Research article
J. Adv. Nutri. Sci. Technol. 1(3): 103-108, 2021 **DOI: 10.15228/ANST.2021.v01.i03.p01**

Isolation and identification of *Mycoplasma capricolum* subsp. *capripneumoniae from* goats

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Abstract

Contagious caprine pleuropneumonia is a transmissible disease in goats caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp). The disease is responsible for remarkable morbidity and mortality in goats. In Pakistan, data regarding its prevalence and incidence is lacking. This research study was aimed at providing the current prevalence rate in Punjab, Pakistan. For this, 50 samples including pleural fluids, nasal swabs and trachea were collected from slaughterhouses, livestock markets and veterinary hospitals around Faisalabad and processed for the determination of Contagious caprine pleuropneumonia by culture and confirmation by polymerase chain reaction. Characteristics fried egg colonies and Giemsa staining were indicative of Mccp. The culture positive samples were then processed to detect 16S rRNA gene of Mccp. Based on cultural and molecular evidence: a) The prevalence of Contagious caprine pleuropneumonia was 40% in slaughterhouse specimens, b) The prevalence of contagious caprine pleuropneumonia was 46.66% in livestock markets and, c) The prevalence of contagious caprine pleuropneumonia was 15% in veterinary hospitals.

***Keywords*:** Mccp, Goat, Polymerase chain reaction

Highlights

- The study was aimed at providing a figure for prevalence of contagious caprine pleuropneumonia in the goat population in Faisalabad Pakistan.
- The detection of the disease organism was confirmed by bacterial culturing and biochemical testing.
- The culture positive samples were further confirmed via qualitative polymerase chain reaction targeting the 16S rRNA gene.
- The prevalence of contagious caprine pleuropneumonia was found to be 40%, 46.66%, and 15% in slaughterhouse specimens, livestock markets, and veterinary hospitals.
- The prevalence was found to be highest in samples collected from younger animals as compared to adult ones.

1. INTRODUCTION

Contagious caprine pleuropneumonia, caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp), is a fatal disease of goats that causes a significant economic loss in the Middle East, Africa, Eastern Europe and Asia (Abd-Elrahman, Khafaga, & Abas, 2020). Mccp belongs to the cluster of *Mycoplasma mycoides* which consists of six mycoplasma species including *M. capricolum, M. capripneumoniae, M. mycoides SC, M. mycoides LC, Mycoplasma sp. Bovine serogroup* 7 and *M. capri* (Shah, et al., 2017, Noah, et al., 2011). *M. capripneumoniae* affect goats only (OIE, 2008). Lesions are confined to the thoracic cavity with straw-colored fluid in the pleural cavity at necropsy and lesions are more specific in lungs and pleura. In severe cases, lungs are consolidated and hepatized with the port wine color (Abraham, et al., 2015). In chronic cases, lung tissues are black and show necrotization (Rahman, et al., 2018). Classical contagious caprine pleuropneumonia lesions are confined to the thoracic cavity while the lesions caused by *Mycoplasma mycoides* LC, *M. capri*, and *M. capricolum* can be in any other parts of the body including the thoracic cavity (El-Deeb, et al., 2017).

Confirmatory diagnosis of *M. capripneumoniae* (Mccp) is very difficult because it has cross-reactivity with other mycoplasma cluster's species (Namazi, Derakhshandeh, Hezaveh, & Eraghi, 2020). Although isolation and identification steps are important in confirmation of *Mycoplasma capricolum* subsp. *capripneumoniae* it also requires advanced laboratory facilities as well as experience to treat this luxuriant bacterium (Kabir and Bari, 2015). Similarly, the use of antibiotics also becomes a hindrance in the isolation of *M. capripneumoniae.* Molecular technique especially PCR is useful in the confirmatory diagnosis of *M. capripneumoniae* (Woubit, et al., 2011). An accurate molecular technique allows to identify Mccp by making multiple copies of 16S gene.

The aim of this study was to provide a baseline prevalence data of Mccp in Faisalabad based on molecular characterization.

2. MATERIALS AND METHODS

2.1. Sample Collection and Processing

Samples were collected aseptically from 50 goats showing respiratory signs and nasal discharge as shown both age-wise and sex-wise in Table 1 and 2, respectively.

Table 1: Age wise sampling of animals from different collection centers

Age	Abattoirs (Lung's exudate)	Livestock markets (Nasal swabs)	Veterinary hospitals (Nasal swabs)
2-6 months	8	9	10
7-12 months	4	3	6
Above 12months	3	3	4
Total	15	15	20

Table 2: Sex wise distribution of the collected samples

Source	Male	Female
Abattoirs	8	7
Livestock markets	7	8
Veterinary hospitals	12	8

Immediately after collection, sample swabs were inoculated in Hay's flick broth poured in McCartney bottles and incubated at 37°C for 24-48 hours following the procedure of Kabir and Bari, 2015. A second passage was given to positive samples in Hay's flick broth in McCartney bottles at 37°C for 24 hours (Ahmad et al., 2020).

2.2. Purification of organism

After second passage, the material was further streaked on Hay's flick agar and placed in anaerobic jar at 37°C for 2-3 days. The colony characters were recorded as there were off-white color growth on agar plates. Colonies were identified under microscope at 100X. Purified colonies were proceeded for Geimsa staining and biochemical characterization via glucose fermentation, phosphatase activity and Tetrazolium reduction tests as described (Parray et al., 2019).

2.3. Molecular characterization

The colonies on the Hay's flick agar media were processed to extract DNA of the organism using GF-1 nucleic acid extraction kit according to manufacturer'sinstructions. Species specific forward and reverse primer against 16S rRNA gene of Mccp, (MmF 5'-CGAAAGCGGCTTACTGGCTTGTT-3') and (MmR 5'-TTGAGATTAGCTCCCCCTCACAG-3') respectively, were used for the confirmation of *Mycoplasma capricolum* subsp. *capripneumoniae* via qualitative polymerase chain reaction. 35 cycles of PCR were carried out under the following conditions mentioned in table 3.

Table 3: Conditions for polymerase chain reaction

Step Name	Temperature	Time
Initial Denaturation	94°C	4 minutes
Denaturation	95°C	45 seconds
Annealing	55°C	1 minute
Elongation	72°C	1 minute
Final elongation	72°C	7 minute

3. RESULTS

A total of 50 samples were collected from abattoirs, livestock market, and veterinary hospitals. The samples were processed for culture and molecular characterization of Mccp. Of the 50 samples processed, 16 samples gave positive result following 2 passages in Hay's flick broth, the positive specimens were indicated by red color (Fig 1).

Fig. 1: Hay's Flick broth showing positive (yellow) and negative (red) samples in McCartney bottles.

Broth positive samples were inoculated then on Hay's flick agar. Off white color growth obtained on gar media was indicative of Mccp (Fig 2).

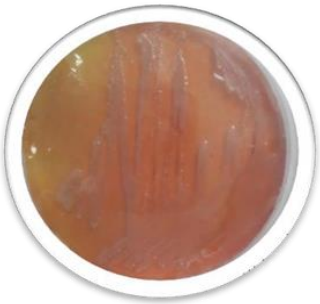

Fig. 2: Off-white colonies on Hay's flick agar.

The colonies were stained with giemsa's stain and analyzed under microscope at 100X magnification to observe fried egg like colonies as shown in figure 3.

Fig 3. Microscopic observation - Fried egg appearance of colonies.

3.1. Source-based isolation of Mccp

Of the abattoir'ssamples, 40 percent were culture positive for Mccp compared to 46.66 percent culture positive in samples from livestock markets and 15 percent culture positive in samples from Veterinary Hospitals (Table 4). The difference in prevalence of the disease between sources was non-significant (Chi square value 4.861, df 2, P value = 0.088).

Table 4: Mccp culture positive cases in Goats in abattoirs, livestock markets and Veterinary Hospitals.

Source of Samples	Number of samples	Number of positive samples	Percentage of positive samples
Abattoirs	15	6	40
Livestock markets	15	7	46.66
Veterinary hospitals	20	3	15
Total samples	50	16	32

3.2. Age-based isolation of Mccp

The prevalence was 39.19 percent in abattoirs relative to 29.41 percent in livestock markets and 20 percent in veterinary hospitals. There was non- significant difference in disease prevalence between different age groups (Table 5) (Chi square 1.293, df 2, P value= 0.524).

Table 5: Age wise distribution of Mccp culture positive in suspect goats.

Age (months)	Number of samples	Number of positive samples	Percentage of positive samples
2 to 6 months	23	9	39.13
6 to 12 months	17	5	29.41
Above 12 months	10	2	20
Total	50	16	-

3.3. Sex-based isolation of Mccp

The prevalence of CCPP was 25.92 percent in male and 39.13 percent in female goats. Prevalence of disease did not significantly differ between male and female (Table 6). (Chi square =0.995, df 1, P-value= 0.319)

Table 6: Sex-based distribution of Mccp culture positive cases in suspect goats.

Sex	Number of samples	Number of positive samples	Percentage of positive samples
Male	27	7	25.92
Female	23	9	39.13
Total	50	16	-

3.4. Molecular Detection of Mccp through PCR

Of the 50 samples, 16 were culture positive for *Mycoplasma capricolum* subsp. *capripneumoniae*. All of these were positive for 16S rRNA gene (fig.4) confirming *Mycoplasma capricolum* subsp. *capripneumoniae*. Product size for this PCR was 548bp that was compared by using 1kb DNA ladder.

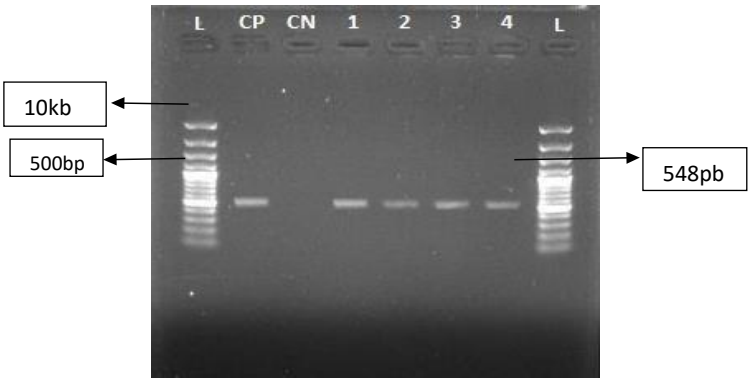

Fig 4. Detection of gene of 16S rRNA of Mycoplasma via Qualitative PCR

4. DISCUSSION

Serologically using the cELISA kit, CCPP has been reported in many countries around the world including Ethiopia 14.6 per cent, Tajikstan 10.1 per cent, Pakistan Diameer 44.2 per cent and Gilgit 2.7 per cent; Mauritius 16 per cent and Kenya 6-90 per cent (ContPeyraud et al., 2014). CCPP is a common disease in goats widely prevalent in Pakistan. In the past, some reports from Punjab (Shahzad et al., 2016), Baluchistan (Awan et al., 2009, Ejaz et al., 2015) and mostly from Khyber Pakhtoon Khaw (KPK) reported cELISA-based seroprevalence. This study has concentrated on *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp) isolation from goats clinically showing fever with respiratory signs. This study finds that (Mccp) can be isolated from a high number of goats clinically showing low grade fever and respiratory signs.

Of the collected samples, 32 percent were culture and PCR positive for Mccp (Table-3). Many studies report seroprevalence rather than Mccp isolation, hence present findings cannot be compared to previous studies. In a study during 2006- 2007, Shahzad et al., (2012) used Latex Agglutination test (LAT) to detect the prevalence of CCPP in different regions of Pakistan but found zero prevalence of Mccp. Similarly, Ejaz et al. (2015) designed a study in goats for molecular prevalence of mycoplasmosis in different districts of Baluchistan and found not a single positive case for Mccp. However, Hussain et al. (2012), in line with the present study. reported a 32 per cent sero-prevalence of CCPP in Beetal goats by counter electrophoresis technique. This diversity of observations could be due to non-specificity or non-sensitivity of the

tests used.

In the present study, Mccp isolation was higher in samples from livestock markets and slaughterhouse compared to samples from Veterinary Hospitals (Table-3).

This could be due to two factors: a) high outlet of non-productive animals suffering from respiratory disease to the livestock markets and slaughterhouse and b) Use of antibiotics could interfere in Mccp isolation in goats brought to the Veterinary Hospitals. No other study is available to compare this finding. (Shahzad et al., 2016), using cELISA, reported 33.33 percent seroprevalence of CCPP.

In the present study, Mccp isolation rate was highest (39.19%) in 2-6 months age group followed by 29.41 per cent in 6-12 months age group and 20 per cent in above 12 months of age group (Table-4). It appears that the probability of isolating Mccp is greater from younger compared to older animals. This hypothesis is supported by Wazir et al. who reported a higher prevalence of Mccp using cELISA in kids (6.73%) and young goats (3.85%) compared to adult goats (2%) in Khyber Pakhtoon Khaw (KPK).

In the present study, Mccp was isolated from 39.13 percent in females compared with 25.92 percent in males (Table-5). This is comparable to Shahzad et al. (2012) and (Teshome, Sori, Sacchini, & Wieland, 2019) reported a higher prevalence in females compared to males in different areas of Pakistan (Thakur, Sharma, Verma, & Kanwar, 2019) reported 16.1 percent prevalence in female compared to 10.7 percent in male.

In Summary, the study suggests that CCPP is widely distributed in goats around Faisalabad. Samples from young animals i.e., less than 6 months of age, may provide higher culture positive samples.

Acknowledgements

I am grateful to my supervisor Prof. Dr. Sajjad-ur-Rehman who provided me with all the facilities and platform to conduct the research. I pay my best regards to Prof. Dr. Ahmed Din Anjum, who helped me in the technical write up. My gratitude is also extended to Prof. Dr. Saqib, who helped me in providing consumables, and some other materials for the conduction of the research.

Conflict of interest

The authors have no conflict of interest to disclose.

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Received: 15th June, 2021 **Accepted: 10nd August, 2021**