

Original Research Article

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## Isolation, Identification and Antibiogram of *Mannheimia haemolytica* Associated with Caprine Pneumonia in the Cauvery Delta Region of Tamil Nadu, India

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### ABSTRACT

A field investigation was made in an organized goat farm near thanjavur city with the history of sudden mortality due to respiratory distress. About 300 tellicherry adult goats are maintained in that farm. Bilateral serous to mucopurulent nasal discharge, lymphadenopathy and respiratory stridor were observed in few animals. Samples such as nasal swab, trans tracheal lavage, blood samples, fecal samples and lymph node aspiration smear were collected from affected animals aseptically for microbiological and parasitological examination. Nasal swabs and tracheal lavages were analyzed for bacteriological examination using standard methods. *Mannheimia haemolytica* could be isolated and confirmed by multiplex PCR using hypothetical protein, Lkt and Lkt2 of leukotoxin D gene and 16S rDNA gene primers. The isolate was found to be susceptible to Cetriaxone, Amikacin and Enrofloxacin while the isolate was resistant to Tetracycline, Gentamicin and Streptomycin. The fecal samples, blood smear and lymph node aspiration smear were negative for parasitic ova, cyst and blood protozoan. The isolation and identification of *M. haemolytica* reflect the possible role causing respiratory disease encountered in goat flocks.

#### Keywords

Tellicherry goat, Pneumonia, *M. haemolytica*, Antibiogram and Multiplex PCR.

#### Article Info

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### Introduction

Pneumonia is defined as inflammation of the pulmonary parenchyma and most commonly encountered respiratory problems in small ruminants throughout the world (Ackermann and Brogden, 2000). In goat herds, pneumonia increases production costs associated with expensive treatments which cause economic burden to small and marginal farmers. Though pneumonia is generally fatal in kids, infections and deaths also happen in adult animals. The respiratory infections contribute 5.6 percent of total diseases of

small ruminants irrespective of etiology (Hindson and Winter, 2002). Respiratory infections of goats are multifactorial (Lacasta *et al.*, 2008). The bacterial, viral, fungal, parasitic and other managemental factors are related with pneumonia in goats. Bacterial agents such as *Pasteurella multocida*, *Mannheimia haemolytica*, *Mycoplasma species*, *Streptococcus pneumoniae*, *Arcanobacterium pyogenes* and *Bordetella parapertussis* cause respiratory infections in small ruminants (Brogden *et al.*, 1998;

Ackermann and Brogden, 2000; Berge *et al.*, 2006; Yener *et al.*, 2009). The pneumonic pasteurellosis in goats are mainly caused by *Mannheimia haemolytica* and *Pasteurella multocida* which leads to significant reduction in growth performance (Brogden *et al.*, 1998, Emikpe *et al.*, 2013). *Pasteurella haemolytica* biotype A was renamed as *Mannheimia* which contain several species including *M. haemolytica*, *M. granulomatis*, *M. glucosida*, *M. ruminalis* and *M. varigena* (Angen *et al.*, 1999a). *M. haemolytica* is a gram-negative coccobacilli, non-motile, non-spore forming, fermentative and bipolar organism (Chen *et al.*, 2002). Diagnosis of *M. haemolytica* by molecular methods yields rapidity and sensitivity compared to conventional cultural and biochemical tests. In the present study pneumonia associated with *M. haemolytica* could be isolated and confirmed by multiplex PCR and antibiotic sensitivity of that isolate was also determined.

## Materials and Methods

### Sample collection

Samples such as nasal swab, trans tracheal lavage, blood samples, fecal samples and lymph node aspiration smear were collected from affected animals aseptically for microbiological and parasitological examination. The samples were transported under cold condition to the laboratory for bacterial isolation.

### Bacteriological examination

The samples were inoculated into blood agar, MacConkey agar, PPLO broth and incubated aerobically for 24 hours at 37°C. Small narrow zone hemolytic colonies were observed in blood agar and colonies were subjected to gram staining and routine biochemical tests were carried out (Quinn *et al.*, 1994). Antimicrobial susceptibility testing can be carried out by disc diffusion method

using tetracycline, sulfadiazine, enrofloxacin, streptomycin, ciprofloxacin, amikacin, ceftriaxone and gentamicin antibiotic discs.

### DNA extraction

DNA was extracted from the suspected colony by thermal lysis method. One or two colonies from the suspicious cultures were picked up and suspended into 1.5 ml of micro centrifuge tube containing 50 µl of molecular grade water. The tube was placed in a boiling water bath for 10 minutes followed by snap chilling and centrifugation at 13000 rpm for 5 minutes. Without disturbing the pellet, the supernatant was removed and stored at -20°C until for further analysis.

### Multiplex PCR

Multiplex PCR was performed in a Thermocycler (Master Cycler nexus gradient) in a total reaction volume of 25 µl containing 12.5 µl ampliqon master mix (2x) and using already published 1 µM of primer sets shown in table 1 (Alexander *et al.*, 2008) and 3 µl of template sample DNA. PCR procedure was performed as follows; 95°C for 15 minutes; 40 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s and final extension of 72°C for 10 minutes.

### Parasitological examination

The fecal samples were screened for parasites affecting the small ruminants by the routine procedures. Further, blood smear and lymph node aspiration smear were examined for blood protozoan parasites.

## Results and Discussion

### Bacterial isolation

*M. haemolytica* (75%) followed by other bacterial agents *Staphylococcus* spp. (10%), *E. coli* (5%) and *Proteus* spp. (10%), were

also isolated from nasal swab and *M. haemolytica* (80%) and *E. coli* (20%) were isolated from trans tracheal lavages shown in table 2. The majority of the isolate was *M. haemolytica*. Further Mycoplasma bacterial growth was not observed in PPLO broth after 14 days of incubation.

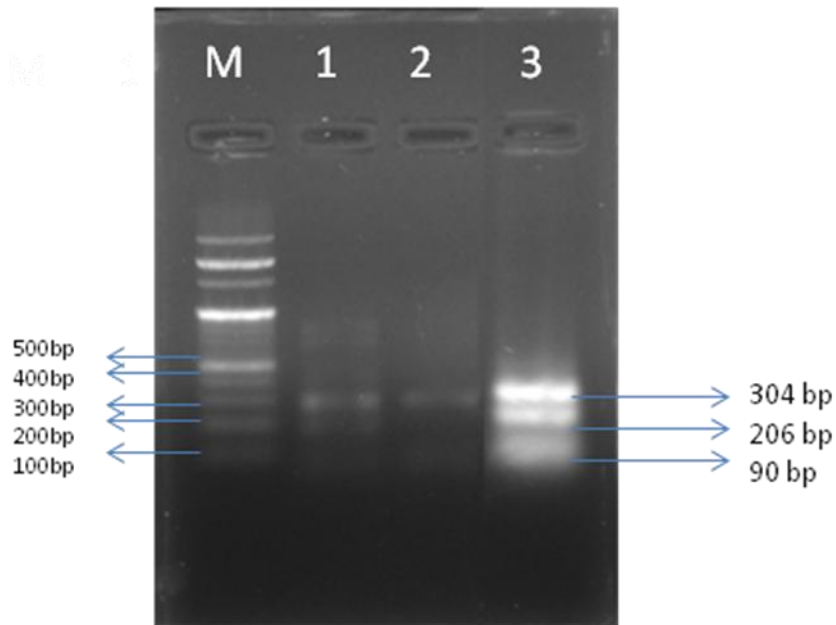
*Mannheimia* spp. isolates identified by culture and biochemical tests were subjected to multiplex PCR using hypothetical protein, Lkt and Lkt2 of leukotoxin D gene and 16S rDNA

gene primer sets which gave positive results for *M. haemolytica* shown in figure 1.

On the basis of these results, *M. haemolytica* was the most common cause of respiratory disease outbreaks in goat flocks.

The antibiotics resistance pattern showed that the isolate was found to be susceptible to Cetriaxone, Amikacin and Enrofloxacin while the isolate was resistant to Tetracycline, Gentamicin and Streptomycin.

**Fig.1** Multiplex PCR for detection and differentiation of *Mannheimia haemolytica* from other species. M- DNA Ladder (100bp), 1- *E. coli*, 2 -*Proteus* spp., 3 - *Mannhemia haemolytica* (HP - 90bp, LKT -206bp, 16S rDNA -304bp)



**Table.1** Primers used in this study

Target gene	Primer sequence	Size	Reference
LKT	GCAGGAGGTGATTATTAAAGTGG CAGCAGTTATTGTCATACCTGAAC	206 bp	Alexander <i>et al.</i> , 2008
LKT2	CTCTCTTTAGAAAAGCTGGAAAAC TTTTGCCAAGTGGTGTATTGC	170 bp	Alexander <i>et al.</i> , 2008
HP	CGAGCAAGCACAATTACATTATGG CACCGTCAAATTCCTGTGGATAAC	90 bp	Alexander <i>et al.</i> , 2008
16S rDNA	GCTAACTCCGTGCCAGCAG CGTGGACTACCAGGGTATCTAATC	304 bp	Alexander <i>et al.</i> , 2008

**Table.2** Frequency and percentage of bacterial species isolated from clinical samples

Samples	Bacterial species	Frequency	Percentage (%)
Nasal swab	<i>Mannheimia spp.</i>	15	75
	<i>Staphylococcus spp.</i>	2	10
	<i>E.coli</i>	1	5
	<i>Proteus spp.</i>	2	10
Trans tracheal lavages	<i>Mannheimia spp.</i>	4	80
	<i>E.coli</i>	1	20

### Parasitological examination

No parasitic ova, cyst and blood protozoan parasites were detected in fecal samples, blood smear and lymph node aspiration smear.

Respiratory tract infection in small ruminants is the major cause of economic burden in marginal and landless farmers in India. Pathogens like bacteria, virus and parasites have been occupied in the development of respiratory disease along with stress factors. Stress factors such as transport, overcrowding, poor housing conditions and environmental changes cause respiratory insult which result in decline in the immune status of the small ruminants and thereby supporting the number of bacterial, viral, and parasitic diseases to break down the tissue defense barriers (Rahal *et al.*, 2014). Due to loss of natural defense mechanism, goats are highly susceptible to *Pasteurella* spp. and *Mannheimia* spp. which are commensals in the respiratory tract. *M. haemolytica* cause important respiratory tract disease in ruminants and small ruminants (Highlander 2001).

The virulence factors associated with pathogenicity of *M. haemolytica* are leukotoxin (LKT), lipopolysaccharide, adhesions, capsule, outer membrane proteins and various proteases. Out of these virulence factors, leukotoxin (LKT) which is species specific, lipopolysaccharide are considered as most important virulence factor for

pulmonary colonization, evasion of immune response and establishment of infection (Singh *et al.*, 2011). In this study, already published primers derived from HP, Lkt and Lkt2 and 16S rDNA genes which amplifying hypothetical protein, different regions of the leukotoxin D gene and universal bacterial sequences were used respectively (Alexander *et al.*, 2008). Isolates were designated as *M. haemolytica* by positive amplification of HP, Lkt, 16S rDNA genes, *M. glucosida* (HP, Lkt, Lkt2, 16S) and *M. ruminalis* (HP, 16S). Molecular methods yield high specificity and sensitivity compared to cultural methods for detection of *Mannheimia* spp. (Catry *et al.*, 2004). Multiplex PCR is employed to distinguish *M. haemolytica* from other bacterial species (Alexander *et al.*, 2008) since serotyping is not a consistent tool for differentiating strains of *Mannheimia* spp. (Jaramillo-Arango *et al.*, 2008).

In the present study, *M. haemolytica* could be isolated from respiratory disease outbreaks in goat flocks in the Cauvery delta region of Tamil Nadu. It is further confirmed by multiplex PCR as a rapid and preferred detection method for differentiation of *M. haemolytica* from other bacterial species compared to cultural and serotyping methods.

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