J. App. Em. Sc: 1(1) 45-50, June 2004

ISOLATION AND IDENTIFICATION OF MYCOPLASMAS FROM PNEUMONIC LUNGS OF GOATS

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ABSTRACT

Fifty pathological samples (lungs) from pleuropneumonia suspected goats were collected from Quetta abattoir and cultured for Mycoplasmas. Subsequent bacteriological investigations revealed 6 isolates. Of these, *Mycoplasma mycoides* subspecies capri (3), *Acholepasma* species (2) and untypable *Mycopasma* (1) were identified on the basis of morphological, cultural and biochemical characteristics. Only 3/6 isolates were sero typed as *Mycoplasma mycoides* subspecies capri.

INTRODUCTION

Caprine pleuropneumonia is world wide in distribution. The exact etiology of the disease complex in this species is not as yet clearly understood although mycoplasmas are generally believed to be important and in addition organisms of the group are known to cause several economically important diseases particularly Contagious Caprine Pleuropneumonia (CCPP) and Contagious Agalactia (CA). In addition to pleuropneumonia, Mycoplasmas are also implicated in a variety of clinical conditions such as arthritis. kerato-conjunctivitis, mastitis and septicaemia. A number of mycoplasmas have been accounted for CCPP. Of these *Mycoplasma mycoides* subspecies capri (Ojo, 1976a), *Mycoplasma* F-38 strain (MacOwan & Minette,1976) and *Mycoplasma mycoides* subspecies mycoides large colony (MmmLC) type (Perreau, 1971) are commonly encountered. On the other hand *Mycoplasma ovipneumoniae* (Perreau, 1977), *Mycoplasma capricolum* (DaMassa et al. 1983), *Mycoplasma arginini* (Mohan & Uzoukwu, 1983) and *Mycoplasma mycoides* subspecies mycoides small colony (MmmSC) (Perreau,1971) are the examples of associative mycoplasmas, which are isolated from diseased lungs of goats either alone or along with other bacteria or viruses. Conversely, *Acholeplasma* and *Ureaplasma* (Atypical mycoplasmas) are found as members of normal microbial flora of mucous membranes and do not seem to be directly pathogenic (Lefevre et al., 1987).

The aetiological diagnosis of CCPP in goats, involving a *Mycopasma* can only be based on bacteriological and serological evidences. Clinical diagnosis of CCPP is confounded by immense variations, which may be observed in symptoms expression, morbidity and mortality, depending on the genetic constitution, management and age of the goats at risk, the novelty of the organism to them, the strain of the organism involved, occurrence of inter current diseases and stress factors. 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.

As the CCPP is a contagious disease, depending on the strain involved, it can inflicts heavy economic losses to goat population, due to its high morbidity and mortality rates. Little work has so far be done on the isolation and identification of caprine *Mycoplasmas* in Pakistan The purpose of the present investigation was to isolate and identify the prevailing *Mycoplasmas* from the respiratory tract of pleuropneumonia suspected goats, slaughtered at Quetta abattoir.

MATERIALS & METHODS

Goats of different breeds slaughtered at Quetta abattoir were clinically examined for CCPP. Fifty goats suspected for caprine pleuropneumonia were marked accordingly. On post-mortem the visceral organs of thoracic cavity were examined carefully. Fifty whole lungs with and without gross lesions were collected and transported to the laboratory. Prior to the excision of the affected portions of lung these were washed thrice with the sterilized normal saline. All the lung samples were store at –20°C until required for isolation study..

The lung tissues samples were taken out of the deep freezer, thawed and washed thrice with sterilized normal saline The affected portion of each of the lung was chopped with the sterilized scissors in pleuropneumonia like organism broth (PPLO broth) after searing with hot spatula.

For the isolation of *Mycoplasmas*, the standard bacteriological procedures were followed (O. I. E manual, 2000). Briefly each of the suspension of lung tissues was inoculated into PPLO broth and agar media. The sub cultured agar plates were incubated at 37°C in CO_2 and moist conditions whereas liquid medium tubes were only incubated at 37°C. The plates were then examined for bacterial colonies through stereoscope from 2 days of subculture to 14 days. Prior to the examination of inoculated liquid medium tubes for the evidence of growth for seven days, these were passaged blindly into fresh medium after three days of initial incubation. Later the materials from the tubes irrespective of the growth were sub cultured onto agar pates and incubated and examined as done before.

The cloning and purification of each of the isolate was done by filtering the liquid culture through the pre-sterilized disposable millipore filter membrane (0.45um) on to PPLO agar plates and incubated at 37°C. The single representative colony from each of the PPLO agar plates was transferred into PPLO broth tubes. The whole procedure was repeated twice to have the pure clone of each of the isolated bacteria.

The pure culture of the isolate was inoculated into PPLO broth (complete medium), PPLO agar without serum and Nutrient Agar. All the sub cultured tubes and plate were incubated accordingly.

For the characterization of the isolates biochemical tests were performed as described by Aluotta et al.(1970), Al-Aubaidi (1972a), Cottew & Yeats (1978), Turkarslan et al (1988) The tests undertaken were glucose fermentation, reduction of triphenyl tetrazolium chloride, methylene blue reduction, casein digestion, phosphatase production, liquefaction of inspissated serum, film and spot production, and arginine decarboxylation. Growth inhibition test (GIT) was performed for the serological characterization of the isolates following the methods described by Clyde (1964), Cottew (1983), and Radwan et al (1985)

RESULTS AND DISCUSSION

Acute or active infection of Caprine Pleuropneumonia is usually common with high morbidity and mortality with typical clinical signs and postmortem lesions. Conversely in this study, at ante mortem examination most of the goats appeared healthy while few were observed emaciated with milder respiratory signs of coughing, sneezing and nasal discharge. At necropsy few of the lungs in the affected goats were noticed having pneumonic lesions. There were small to large reddish areas of lung consolidation, varying degree of hepatization and in few cases thickened pleurae adherent to the inner chest wall were observed. The

pericardium was normal and no pleural fluid was found in any case. The milder clinical signs and postmortem lesions may be an indication of chronic infection or could be due to the prevalence of mild patho-type of Mycoplasma species. Almost similar clinical and postmortem lesions were reported in most of the chronic cases of Caprine Pleuropneumonia by Al-Aubaidi (1972a), Buxton and Fraser (1977), (Sharma (1978), Cottew (1979) and Simos (1987).

Of the 50 lung samples processed, 6 isolates (table III) were recovered and identified on the basis of growth characteristics (table I), biochemical tests (table II) and serological reaction (table I) These isolates were grouped into 3 types. Type I comprised of 3 isolates which on PPLO agar produced large, and flat colonies that could be seen with naked eye. Microscopic examination revealed typical fried egg type colonies with proportionally small central papilla with entire periphery. All of these isolates produced great turbidity in PPLO broth medium. Biochemically these isolates were positive for glucose fermentation, tetrazolium reduction, casein digestion, liquefaction of inspissated serum, and methylene blue reduction tests. On these parameters, type I isolates were declared as *Mycoplasma mycoides* subspecies capri Similar characteristics for *Mycoplasma mycoides* subspecies capri have been reported by Barber and Ydloutsching (1970), Buchanan and Gibbons (1975), Awan (1985), Ojo (1976) and Turkarslan(1988). Growth inhibition test was also performed for these 3 isolates using standard antiserum against *Mycoplasma mycoides* subspecies Capri (PG3 Strain) .The inhibition zones were greater than 2mm which is in agreement with the results reported by Radwan et al.,(1985)., Awan,(1985)

Type II designated group included 2 isolates. They produced large, granular fried egg type colonies on PPO agar with proportionally large central papilla with irregular periphery. The turbidity in PPLO liquid medium was moderate by these isolates. Bio- chemically these isolates were negative for all the tests employed except glucose fermentation and tetrazolium reduction. Type II isolates were found capable of growing in PPLO medium with and without equine serum. These were declared as Acholeplasma species. Similar findings were also reported by Wilson and Miles (1975), Benerje et al (1979) and Awan (1985). The GIT was not performed because standard antiserum against Acholeplasma coud not be found.

There was only one isolate in type III category. This isolate produced small, smooth, center less colonies on PPLO agar with fair or little turbidity in PPLO broth. This isolate though biochemically similar to Acholeplasma but different colonial appearance and inability to grow on PPLO medium without serum. excluded it from type II group. This isolate was classified as unidentified Mycoplasmas. Growth inhibition test could not be done due to these reasons.

Further work on the pathogenicity of these isolated Mycoplasmas in goats under local conditions should be carry out. The vigorous surveillance studies for the prevailing mycoplasma species particularly F-38 biotype, designated as *Mycoplasma capricolum subspecie capripneumoniae* (Mccp) (Nicholas, 2002) should be planned out, in order to highlight the extent of infection, the carrier animals and preparation of effective live or killed vaccine against Contagious Caprine Pleuro-pneumonia . Moreover the role of imported animals in the transmission of mycoplasmal diseases in Balochistan should be investigated by strict and well organized Quarantine measures.

Table I

Biochemical Characteristics of the Isolates

Sample Number	Isolate Number	A	B	C	D	E	F	G	H	Isolate Identification
135	1	-	+	+	+	+	+	-	+	*Mycoplasma mycoides subsp capri*
180	2	-	+	+	+	-	-	-	-	*Acholeplasma*
273	3	-	+	+	+	+	+	-	+	*Mycoplasma mycoides subsp capri*
322	4	-	+	+	+	-	-	-	-	*Untypable Mycoplasma*
349	5	-	+	+	+	+	+	-	+	*Mycoplasma mycoides subsp capri*
453	6	-	+	+	+	-	-	-	-	*Acholeplasma*

A: Arginin decarboxylation **B: Glucose fermentation**
C:Tetrazolium reduction **D: Casein digestion**
E: Phosphate production **F: Serum liquefaction**
G: Film & spot reaction **H: Methylene blue reduction**

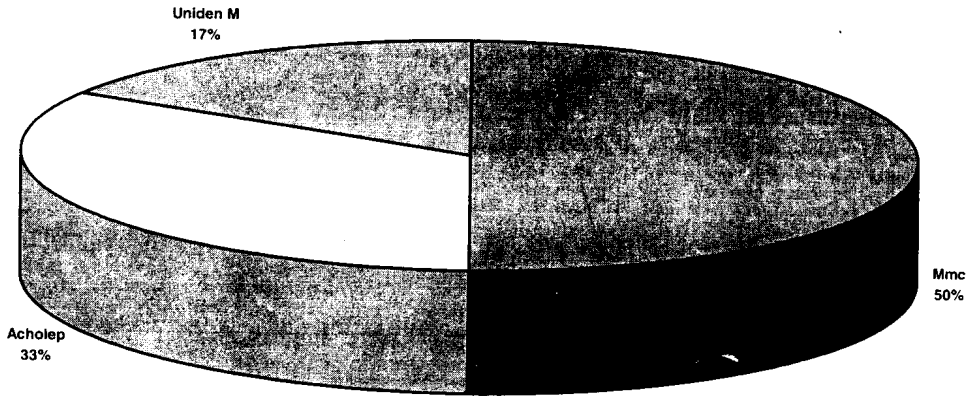
Table.II

Growth & Serological Characteristics of the Isolates

Isolate Number	Morphology	Equine serum Requirement	Turbidity in PPLO broth	Recovery site	Growth inhibition test	Type	Identification
1, 3, 5	Large flat, typical fried egg colonies	Yes	Great	Lungs	Inhibited with PG3 antiserum	1	*Mycoplasma mycoides subsp capri*
2, 6	Medium, non fried egg colonies with larger central papilla	No	Moderate	Lungs	Not done	2	*Acholeplasma*
4	Small smooth raised, center less colonies	Yes	Fair	Lungs	Not done	3	Untypable *Mycoplasma sp.*

Table III

Distribution of the Isolates



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