

# ISOLATION AND CHARACTERIZATION OF MYCOPLASMA AND ACHOLEPLASMA FROM PNEUMONIC LUNGS OF GOATS SLAUGHTERED AT FAISALABAD ABATTOIR

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## ABSTRACT

Among the goats slaughtered at Faisalabad abattoir, pneumonic lungs were collected from 50 animals suspected for contagious caprine pleuropneumonia (CCPP). These samples were subjected to bacteriological studies and 8 isolates were recovered. On the basis of morphological, biochemical and serological characteristics, these were identified as *Mycoplasma mycoides* subspecies capri (4), acholeplasmas (2) and unidentified mycoplasmas (2).

## INTRODUCTION

The economic importance of goat farming is increasing in many parts of the world and at the same time, caprine infectious diseases are becoming more and more important. Among these, contagious caprine pleuropneumonia (CCPP) and related mycoplasmoses in goats have attracted particular interest. The disease is world-wide in distribution but definite aetiology of the disease complex is not yet clearly understood. Among various organisms, most of the times, mycoplasmas are recovered from caprine pneumonic lungs (Nascimento *et al.*, 1986). Mycoplasma associated pulmonary infections of goats are often associated with *Mycoplasma mycoides* subspecies capri, *Mycoplasma mycoides* subspecies mycoides (LC-type) and *M. capricolum*. Others, such as *Mycoplasma ovipneumoniae* are not pathogenic except when the animal is immune compromised, or like *Mycoplasma arginini* merely invade tissues without actual pathogenicity (Lefevre *et al.*, 1987).

Clinical picture of the affected animals does not permit the differentiation among various mycoplasma infections and the diagnosis can only be based on the isolation of the organisms in the laboratory.

The present investigations were carried out to isolate and characterize the mycoplasma from suspected pleuropneumonia cases as well as to record their susceptibility to various antimicrobial agents *in vitro*. It was anticipated to determine the prevalent mycoplasma species in goats and their *in vitro* susceptibility to common antibacterial agents.

## MATERIALS AND METHODS

From the goats slaughtered at Faisalabad abattoir,

50 lungs showing pneumonic lesions were collected for bacteriological studies. The tissue samples were washed twice with sterilized normal saline and stored in deep freezer at -20°C as soon as possible. The isolation of caprine mycoplasmas was carried out using PPLO medium (broth & agar) containing horse serum (15%), yeast extract (1%), thallos acetate (0.05%) and ampicillin (0.001%). Selected tissues were minced using sterilized scissors in PPLO broth (1:9 W/V). One ml of this suspension was also streaked on PPLO agar plate. The plates were fixed with adhesive tape and incubated at 37°C in candle jar under moist conditions (Levinson and Jawetz, 1996).

These plates were observed microscopically (24 X) for growth after every two days up to 14 days. All the inoculated broth tubes were passaged blindly into fresh PPLO broth following three days of incubation and were examined daily for a week for the evidence of growth. The materials from the tubes with and without growth were streaked on PPLO agar plates and incubated accordingly. While those with no visible growth on PPLO plates were again passaged in PPLO broth. In case of positive culture, repeated sub-culturing was done on fresh medium 2-3 times to achieve purity of the isolates. For cloning and purification purposes, the broth cultures were also filtered through 0.5 µm (APD) millipore filter. The filtrate was streaked on the solid medium and incubated at 37°C as mentioned above. The filtration and plating was repeated twice.

The purified cultures were also inoculated on nutrient agar as well as PPLO medium without serum to know the sterol dependency of the isolates. For the characterization of the isolates, various biochemical parameters were recorded (Cottew and Yeats, 1978; Turkarslan, 1988).

Table 1 : Biochemical characteristics of the isolates

Parameters	Isolates							
	1	2	3	4	5	6	7	8
Arginine Decarboxylation test	-	-	-	-	-	-	-	-
Glucose Fermentation test	+	+	+	+	+	+	+	+
Tetrazolium Reduction test	+	+	+	+	+	+	+	+
Casein Digestion Test	-	+	-	-	+	+	-	+
Phosphatase Production Test	-	-	-	-	-	-	-	-
Inspissated Serum Liquefaction Test	-	+	-	-	+	+	-	+
Film & Spot Reaction Test	-	-	-	-	-	-	-	-
Methylene Blue Reduction	-	+	-	-	+	+	-	+

*Mycoplasma mycoides* subspecies capri was procured from Veterinary Research Institute, Lahore. Antiserum was raised in rabbits after four repeated inoculations (Singh and Srivastava, 1993). Growth inhibition test (GIT) was also carried out for the serological characterization of mycoplasma isolates (Cottew, 1983; Radwan *et al.*, 1985). The identified isolates were tested *in vitro* for antimicrobial sensitivity against Tetracycline (Pfizer), Chloromycetin (Park Davis), Neomycin (Oxoid), Carbenicillin (Pfizer), Kanamycin (Lilly), Sulphatriada and Cephalexin (Oxoid). Zones of inhibition of various antimicrobial agents were measured in millimeters.

## RESULTS AND DISCUSSION

Of the 50 tissue samples, 8 exhibited observable growth in PPLO broth (complete medium), based on morphological, cultural and biochemical characteristics, these isolates were divided into three groups, each being distinct in its characteristics regarding arginine decarboxylation, glucose fermentation, casein digestion etc. (Table 1).

Group 1 included 4 isolates which produced large, entire, typical fried-egg like colonies having a proportionately small central papilla on PPLO agar plates. While in broth, these strains produced greater turbidity as compared to isolates in other groups. They fermented glucose, reduced tetrazolium and methylene blue, digested casein and liquefied inspissated horse serum. They did not produce phosphatase and were negative for film and spot reaction and arginine decarboxylation. These characteristics are peculiar to *Mycoplasma mycoides* as reported by various research workers (Barber and Yedloutsching, 1970; Buchanan and Gibbons, 1975; Ojo, 1976; Awan, 1985; Turkarslan, 1988). Growth inhibition tests using specific antisera further facilitated to conclude that these

isolates were *Mycoplasma mycoides* subspecies capri. This species is the most common cause of the CAPP, therefore, control policies should be mainly directed against this organism.

Group 2 comprised two isolates which produce and large, typical fried-egg type colonies with proportionately wide central nipple and rough, granular periphery. The turbidity produced in liquid medium was obvious. Growth was also observed in serum free PPLO medium. The biochemical reactions showed them to be positive for glucose fermentation and tetrazolium reduction, while negative for rest of the tests. Typical colony characteristics and growth on serum free PPLO medium supported the conclusion that the isolates were *Acholeplasma* as has also been reported by Parker and Collier (1990) and Awan (1985).

The remaining two isolates (Group 3) produced centre-less, small, raised and smooth colonies on solid medium. They were unable to grow in the absence of serum. The isolates were positive to glucose fermentation and tetrazolium reduction. They neither digested casein nor produced film and spot reaction on egg yolk medium. The colonial morphology and biochemical reactions of the members of 3rd group placed them in the *Mycoplasma* species. Growth inhibition test could not be carried out due to unavailability of specific antisera. Since serological evidence could not be gathered as such, these isolates were reported as unidentified mycoplasmas (Awan, 1985).

Comparative studies with available antibacterials were carried out on eight cultures of *Mycoplasma* and *Acholeplasma* of 3 types. The organisms did not show great type-dependent sensitivity. Most of the cultures were highly sensitive to Tetracycline and weakly sensitive to Cephalexin and Kanamycin. Barber and Yedloutsching (1970), Kovalenko *et al.* (1978) and Tariq (1980) also reported high efficacy of tetracycline

as a curative agent in the event of *Mycoplasma* infections.

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