRACT: Contagious caprine pleuropneumonia (CCPP) is caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) which belongs to the *Mycoplasma mycoides* cluster, a group of five closely related *Mycoplasmas*, pathogenic to ruminants. The true lesions of CCPP are restricted to the alveolar tissues of infected goats, which distinguish it from other respiratory diseases of small ruminants caused by members of the *Mycoplasma mycoides* cluster. The typical signs of CCPP are an accumulation of pleural fluid, unilateral hepatisation, adhesions, pleurisy and pleuropneumonia which clearly differentiate it from “MAKePS” syndrome caused by *Mycoplasma mycoides* subsp. *capri* (*Mmc*). The available literature on CCPP shows that so far in Pakistan, the true causative agent (*Mccp*) of this disease has only been isolated in the Pashin District of Balochistan and that the disease is more frequently confused with other respiratory diseases of goat caused by the *Mycoplasma mycoides* cluster. The lack of suitable techniques and extensive knowledge in the field is a big limitation for the isolation and characterisation of *Mccp* from prevailing CCPP-like cases in the goat population of Pakistan.

Keywords: goat; *Mycoplasma mycoides* cluster; *Mycoplasma capricolum* subsp. *capripneumoniae*; diagnostic tests; respiratory diseases

List of abbreviations

CBPP = contagious bovine pleuropneumonia, **CCPP** = contagious caprine pleuropneumonia; **CFT** = complement fixation test, **ELISA** = enzyme linked immunosorbent assay, **FAO** = Food and Agriculture Organization, **GIT** = growth inhibition test, **IFA** = indirect fluorescent antibody, **IHA** = indirect Haemagglutination, **Mcc** = *Mycoplasma capricolum* subspecies *capricolum*, **Mccp** = *Mycoplasma capricolum* subsp. *capripneumoniae*, **MLSA** = multi locus sequence analysis, **Mmc** = *Mycoplasma mycoides* subsp. *capri*, **MmmLC** = *Mycoplasma mycoides* subsp. *mycoides* large colony, **MmmSC** = *Mycoplasma mycoides* subsp. *mycoides* small colony, **OIE** = Office International des Epizooties, **PCR** = polymerase chain reaction

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1. Brief introduction to contagious caprine pleuropneumonia

Contagious caprine pleuropneumonia (CCPP) is a highly fatal caprine disease firstly reported in Algeria in 1873 (McMartin et al. 1980). It is a devastating disease of goats (Bascunana et al. 1994) included in the list of notifiable diseases of the Office International des Epizooties (OIE) (Manso-Silvan et al. 2011). CCPP is a major threat to the goat farming industry in developing countries (Lorenzon et al. 2002) and is pandemic in Africa, the Middle East and Asia (Manso-Silvan et al. 2011; Nicholas and Churchward 2012). Previously, only 20 countries have reported the isolation of the *Mccp* organism due to scarcity of laboratory expertise (Nicholas 2002a) but it has now been isolated in China (Chu et al. 2011), Mauritius (Srivastava et al. 2010), and Tajikistan (FAO 2012), by applying advanced “microorganism-detecting protocols” such as PCR. In Pakistan the causative agent of CCPP has only been isolated from sick goats in the Pashin District of Balochistan (Awan et al. 2010). CCPP is a major cause of economic losses in the goat industry globally as these intracellular bacteria can infect domestic as well as wild breeds of goat (Nicholas 2002a; Arif et al. 2007; Ostrowski et al. 2011), with 100% morbidity and 60–80% mortality rates (Rurangirwa and McGuire 2012; OIE 2009). This disease is characterised by fibrinous pleuropneumonia with increased straw coloured pleural fluid in the infected lung (Rurangirwa and McGuire 2012). *Mccp* has been reported to affect only goat species (Thiaucourt and Bolske 1996) and does not infect sheep (McMartin et al. 1980). However, in contrast to these findings, F38 has been isolated from healthy sheep that were in contact with CCPP-positive goats in Africa (Litamoi et al. 1990; Bolske et al. 1996). The presence of a distinct Asian cluster strongly indicates that CCPP was not recently imported to continental Asia but has been endemic in the area for a long time (Manso-Silvan et al. 2011).

2. Causative agent and its relationship to other *Mycoplasmas*

CCPP is caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*) formerly known as the F38 strain of *Mycoplasma* (Leach et al. 1993; Nicholas 2002b), which was first isolated and char-

acterised in 1976 (MacOwan 1976). The *Mccp* organism is genetically and antigenically very closely related to *Mycoplasma capricolum* subspecies *capricolum* (*Mcc*), the causative agent of respiratory pneumonia in goats and sheep (Bascunana et al. 1994; Monnerat et al. 1999). *Mccp* is a member of the so-called *Mycoplasma mycoides* cluster (Heldtander et al. 2001), and is difficult to identify through ordinary procedures, as it belongs to a group of five closely related ruminant pathogenic *Mycoplasmas* (Manso-Silvan et al. 2011; Thiaucourt et al. 2011), that share similar serological cross reactions and similar biochemical features sometimes leading to the erroneous diagnosis of CCPP (Cottew et al. 1987; Erno 1987; Adehan et al. 2006). F38 was taxonomically reported as a subspecies of *Mycoplasma capricolum* and was recently renamed as *Mycoplasma capricolum* subsp. *capripneumoniae* (Leach et al. 1993). Before the isolation and identification of *Mccp*, *Mycoplasma mycoides* subsp. *capri* (*Mmc*) was considered to be the causative agent of CCPP (Edward 1953; Jonas and Barber 1969; MacOwan 1976; Littlejohns and Cottew 1977). *Mycoplasma mycoides* subsp. *mycoides* Large Colony (*MmmLC*) and *Mmc* infections have a systemic manifestation and are sometimes referred to as the “MAKePS” (mastitis, arthritis, keratitis, pneumonia, and septicemia) syndrome (Thiaucourt and Bolske 1996). *MmmLC* has recently been reclassified as a serovar of *Mmc* (Manso-Silvan et al. 2009). *Mycoplasma mycoides* subsp. *mycoides* Small Colony (*MmmSC*) causes contagious bovine pleuropneumonia (CBPP) in cattle (Thiaucourt et al. 2011; Fisher et al. 2012).

3. Genetic diversity of *Mycoplasma capricolum* subsp. *capripneumoniae*

Mycoplasmas are the smallest free-living fastidious bacteria. They are about 300 nm in diameter, bound by a triple layered membrane and unlike conventional bacteria they don't have a rigid cell wall of murin (Robinson and Bebear 1997). Their genome size is only one sixth to one third of that of *Escherichia coli* (Bascunana et al. 1994). *Mycoplasmas* are phylogenetically related to gram positive bacteria with low G + C content (Razin et al. 1983; Bascunana et al. 1994). The *Mycoplasma mycoides* cluster has two rRNA operons in which intraspecific variations have been demonstrated (Heldtander et al. 2001). *Mccp* was once thought

Table 1. Pathogenic *Mycoplasmas* of ruminants

Mycooides subgroup Genome (1.5 Mbp)	Host	Disease	Capricolum subgroup Genome (1.2 Mbp)	Host	Disease
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> SC	cattle	CBPP	<i>M. capricolum</i> subsp. <i>capricolum</i>	goat, sheep	MAKePS
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> LC	goat/sheep	pneumonia/contagious agalactia	<i>M. capricolum</i> subsp. <i>capripneumoniae</i>	goat	CCPP
<i>Mycoplasma mycoides</i> subsp. <i>capri</i>	goat, sheep	MAKePS			
<i>Mycoplasma leachii</i>	cattle	mastitis, arthritis			

Adapted from Thiaucourt and Bolske (1996), Nicholas (2002b), Genesig Standard Handbook (2004)

MAKePS = mastitis, arthritis, keratitis, pneumonia, and septicaemia, CBPP = contagious bovine pleuropneumonia,

CCPP = contagious caprine pleuropneumonia, LC = large colony, SC = small colony

to be a homogenous taxon (Abu-Groun et al. 1994; Nicholas 2002a; Manso-Silvan et al. 2007), but the discovery of two molecular markers showed some degree of heterogeneity among strains that opened a further channel for studies on the molecular epidemiology of CCPP (Manso-Silvan et al. 2011). Polymorphisms in *Mccp* strains can be used as epidemiological markers for CCPP in smaller geographical areas and to study the molecular evolution of this species (Heldtander et al. 2001). Eleven polymorphic positions were observed in the sequence of 2400 bp long fragments, obtained from 19 *Mccp* strains from various geographical locations (Lorenzon et al. 2002). Similarly, in molecular typing a good correlation between MLSA (multi locus sequence analysis) groups and geographic origins of the *Mccp* strains was observed (Manso-Silvan et al. 2011).

4. Contagious caprine pleuropneumonia transmission

CCPP is transmitted directly by an aerogenic route through contaminated droplets (Thiaucourt et al. 1996). The outbreak of the disease follows the introduction of an infected animal into a group of susceptible goats (OIE 2009). The disease is readily contagious and a short period of contact is enough for successful transmission through coughing (Thiaucourt and Bolske 1996; OIE 2009). No evidence of indirect contact has been shown as the organism is highly fragile in the environment (Thiaucourt and Bolske 1996). It is quickly inactivated within 2 min at 60 °C but can survive for more

than 10 years in frozen infected pleural fluid (OIE 2009). Disease outbreak may occur after heavy rain, animal transportation over a long distance (OIE 2009), poor climatic conditions and primary infections (Thiaucourt and Bolske 1996). Formaldehyde can inactivate *Mccp* in 30 s at a concentration of 0.05%. A solution of 1.0% phenol can inactivate the organism within 3 min (OIE 2009).

5. Incubation period of *Mycoplasma capricolum* subsp. *capripneumoniae*

Mccp normally has a very short incubation period in the lungs (three to five days) but this may be prolonged (three to four weeks) depending on predisposing factors (Thiaucourt and Bolske 1996). In primary infected goats, CCPP last for about two days with high mortality (McMartin et al. 1980) while in other cases it may last for several days (OIE 2009). However, in the experimental infection model of March et al. (2002), *Mccp* was not isolated from the infected lungs of goats (eight week post infection) due to the development of humoral immunity (IgG, IgM).

6. Clinical picture of contagious caprine pleuropneumonia

The typical clinical signs of CCPP are hyperpyrexia (41–43 °C), high morbidity and mortality rates in susceptible herds irrespective of age and sex, dyspnea sometimes with grunting and snoring, continuous nasal discharges, anorexia and abor-

tion (Nicholas 2002a; OIE 2009). In *per acute* cases, goats may die within one to three days with minimal clinical signs (OIE 2008). Typical CCPP lesions occur in the thoracic cavity only (Mondal et al. 2004), and sometimes affect one lung with abundant pleural exudates and conspicuous pleuritis (Thiaucourt and Bolske 1996). Coughing is irregular and nasal discharge is often absent initially (OIE 2009). Affected lungs degenerate into a voluminous abscess as a consequence of secondary bacterial infection (Thiaucourt and Bolske 1996). Affected lungs become hepatized and take on a port wine colour (Thiaucourt and Bolske 1996), with pea sized yellow nodules surrounded by congestion (OIE 2009). The pleural cavity contains an excess of straw coloured fluid with fibrin flocculations (Kaliner and MacOwan 1976; Wesonga et al. 1993; OIE 2008; Rurangirwa and McGuire 2012). Adhesions between the lung and the pleura are very common and often very thick (MacOwan and Minette 1977). In sub-acute or chronic cases, the symptoms are very similar to acute cases, but weak in nature (Thiaucourt and Bolske 1996).

7. Diagnostic tests

Confirmatory diagnosis is based on the isolation of *Mccp* from clinical samples of lung (Nicholas and Churchward 2012). The ideal sample for *Mccp* isolation is pleural fluid obtained from a recently slaughtered or live infected goat (Thiaucourt et al. 1996). Unlike the true CCPP caused by *Mccp*, other *Mycoplasma* infections can spread beyond the thoracic cavity (OIE 2008). In the laboratory, the major problem in *Mccp* isolation is its slow growth and frequent contamination of the culture by other *Mycoplasmas* (Thiaucourt et al. 1996; Nicholas and Churchward 2012). Under an ordinary microscope, the organism has a branching, filamentous morphology in exudates, impression smears or tissue sections, while other caprine *Mycoplasmas* usually appear as short filamentous organisms (OIE 2009). *Mccp* and other members of the *Mycoplasma mycoides* cluster cross react in the serological test and share biochemical and genetic similarities, so biochemical and growth inhibition tests are not reliable and specific (Awan et al. 2009; OIE 2009). The best and most accurate diagnostic method is molecular typing of *Mccp* (Woubit et al. 2004).

7.1. Culturing

A number of media have been used for the general growth and isolation of *Mycoplasma*. *Mycoplasma* agar and broth media (Oxoid; Sigma), are used for the selective isolation of *Mycoplasma* spp. An agar non-selective media under the product code name CC1A (*Mycoplasma* Experience Ltd. Product), is available that allows the development of *Mccp* as red colonies over seven days of incubation (MEPG [online](#)). *Mccp* has been successfully grown and isolated from infected lungs through culturing on Hayflick medium broth (H25P) by Balikci et al. (2008), Cetinkaya et al. (2009) and Noah et al. (2011). Similarly modified Hayflicks media have been used for the growth and isolation of *Mccp* organisms (Manso-Silvan et al. 2011). Other than *Mccp* (five to seven days *in vitro* growth), all *Mycoplasma mycoides* cluster members grow within 24–48 h *in vitro*, producing colonies 1–3mm in diameter (Thiaucourt et al. 1996).

7.2. Biochemical tests

For preliminary screening, a limited number of biochemical tests are available based on nutritional capabilities of *Mccp* or specific enzyme activities (Noah et al. 2011). Digitonin sensitivity distinguishes *Mycoplasmas* from acholeplasmas, and serum digestion distinguishes members of the *Mycoplasma mycoides* cluster from all other small ruminant *Mycoplasmas* (FAO 2012). Phosphatase production separates *Mcc* from other members of the *Mycoides* cluster, while metabolic differences (such as maltose positive reaction for *Mccp*) allow differentiation between *Mcc* and *Mccp* (Bradbury 1983). The interspecies variation in some biochemical reactions is often remarkable, rendering their application valueless (Jones 1989; Rice et al. 2000). The lack of arginine catabolism in *Mccp* may help to differentiate it from *Mcc* (Noah et al. 2011), but in some strains of *Mcc* arginine catabolism is reported to be lacking or very difficult to detect (Jones 1992; Leach et al. 1993; Rurangirwa 1996).

7.3. Serological tests

Quite a few serological tests are available that are used in the field for the confirmatory diagnosis of CCPP. Indirect haemagglutination (IHA) and com-

plement fixation tests (CFT) are used to assay the antibody response of goat to *Mccp* (daMassa et al. 1992). The CFT used for the detection of CCPP (MacOwan 1976; MacOwan and Minette 1977) is more specific, though less sensitive than the IHA (Muthomi and Rurangirwa 1983; daMassa et al. 1992). The IHA specificity for the *Mycoplasma mycoides* cluster has been evaluated and the results were found to show cross-reactivity between these organisms (Jones and Wood 1988; Litamoi et al. 1989; daMassa et al. 1992). The latex agglutination test which detects serum antibodies in CCPP-infected goats is more sensitive than CFT and can be performed in field conditions using whole blood or undiluted serum with a prompt result (Cho et al. 1976). An indirect enzyme-linked immunosorbent assay (ELISA) has been developed to screen goat serum at a single dilution of antibody to *Mccp* (Wamwayi et al. 1989). The specificity and suitability of ELISA for large scale testing make it an appropriate tool for epidemiological investigation of CCPP (OIE 2008). Direct antigen detection and blocking ELISA detects antibodies in the serum of naturally or artificially CCPP-infected goats (Wamwayi et al. 1989). Direct and indirect fluorescent antibody tests are the simple, reliable and rapid serological methods applied to clinical samples for the identification of most *Mycoplasmas* (Thiaucourt et al. 1996b). Among many, the indirect fluorescent antibody (IFA) test is the most commonly used and is applied to unfixed *Mycoplasma* colonies on agar (OIE 2008).

The growth inhibition test (GIT) is the least sensitive and simplest of the tests available for CCPP diagnosis (OIE 2008). It depends on the direct inhibition of *Mycoplasma* growth on solid media by specific hyper immune serum, and detects primary surface antigens (Dighero et al. 1970; Rosendal and Black 1972). The GIT is particularly useful in identifying *Mccp* because they appear to be serologically homogeneous, and anti-serum to the type strain produces wide inhibition zones (OIE 2008).

7.4. Molecular diagnostic tests

Until recently, isolation was the only way to confirm the presence of CCPP. A DNA probe which differentiates *Mccp* from other members of the *Mycoplasma mycoides* cluster was developed

(Taylor et al. 1992). PCR-based diagnostic systems are used for the rapid detection, identification and differentiation of the *Mycoplasma mycoides* cluster members to the serovar and strain level (Taylor et al. 1992). Sequencing of the gene for 16S ribosomal RNA has also been used to develop a PCR-based test where the final identification of *Mccp* is made depending on the pattern of the products after digestion of the PCR product with the restriction enzyme PstI (Bascunana et al. 1994; Bolske et al. 1996). Species identification based on PCR of the 16S rRNA genes and restriction at positions where unique differences occur between the two operons has been demonstrated previously for *Mccp* (Bascunana et al. 1994). An improved resolution method, MLSA (multi-locus sequence analysis) based on the analysis of several genetic markers has also been used for the identification of *Mccp* (Manso-Silvan et al. 2011). Sequence-based genotyping methods for bacterial typing are technically simple, objective oriented and portable (Van Belkum et al. 2007); moreover they allow direct amplification and sequencing of the organism from clinical material (Manso-Silvan et al. 2011).

8. Treatment and prophylaxis

The duration of the disease varies according to the environmental circumstances (OIE 2009), however, the infected goat can survive for more than one month or even recover if placed in good rearing conditions coupled with proper treatment (Thiaucourt et al. 1996). A number of antibiotics and vaccines have been mentioned in the treatment and control of CCPP. In one case report, streptomycin-treated goats suffering from natural and experimental CCPP recovered on the third day of treatment and became completely immune to reinfection with *Mccp* (Rurangirwa and McGuire 2012). An administration of long acting oxytetracycline stopped morbidity and mortality, and controlled further CCPP spread immediately (Giadinis et al. 2008). Danofloxacin was found to be highly effective in the treatment of clinical CCPP in goats Ozdemir et al. 2006). Commercially available vaccines such as Pulmovac and Capridoll (live) and CCPPV (killed) are produced in Turkey and Ethiopia, respectively. Caprivax is an inactivated CCPP vaccine prepared from an *Mccp* strain by the Kenya Veterinary Vaccine Production Institute, Nairobi. The inactivated *Mycoplasma* strain F38-saponin vaccine in natural CCPP cases showed 100% protection (Litamoi et al. 1989).

9. The term contagious caprine pleuropneumonia is erroneously used in Pakistan

Goat farming in Pakistan is of benefit to the community in the shape of milk, meat, skin, mohair and manure on a small scale (Rahman et al. 2003). Knowledge and understanding of the epidemiological profile of animal diseases, is critical for evaluating and addressing the veterinary health needs of the livestock population of a local area (Khan 2010). Of the many small and large ruminant diseases in Pakistan, the goat population is prone to a huge number of systemic infectious diseases, for which harsh weather, poor husbandry practices, improper transportation and lack of proper quarantine measurements are some of the predisposing factors (Tariq 1980). The small ruminant production system in the country is nomadic, transhumant and sedentary (Ishaque 1993; Awan et al. 2010), sometimes leading to endemic diseases becoming pandemic. Every year an outbreak of respiratory diseases occurs in the goat population with an alarming rate of morbidity and mortality but the lack of advanced techniques is a big hindrance in its proper diagnosis. The first report on CCPP in Pakistan was reported by Awan et al. (2010), who isolated *Mccp*, *Mcc* and *Mycoplasma putrefaciens* (*Mp*) from clinically sick goats in the Pashin district of Balochistan, Pakistan. The results of the field diagnosis and conventional laboratory isolation reports of Sadique et al. (2012a,b,c) in the Khyber Pukhtoonkhwa region of Pakistan suggested the causative agent for CCPP was *Mmc*, but according to the extensive literature available on CCPP, the causative agent of typical CCPP is only *Mccp* and this disease should not be confused with other types of pneumonia and pleuropneumonia caused by other *Mycoplasma* (Nicholas 2002a). In the same studies of Sadique et al. (2012a), the causative agent *Mycoplasma mycoides capri* (*Mmc*) was also isolated from other organs such as liver, kidneys, heart, intestine etc. with a wide range of variations in the percentage of clinical and histopathological signs of the disease based on goat farming areas. According to OIE (2009), unlike CCPP, which is confined to the thoracic cavity, disease caused by other *Mycoplasmas* of the mycoides cluster is accompanied by prominent lesions in other organs and/or parts of the body besides the thoracic cavity. *Mmc* is a more ubiquitous pathogen in small ruminants causing pneumonia, mastitis, arthritis,

septicaemia and keratitis (MAKePS) and is also found as a saprophyte in the ear canal (Thiaucourt et al. 2011). After a long confusion over the causative agent of CCPP, *Mccp* has been demonstrated to be the causative agent which distinguishes true CCPP from pleuropneumonia caused by other members of the *Mycoplasma mycoides* cluster which are often associated with other pathologies (Manso-Silvan et al. 2011). In the studies of Hernandez et al. (2006), *Mmc* was isolated as a causative agent from an outbreak of respiratory mycoplasmosis in goats in which the clinicopathological conditions resembled CCPP. The research published by Rahman et al. (2006), Ettorre et al. (2007), Awan et al. (2009), De la Fe et al. (2010), Goncalves et al. (2010) and Ogor et al. (2011) deal with other *Mycoplasma* species/subspecies which should not be confused with classical CCPP caused by *Mccp*. Similarly the survey conducted by Khan (2010) does not mention the isolation of the causative agent of CCPP in Pakistan and the study was a more generalised survey mainly based on signs and symptoms of livestock diseases. Nothing has been mentioned regarding CCPP in the articles of Zahur et al. (2006) and Afzal (2010), published in the Pakistan Veterinary Journal which has been wrongly cited in a literature review as a report on CCPP. Hussain et al. (2012) isolated *Mycoplasma mycoides* from sick goats showing respiratory symptoms with 9.17% mortality and the respiratory disease was given the name CCPP; this does not comply with the classical definition of CCPP.

A greater confusion has been created from the literature available on goat respiratory diseases in Pakistan, as there is no evidence of the molecular diagnosis of the typical causative agent (*Mccp*) of CCPP in Pakistan except for the research published by Awan et al. (2010). The pronounced serological cross reactions between *Mccp*, *Mc* and *Mycoplasma leachii* make it difficult to diagnose CCPP accurately (daMassa et al. 1992; Bonnet et al. 1993). Disease caused by *Mmc* and *MmmLC* is still referred to erroneously as CCPP in some countries (Nicholas 2002a).

10. Conclusions and need for practical research

From the available literature, it is clearly concluded that CCPP is caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (*Mccp*), a fastidious bacteria of the *Mycoplasma mycoides* cluster. Only one

published report mentions the isolation of *Mccp* in Pakistan and the term CCPP is erroneously intermingled with other respiratory diseases of goat in the country. The epidemiology of this disease in Pakistan is still unclear as CCPP-like outbreaks occur in the goat industry, but, so far, the causative agent (*Mccp*) has not been isolated and typed. It is the responsibility of the livestock industry to implement advanced technologies for the molecular typing of the causative agent of CCPP or CCPP-like diseases in goats in Pakistan.

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