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Research Article

Insights into Clinical Features and Antibiotic Response in MCCP Infection among Small Ruminants

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ABSTRACT

This study documents the clinical manifestations observed during sample collection from diseased small ruminants. The samples and clinical data were collected from CCPP suspected sheep and goat flocks. The culturing of Mycoplasma was performed using Modified Hay Flick medium. Mccp species were identified by biochemical profile. Antibiogram of the isolated strains was performed according to CLSI using various antibiotics including ampicillin, ceftiofur sodium, gentamicin, cloxacillin, tylosin, metronidazole, oxytetracycline, enrofloxacin and ciprofloxacin. Among the 385 culture-positive samples, fever (91.1%) and nasal discharge (88.3%) were the most prevalent symptoms, followed by cough (82.3%), dyspnea (63.6%), anorexia (61.6%), and weakness (56.8%). Contingency coefficient analysis revealed a strong association between Contagious Caprine Pleuropneumonia (CCPP) and clinical manifestations; temperature, nasal discharge, moderate association with cough and dyspnea, and weak association with anorexia and weakness. Additionally, the study assessed the in-vitro susceptibility of Mccp isolates to various antibiotics. Enrofloxacin demonstrated the highest effectiveness, followed by ciprofloxacin and gentamicin. Tylosin, metronidazole, and oxytetracycline showed moderate effectiveness, while ceftiofur sodium and cloxacillin were completely ineffective. In summary, clinical severity such as elevated temperature, nasal excretions, and respiratory distress was notable during the infection produced by prevailing field Mccp strains. The promising antibiotics response was determined for enrofloxacin, gentamicin and enrofloxacin against the isolated Mccp species from various regions of province KPK, Pakistan.

Keywords: Mycoplasma, Small ruminants, Clinical features, Antibiogram.

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INTRODUCTION

Mycoplasma infections widespread outbreak in herd leading to major production and economic losses across Pakistan. CCPP disease holds considerable economic importance and has been linked to reduced goat production across the country. Infectious disease known as contagious caprine pleuropneumonia infect sheep and goat folcks, pose great threat to livestock farming industry (Fauzia *et al.*, 2016; Sadique *et al.*, 2012; Shah *et al.*, 2017; Shahzad *et al.*, 2012; Awan *et al.*, 2010; Ur-Rahman *et al.*, 2006).

Diagnosing CCPP during natural outbreaks can be challenging due to mixed infections and similar clinical features with other diseases like pasteurellosis and Pest des petits ruminants (PPR).

The infection progresses slowly, with an incubation period of 5 to 28 days depending on the infecting species. Initially, goats infected with Mccp infected herds suffer high fever (105.8°F) and reluctant to walk. In sheep and goat, CCPP is clinically manifested by fever, pneumonia, mastitis, lacrimation, conjunctivitis, arthritis, cough, nasal discharges and rare abortion (Rahman *et al.*, 2021; Samiullah, 2013). As the infection advances, respiratory symptoms become more noticeable, characterized by painful, deep breathing and frequent coughing. In the later stages, additional symptoms such as diarrhea, nervous signs, increased salivation, and mucopurulent nasal discharge appear, causing the animals to be reluctant to move and exhibit lameness (Sadique *et al.*, 2012).

The disease produce direct losses in terms of reduced milk, meat production, high mortality rates, the expenditure associate to diagnosis, treatment, and control of infections in small ruminant flocks. Indirect losses occur due culling, poor marketing and trade barriers (Regassa *et al.*, 2010; Yatoo *et al.*, 2018).

Irrational use of broad-spectrum antibiotics and.or treatment failure may lead to the spread of the infection (Abraham *et al.*, 2015). Antibiotics such as linezolid, sulfonamides, nalidixic acid, polymyxin, rifampin, and trimethoprim have been found ineffective against Mollicutes due to their different modes of action (Chernova *et al.*, 2016). Due to Mollicutes has lack a cell wall, rendering antibiotics that target cell walls including beta-lactam antibiotics, glycopeptides and fosfomycin, become less effective. Moreover, the improper dose and irrational use of drugs in veterinary practice may lead to a great risk of promoting the development of antibiotic resistance (Chernova *et al.*, 2016). Considering the widespread of CCPP infections across the province, this study was designed to evaluate the clinical manifestations and the disease severity association. In addition antibiotic response was determined for the isolated Mycoplasma pathogens in goat and sheep flocks in various regions of Province KPK, Pakistan.

MATERIALS AND METHODS

Study Area

The research performed in various areas of province Khyber Pakhtunkhwa, that categorized into four distinct regions due to differences in climate and topography. These regions consisted of the northern (Abbottabad), southern (Kohat, Bannu, Karak, Dera Ismail Khan), central (Buner, Charsadda, Mardan, Nowshera, Peshawar), and newly merged tribal territories (Kuram, Orakazia, Bajur, North Waziristan, South Wazirstan).

Sample and Clinical Data Collection

A total of 1600 samples were gathered from sheep and goats in specific districts of Khyber Pakhtunkhwa. The samples, including nasal and throat swabs, lung tissue, and pleural fluid, were collected randomly, adhering to the standard procedure outlined by Oie (2014) across various districts in the northern, southern, central, and newly merged tribal regions (Table 1). A pre-designed questionnaire was utilized to gather information on history, animal demographics, and clinical features of sampled animals. Nasal and throat samples were obtained from diseased animals, while lung tissue and pleural fluid were collected from deceased animals. Subsequently, the samples were dispatched to the Veterinary Research Institute, Peshawar, for screening and further analysis.

Table 1. Collection of samples for isolation of Mycoplasma species from small ruminants from different regions of Khyber Pakhtunkhwa.

Region	Nasal swabs				Throat swabs				Lungs tissue				Pleural fluid				Total
	Sheep		Goat		Sheep		Goat		Sheep		Goat		Sheep		Goat		
	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	
Northern	40	40	40	40	40	40	40	40	10	10	10	10	10	10	10	10	400
Southern	40	40	40	40	40	40	40	40	10	10	10	10	10	10	10	10	400
Central	40	40	40	40	40	40	40	40	10	10	10	10	10	10	10	10	400
Merged	40	40	40	40	40	40	40	40	10	10	10	10	10	10	10	10	400
Total	160	160	160	160	160	160	160	160	40	40	40	40	40	40	40	40	1600

M=Male, F=Female

Media Preparation / Bacterial Culture

The culturing of Mycoplasma was performed using Modified Hay Flick medium, following the standard protocol outlined by OIE (2014). This medium comprises two components: Part A (Autoclaved Section) and Part B (Membrane-filtered Section). Part A consists of Bacto PPLO broth without crystal violet, autoclaved at 121°C for 15 minutes. Part B contains inactivated horse serum blended with yeast extract, glucose, sodium pyruvate, thallium

acetate, ampicillin, and phenol red. Both sections were aseptically mixed for broth preparation, while 0.9% PPLO agar was added to the broth for agar preparation.

Culture, Sub-culture and Microscopic Examination

The swabs were placed in 3 ml of PPLO medium and then incubated at 37°C with 5% carbon dioxide for a period of 7-15 days. Positive cultures exhibited turbidity, a swirling movement, and a color change from red to yellow, while negative cultures were discarded. The positive cultures underwent filtration (0.45µm membrane) and were streaked onto PPLO agar, followed by incubation at 37°C for 3-15 days. Mycoplasma colonies were inspected daily under a stereo microscope. The isolated colonies were purified and preserved in PPLO broth for further studies (Oie, 2014; Whitford *et al.*, 1994).

Biochemical Profile

For isolation and identification of Mycoplasma species (Mccp strains) various biochemical techniques such as phosphatase test, film and spot formation, tetrazolium reduction test, digitonin sensitivity, glucose breakdown cascade and urea hydrolyzation were performed using methods recommended by Shahzad *et al.* (2012) and Houshaymi *et al.* (2002).

Antibiotic Susceptibility Profiling

Antibiogram of isolated Mccp species identified from different areas of KPK Province, was performed. Antibiogram of isolated Mycoplasma strains performed to determine the efficacy of various commonly used antibiotics such as ampicillin, ceftiofur sodium, gentamicin, cloxacillin, tylosin, metronidazole, oxytetracycline, enrofloxacin and ciprofloxacin. The antibiotic sensitivity pattern of Mycoplasma species was performed using the procedure prescribed by Clinical and Laboratory Standards Institute (CLSI, 2010). Mycoplasma colonies suspension was uniform spread on Muller Hinton agar containing 5% sheep blood and left to dry. The antibiotic discs were placed using disc dispenser and incubated at 37°C for 24 hours. The antibiotics produced Zone of inhibition was measured (mm) and analyzed accordingly.

Statistical Analysis

Clinical conditions of Contagious Caprine Pleuropneumonia (CCPP) infections in small ruminants and its association and severity determind using Chi-square and Binary logistic regression analyses. Contingency coefficient values were performed to evaluate the correlation between disease severity and clinical features. ANOVA was used to observe antibiotics produced zones of inhibition. The LSD multiple comparisons performed to calculate means using Statistics 8.1 (Lawrence *et al.*, 2017).

RESULTS

Clinical features recorded during the survey indicated that laboratory diagnosed (385 positive samples), the majority of ruminants were suffering with high fever (91.1%) and nasal excretions (88.3%). Cough was observed in 82.3% of cases, while dyspnea, anorexia, and weakness were reported in 63.6%, 61.6%, and 56.8% of animals, respectively. Contingency coefficient values revealed a strong association between CCPP and temperature and nasal discharge, a moderate association with cough and dyspnea, and a weak association with anorexia and weakness (Table 2).

Table 2: Clinical presentation of Contagious caprine pleuropneumonia (CCPP) in small ruminants of Khyber Pakhtunkhwa.

Sign symptoms	Status	Positive (n=385)	Percent (%)	*Contingency co-efficient value	Severity level
Temperature	Yes	351	91.1	0.616	Strong
	No	34	8.83		
Nasal discharge	Yes	340	88.3	0.612	Strong
	No	45	11.6		
Cough	Yes	317	82.3	0.533	Moderate
	No	68	17.6		
Dysponea	Yes	245	63.6	0.438	Moderate
	No	140	36.3		
Anorexia	Yes	237	61.6	0.317	Weak
	No	148	38.4		
Weakness	Yes	219	56.8	0.264	Weak
	No	166	43.1		

*Contingency coefficient values were 0.2-0.39 for a weak, 0.4-0.599 for a moderate, and 0.6-0.799 for a strong relationship

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For the antibiogram profile, various antibiotics were utilized to assess the in-vitro susceptibility of Mccp isolates. The zone of inhibition observed for different antibiotics was highest for enrofloxacin (18.6a±1.18), followed by ciprofloxacin (15.45b±0.79) and gentamicin (13.1b±0.68) against the isolated Mycoplasma species (Table 3). Tylosin (8.05c±0.82), metronidazole (7.18c±0.55), and oxytetracycline (5d±0.91) exhibited moderate effectiveness against the isolated Mccp strains. Ceftiofur sodium and cloxacillin were found to be completely ineffective against the organism. The sensitivity level was interpreted as quite sensitive for tylosin and ampicillin. Overall, the isolated Mycoplasma species demonstrated high sensitivity to ciprofloxacin, gentamicin, and enrofloxacin, while no sensitivity was observed for ceftiofur sodium and cloxacillin.

Table 3: Antibiogram profile of different antibiotics used in the current study for Invitro analysis.

Antibiotics used	Zone of inhibition (mean±SD)	Sensitivity level	Interpretation
Tylosin	8.05^{c}±0.82	+++	Moderately effective
Gentamycin	13.1^{b}±0.68	++++	Highly effective
Ciprofloxacin	15.45^{b}±0.79	++++	Highly effective
Enrofloxacin	18.6^{a}±1.18	++++	Highly effective
Ceftofer Sodium	0^{e}±0.00	-	Resistant
Cloxacillin	0^{e}±0.00	-	Resistant
Ampicillin	9.23^{c}±0.65	+++	Quite effective
Oxytetracycline	5.06^{d}±0.91	++	Moderately effective

Means of zone of inhibition with distinct superscripts are significantly disparate at a significance level of α = 0.05.

Pathogenic mycoplasmas have a predilection for the mucosa of the respiratory, ophthalmic, and vaginal tracts, where they colonize mucosal surfaces. This colonization is particularly pronounced in hosts with compromised immune defenses, leading to the development of severe clinical manifestations (Shahzad *et al.*, 2012; Whitford *et al.*, 1994).

The prevalence of CCPP was assessed across various climatic regions in Khyber Pakhtunkhwa, Pakistan. The northern region exhibited the highest prevalence, followed by the central region, the newly merged tribal districts zone, and finally the southern region. These findings regarding distribution of Mycoplasma infections may in relation to carrier animals, climate conditions, agro-ecological diversity, population density and husbandry practices in different areas (Sherif *et al.*, 2012; Yousuf *et al.*, 2012; Shah *et al.*, 2017).

The clinical features related to prevailing CCPP infections in sheep and goat population in the study region. The severity of the clinical manifestation, high fever and nasal excretion were most common symptoms/ signs, observed in the majority of animals. Symptoms high fever and nasal discharges likely to show strong relationship to the disease, indicates these clinical features may serve as reliable indicators for presence of the infection in field conditions. In addition cough, anorexia dyspnea and weakness were prominent features noted in the infected herds. The moderate severity of symptoms like cough, anorexia dyspnea and weakness may not be consistently occur but are still effective in indicator for clinical diagnosis of the disease. A high prevalence of clinical signs associated with CCPP among the diseased animals. Fever and nasal discharge were the most common manifestations, observed in the majority of cases. Coughing was also prevalent, followed by dyspnea, anorexia, and weakness. The strong association between CCPP and fever, as well as nasal discharge, indicates these symptoms may serve as reliable indicators conjugate with other clinical features for the presence of the disease. These results highlight the valuable information associate to clinical manifestations the disease for timely intervention and accurate diagnosis. In addition, previous research demonstrated that clinical features are associated to surface antigen of protein of Mycoplasma that trigger the inflammatory response. Also, weakness and anorexia are correlated to insufficient changes in weight and strength (Shah *et al.*, 2017; Oie, 2014; Shahzad *et al.*, 2012).

In small ruminants, CCPP treatment usually depends on the type antibiotic therapy. However, CCPP in small ruminants growing concerns due to the emergence of antibiotic resistance of the causative pathogen. Developing of antibiotic resistance of the organism is a serious issue and hampers the disease management, effective prevention and control of Mycoplasma infections sheep and goat flocks (Gautier-Bouchardon *et al.*, 2002). Nevertheless, the challenge incurred from the pathogen ability to develop resistance, that may increase due to indiscriminate use of antibiotics (Shah *et al.*, 2017; Bébéar and Pereyre, 2005; Gautier-Bouchardon *et al.*, 2002).

The findings of this study regarding antibiotic resistance indicated variations in potency among different antibiotics

used against isolated Mccp strains. Enrofloxacin exhibited the highest antibiotic resistance, followed by ciprofloxacin and gentamicin, as evidenced through form formation of large zones of inhibition. The results of current research concurrent with previous studies, demonstrated that ciprofloxacin efficient activity against Mycoplasma species (Rahman *et al.*, 2021; Antunes *et al.*, 2015; Riaz Mustafa *et al.*, 2013; Antunes *et al.*, 2007). Enrofloxacin have shown proven antibiotic response and regarded as the most effective antibiotic for treatment of CCPP and other diseases in livestock and poultry (Hernandez *et al.*, 2006; Loria *et al.*, 2003; Hannan, 2000). Gentamicin had been recognized for high efficacy against the pathogen (Taylor-Robinson and Bebear, 1997). In this study, oxytetracycline, metronidazole and Tylosin showed moderate antibiotic response against the isolated Mycoplasma species. Previously, tt had been illustrated that Mycoplasma cells have developed resistance to spectinomycin, tylosin and oxytetracycline and hinders in treatment, prevention and control of the pathogen (Hernandez *et al.*, 2006; Ayling *et al.*, 2005). In comparison to findings to this research, the promising efficiency of Metronidazole demonstrated against *Mycobacterium girerdii* infections (Costello *et al.*, 2017).

In current research cloxacillin ceftiofur sodium were shown complete ineffective response against the isolated pathogens. In contrast to our results, Jin *et al.* (2014) detected the marked efficiency of ceftiofur sodium against Mycoplasma species. The antibiogram of Mycoplasma species to cloxacillin is conncurent to response detected by Egwu (1992). The results in this study explore the significant choice of appropriate medicine for the effective treatment and control of Mccp infections.

CONCLUSION

This study highlights the clinical manifestations of Mccp infection in small ruminants, with fever and nasal discharge being the most prevalent symptoms. Contingency coefficient analysis revealed varying degrees of association between CCPP and different clinical signs. Enrofloxacin, ciprofloxacin, and gentamicin demonstrated the highest effectiveness against Mccp isolates, emphasizing their potential for treatment. However, ceftiofur sodium and cloxacillin were found to be ineffective, underscoring the importance of antibiotic selection based on susceptibility testing.

AUTHOR CONTRIBUTIONS

All authors contributed equally to this research.

COMPETING OF INTEREST

The authors have not declared any conflict of interest.

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