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EVALUATION OF ANTIMICROBIAL TREATMENT OUTCOMES IN NATURAL MCCP OUTBREAKS AND THEIR RELATIONSHIP WITH CLINICAL MANIFESTATIONS: A CROSS-SECTIONAL STUDY

Original Article

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ABSTRACT

Background: Contagious caprine pleuropneumonia is a highly contagious and economically devastating disease of sheep and goats caused by *Mycoplasma capricolum* subsp. *capripneumoniae*. The disease remains endemic in many developing regions, where limitations in early diagnosis and inappropriate antibiotic use complicate control efforts. Overlapping clinical signs with other respiratory infections and the increasing emergence of antimicrobial resistance further highlight the need for region-specific clinical and therapeutic evidence to support effective disease management.

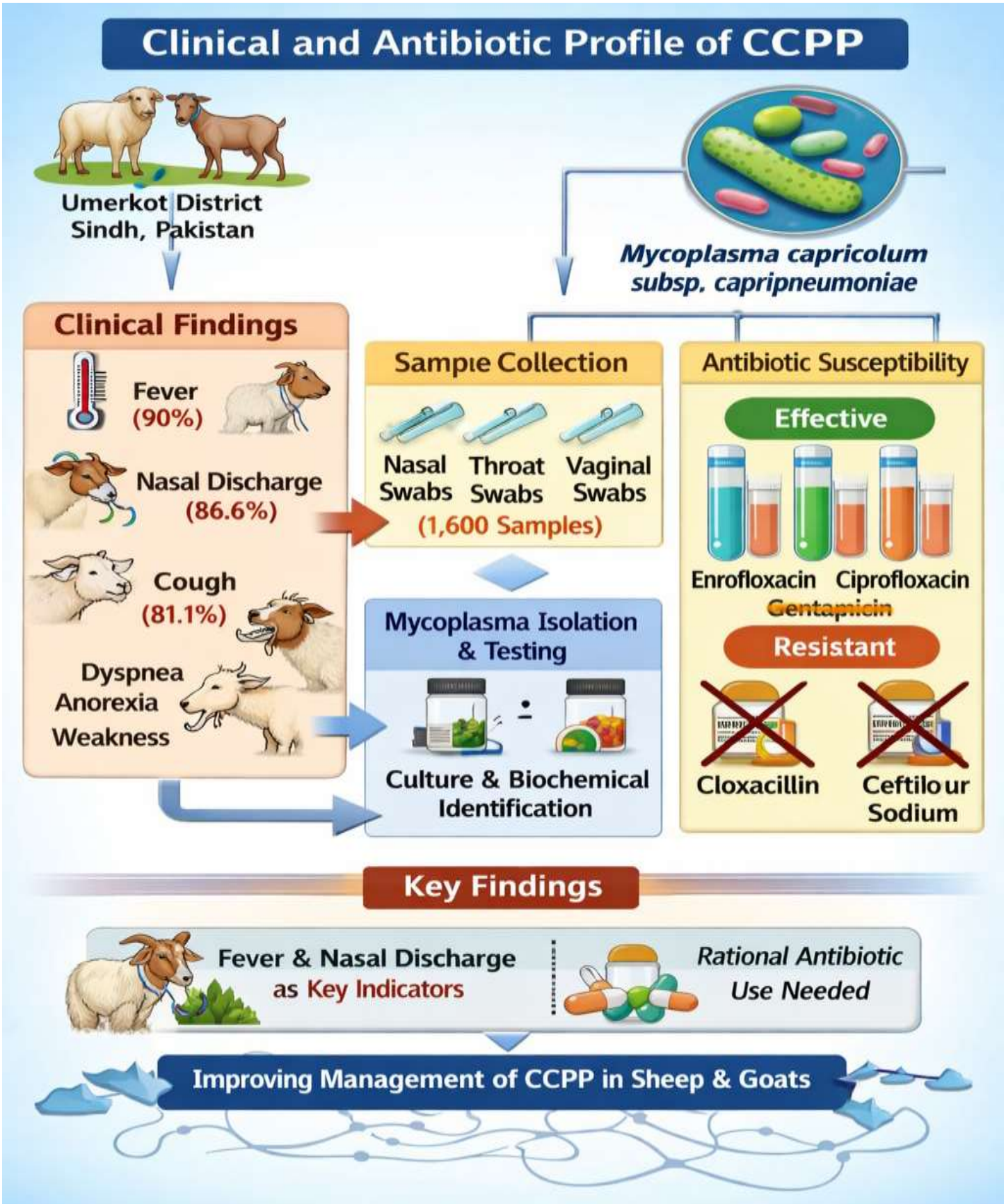
Objective: The study aimed to evaluate the clinical manifestations associated with contagious caprine pleuropneumonia and to determine the antibiotic susceptibility profile of *Mycoplasma capricolum* subsp. *capripneumoniae* isolates recovered from naturally infected sheep and goats.

Methods: A cross-sectional study was conducted in four talukas of Umerkot District, Sindh, Pakistan. A total of 1,600 samples, including nasal, throat, and vaginal swabs, were collected from clinically suspected sheep and goats. Samples were cultured on Modified Hayflick medium for isolation of *Mycoplasma* species, followed by biochemical confirmation. Clinical and epidemiological data were recorded using a structured questionnaire. Antibiotic susceptibility testing was performed using the disc diffusion method against nine commonly used antimicrobials. Statistical analyses included Chi-square tests, binary logistic regression, contingency coefficient analysis, and one-way ANOVA.

Results: Out of the examined animals, 402 were clinically positive for contagious caprine pleuropneumonia. Elevated body temperature was observed in 90.05% of cases and nasal discharge in 86.56%, both showing strong associations with disease severity. Cough (81.09%) and dyspnea (64.17%) demonstrated moderate associations, while anorexia (60.69%) and weakness (57.46%) showed weaker correlations. Antibiotic susceptibility testing revealed the highest mean zones of inhibition for enrofloxacin (19.14 ± 1.09 mm), followed by ciprofloxacin (16.02 ± 0.83 mm) and gentamicin (13.58 ± 0.71 mm). Moderate inhibitory responses were recorded for ampicillin, tylosin, and oxytetracycline, whereas complete resistance was observed against cloxacillin and ceftiofur sodium.

Conclusion: Fever and nasal discharge emerged as the most reliable clinical indicators for early field diagnosis of contagious caprine pleuropneumonia. Fluoroquinolones and gentamicin demonstrated superior in-vitro efficacy against *Mycoplasma capricolum* subsp. *capripneumoniae*, while β-lactam antibiotics were ineffective. These findings emphasize the importance of evidence-based antibiotic selection and rational antimicrobial use to improve disease control and limit the progression of antimicrobial resistance in small ruminant production systems.

Keywords: Antibiotic resistance, Contagious caprine pleuropneumonia, Goats, Mycoplasma capricolum, Pakistan, Sheep, Zoonotic bacterial infections.



INTRODUCTION

Contagious caprine pleuropneumonia (CCPP) is a highly infectious and frequently fatal respiratory disease of small ruminants caused by *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp). The disease is internationally recognized as a major transboundary animal health problem because of its ability to spread rapidly within susceptible herds, trigger explosive outbreaks, and cause severe economic disruption, particularly in arid and resource-limited regions where small ruminant farming is central to livelihoods (1). Beyond its veterinary importance, CCPP directly threatens food security and household income by undermining meat and milk production systems that support vulnerable rural populations. In Pakistan, CCPP is considered endemic and represents a persistent constraint to the productivity and sustainability of the goat and sheep sector. Recurrent outbreaks of mycoplasmal infections have been associated with substantial population losses, reduced growth performance, and declining herd value, collectively exerting long-term pressure on the national livestock economy (2). The disease affects both goats and sheep and typically follows an incubation period of 5–28 days, after which animals may develop high fever, lethargy, and reluctance to move. As the disease progresses, clinical signs commonly include pneumonia, coughing, nasal discharge, lacrimation, and conjunctivitis, with occasional involvement of joints and mammary tissue; abortion, however, is reported infrequently (3). In severe or advanced cases, animals may exhibit pronounced respiratory distress along with systemic manifestations such as diarrhea, nervous signs, and lameness, often culminating in death if untreated (4). The economic burden of CCPP is multifaceted. Direct losses arise from high morbidity and mortality rates, reduced meat and milk yields, and expenditures related to diagnosis and treatment. Indirect losses further compound this burden through forced culling, depreciation of animal market value, disruption of trade, and restrictions on animal movement imposed during outbreaks (5). For smallholder farmers, these combined losses can be devastating, reinforcing cycles of poverty and limiting investment in improved animal health practices.

Despite its importance, accurate field diagnosis of CCPP remains challenging. The clinical presentation often overlaps with other common respiratory diseases of small ruminants, particularly pasteurellosis and Peste des Petits Ruminants (PPR), and the situation is frequently complicated by co-infections. This diagnostic uncertainty often leads to empirical treatment strategies and irrational use of antibiotics. Management is further complicated by the unique biology of mycoplasmas, which lack a cell wall and are therefore inherently resistant to commonly used beta-lactam antibiotics (6-8). Although antimicrobials such as tetracyclines and macrolides are commonly employed, variable therapeutic responses, treatment failures, and emerging antimicrobial resistance have been increasingly reported. The inappropriate use of broad-spectrum antibiotics, incorrect dosing regimens, and reliance on ineffective drugs such as sulfonamides or trimethoprim not only compromise treatment outcomes but may also facilitate the silent spread of infection within and between flocks (9,10). Sindh province represents a major hub of small ruminant production in Pakistan; however, detailed epidemiological, clinical, and therapeutic studies on CCPP from this region remain limited, particularly at the district level. Umerkot district, characterized by its substantial goat and sheep population and traditional livestock farming systems, is especially vulnerable to infectious respiratory diseases. The lack of localized data on disease presentation, severity patterns, and treatment response hampers evidence-based decision-making and the development of effective control strategies tailored to local conditions. In this context, the present study was designed to address a critical knowledge gap by investigating natural outbreaks of CCPP in four talukas of District Umerkot, Sindh. The central research objective was to systematically evaluate the clinical manifestations of CCPP and their association with disease severity under field conditions, and to assess the in-vivo antibiotic response and therapeutic efficacy against Mccp infections in local goat and sheep flocks, thereby generating practical evidence to inform improved diagnosis, rational treatment, and disease management strategies in endemic settings.

METHODS

This cross-sectional field and laboratory-based study was conducted in Umerkot District, Sindh Province, Pakistan, an area characterized by arid climatic conditions and a high dependence on small ruminant production. To ensure geographic and management diversity, the district was stratified into four talukas—Umerkot, Samaro, Pithoro, and Kunri—based on their agro-climatic features, livestock density, and husbandry practices. These talukas were selected to enable a representative assessment of the distribution of *Mycoplasma* infections and clinical manifestations of contagious caprine pleuropneumonia (CCPP) across the district. The study population comprised sheep and goats presenting with clinical suspicion of CCPP during natural outbreaks. Animals of both sexes and varying ages were included if they exhibited one or more clinical signs suggestive of CCPP, such as fever, respiratory distress, nasal discharge, coughing, or lethargy. Apparently healthy animals and those under active antibiotic treatment at the time of sampling were excluded to minimize diagnostic bias. A total of 1,600 samples were collected using a random sampling approach, with 400 samples obtained from each taluka. The

samples consisted of nasal swabs, throat swabs, and vaginal swabs collected aseptically following standard procedures recommended by the World Organization for Animal Health (OIE) guidelines (OIE, 2014). Detailed clinical and epidemiological information, including animal demographics, herd history, management practices, and observed clinical signs, was recorded using a structured questionnaire administered to livestock owners and field veterinarians. All swab samples were collected from animals suspected of CCPP, appropriately labeled, and preserved under cold chain conditions. The samples were transported to the Central Veterinary Diagnostic Laboratory (CVDL), Sindh, Tando Jam, for bacteriological processing and analysis. Isolation and cultivation of *Mycoplasma* species were performed using Modified Hayflick medium as described by OIE protocols (OIE, 2014). The medium was prepared in two components: Part A, consisting of Bacto PPLO broth without crystal violet, which was autoclaved at 121 °C for 15 minutes, and Part B, which contained membrane-filtered inactivated horse serum, yeast extract, glucose, sodium pyruvate, thallium acetate, ampicillin, and phenol red. Both components were aseptically combined to prepare the broth medium, while PPLO agar supplemented with 0.9% agar was used for solid media preparation.

Swab samples were inoculated into 3 ml of PPLO broth and incubated at 37 °C in a humidified atmosphere with 5% CO_2 for 7–15 days. Cultures were monitored daily, and growth was provisionally identified based on characteristic turbidity, swirling movement, and a color change of the medium from red to yellow. Cultures showing no growth after the incubation period were considered negative and discarded. Positive broth cultures were passed through a 0.45 µm membrane filter and subsequently streaked onto PPLO agar plates, which were incubated at 37 °C for 3–15 days. Developing colonies were examined daily under a stereomicroscope for typical "fried-egg" morphology. Purified colonies were sub-cultured and preserved in PPLO broth for further identification and characterization (11,12). Biochemical characterization was carried out to confirm the identity of *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp). The tests included phosphatase activity, film and spot formation, tetrazolium reduction, digitonin sensitivity, glucose fermentation, and urea hydrolysis. All biochemical assays were performed following standardized methods described in previous studies (13). Antibiotic susceptibility profiling of confirmed Mccp isolates was conducted to evaluate their in-vitro response to commonly used antimicrobial agents. The antibiotics tested included ampicillin, ceftiofur sodium, gentamicin, cloxacillin, tylosin, metronidazole, oxytetracycline, enrofloxacin, and ciprofloxacin. Susceptibility testing was performed using the disc diffusion technique in accordance with Clinical and Laboratory Standards Institute guidelines (CLSI, 2010). A standardized suspension of *Mycoplasma* colonies was uniformly spread on Mueller–Hinton agar supplemented with 5% sheep blood. Antibiotic discs were applied using a disc dispenser, and plates were incubated at 37 °C for 24 hours. Zones of inhibition were measured in millimeters and interpreted based on established criteria.

Statistical analysis was performed to examine the relationship between clinical manifestations and disease severity in small ruminants affected by CCPP. Chi-square tests and binary logistic regression analyses were used to assess associations between categorical variables, while contingency coefficient values were calculated to determine the strength of correlations between clinical signs and disease severity. One-way analysis of variance (ANOVA) was applied to compare mean zones of inhibition among different antibiotics, and post-hoc comparisons were conducted using the least significant difference (LSD) test. All analyses were carried out using Statistics software version 8.1, with statistical significance set at $p < 0.05$ (14). Ethical approval for the study was obtained from the relevant institutional ethical review committee for animal research, and all procedures were conducted in accordance with national and international guidelines for the ethical use of animals in research. Informed verbal consent was obtained from livestock owners prior to sample collection, and animal handling was performed to minimize stress and discomfort.

Table: Collection of samples for isolation of Mycoplasma species from small ruminants from different regions of Umerkot

Region	Nasal Swab Sheep (M/F)	Nasal Swab Goat (M/F)	Throat Swab Sheep (M/F)	Throat Swab Goat (M/F)	Vaginal Swab Sheep (M/F)	Vaginal Swab Goat (M/F)	Total Samples
Umerkot	42/38	41/39	40/40	39/41	12/08	11/09	400
Samaro	41/39	40/40	42/38	41/39	11/09	10/10	400
Pithoro	40/40	42/38	41/39	40/40	10/10	12/08	400
Kunri	39/41	40/40	40/40	42/38	09/11	10/10	400
Total	162/158	163/157	163/157	162/158	42/38	43/37	1600

RESULTS

A total of 1,600 samples were collected from sheep and goats across four talukas of Umerkot District, with equal representation from Umerkot, Samaro, Pithoro, and Kunri. Each taluka contributed 400 samples, ensuring balanced geographic coverage of the study area. The overall sampling included nasal swabs, throat swabs, and vaginal swabs obtained from both male and female animals. Cumulatively, nasal swabs accounted for 640 samples from sheep and 640 from goats, throat swabs comprised 640 samples from sheep and goats combined, while vaginal swabs represented 160 samples collected exclusively from female animals. This uniform distribution allowed for a comprehensive assessment of *Mycoplasma* isolation across different animal species, sexes, anatomical sites, and agro-climatic settings within the district. Out of the total sampled population, 402 animals were identified as clinically positive for contagious caprine pleuropneumonia. Among these affected animals, elevated body temperature was the most frequently observed clinical finding, recorded in 362 cases, corresponding to 90.05% of clinically positive animals. This sign demonstrated a strong association with disease severity, as indicated by a contingency coefficient value of 0.621. Nasal discharge was observed in 348 animals (86.56%) and also showed a strong association with disease severity, with a contingency coefficient of 0.605. Cough was reported in 326 animals (81.09%) and exhibited a moderate association with disease severity (contingency coefficient = 0.547). Dyspnea was present in 258 cases (64.17%), likewise showing a moderate association (contingency coefficient = 0.452). In contrast, anorexia and generalized weakness were recorded in 244 (60.69%) and 231 animals (57.46%), respectively, and both showed weaker associations with disease severity, with contingency coefficient values of 0.332 and 0.281. Collectively, pyrexia, nasal discharge, and coughing emerged as the most prevalent and strongly associated clinical manifestations of CCPP in small ruminants from the study area. In vitro antibiotic susceptibility testing of *Mycoplasma* isolates revealed marked variability in antimicrobial effectiveness. Enrofloxacin demonstrated the highest antibacterial activity, producing a mean zone of inhibition of 19.14 ± 1.09 mm. Ciprofloxacin and gentamycin also showed strong activity, with mean inhibition zones of 16.02 ± 0.83 mm and 13.58 ± 0.71 mm, respectively, and were categorized as highly effective. Ampicillin and tylosin exhibited intermediate activity, with mean zones of inhibition measuring 9.67 ± 0.59 mm and 8.42 ± 0.76 mm, respectively, indicating quite to moderately effective responses. Oxytetracycline showed comparatively lower efficacy, with a mean inhibition zone of 5.44 ± 0.88 mm. In contrast, ceftiofur sodium and cloxacillin showed complete resistance, as no inhibitory zones were observed against the isolates. Statistical analysis confirmed significant differences in mean zones of inhibition among the tested antibiotics at $\alpha = 0.05$, highlighting substantial variation in antimicrobial susceptibility profiles of the isolated *Mycoplasma* strains.

Table 1: Clinical presentation of Contagious Caprine Pleuropneumonia (CCPP) in small ruminants of Umerkot District, Sindh, Pakistan

Sign / Symptoms	Status	Positive (n = 402)	Percent (%)	Contingency coefficient value	Severity level
Temperature	Yes	362	90.05	0.621	Strong
	No	40	9.95		
Nasal discharge	Yes	348	86.56	0.605	Strong
	No	54	13.43		
Cough	Yes	326	81.09	0.547	Moderate
	No	76	18.90		
Dyspnea	Yes	258	64.17	0.452	Moderate
	No	144	35.82		
Anorexia	Yes	244	60.69	0.332	Weak
	No	158	39.30		
Weakness	Yes	231	57.46	0.281	Weak
	No	171	42.53		

Contingency coefficient values: 0.20–0.39 = Weak, 0.40–0.59 = Moderate, 0.60–0.79 = Strong association.

Table 2: Antibiogram profile of different antibiotics used for in vitro analysis of Mycoplasma isolates

Antibiotics	Zone of inhibition (mean ± SD)	Sensitivity level	Interpretation
Tylosin	$8.42^{c} \pm 0.76$	+++	Moderately effective
Gentamycin	$13.58^{b} \pm 0.71$	++++	Highly effective
Ciprofloxacin	$16.02^{b} \pm 0.83$	++++	Highly effective
Enrofloxacin	$19.14^{a} \pm 1.09$	++++	Highly effective
Ceftiofur sodium	$0^{e} \pm 0.00$	– –	Resistant
Cloxacillin	$0^{e} \pm 0.00$	– –	Resistant
Ampicillin	$9.67^{c} \pm 0.59$	+++	Quite effective
Oxytetracycline	$5.44^{d} \pm 0.88$	++	Moderately effective

Means of zones of inhibition bearing different superscripts differ significantly at $\alpha = 0.05$.

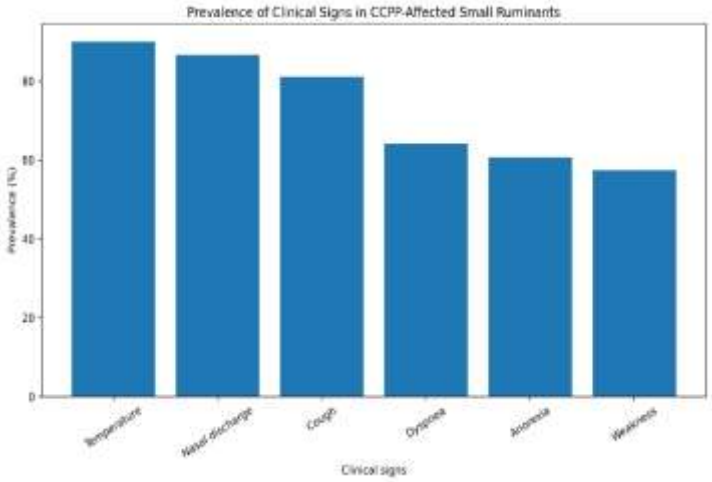


Figure 1 Prevalence of Clinical Signs in CCPP Affects Small Ruminants

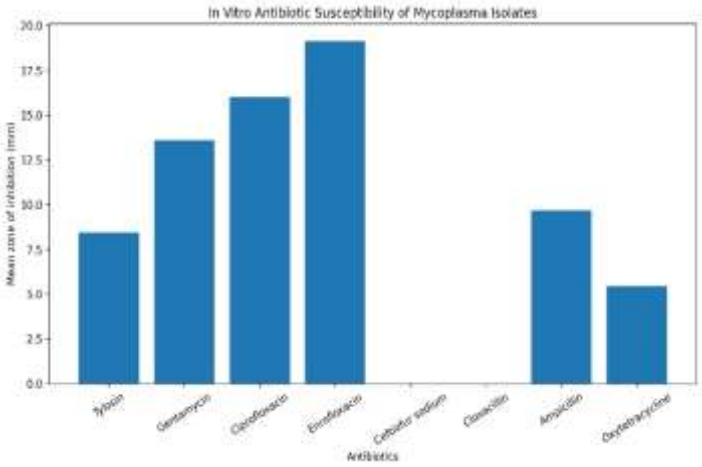


Figure 2 In Vitro Antibiotic Susceptibility of Mycoplasma Isolates

DISCUSSION

Pathogenic *Mycoplasma* species are known to exhibit a marked tropism for mucosal surfaces of the respiratory, ocular, and urogenital tracts, where their ability to adhere to epithelial cells facilitates persistent colonization and tissue damage. This pathogenic behavior is particularly pronounced in immunocompromised animals, often resulting in severe and rapidly progressive clinical disease (15,16). The findings of the present study are consistent with this biological behavior, as *Mycoplasma capricolum* subspecies *capripneumoniae* was frequently associated with overt respiratory involvement and systemic illness in naturally infected small ruminants. The observed spatial variation in disease prevalence across different talukas of Umerkot District further highlights the complex interaction between host, pathogen, and environmental factors. Variability in climatic conditions, animal density, agro-ecological characteristics, movement of carrier animals, and differences in husbandry and biosecurity practices likely contributed to the uneven distribution of CCPP across the study area, a pattern that has also been reported in endemic regions elsewhere (14,17). Clinically, elevated body temperature and nasal discharge emerged as the most consistent and strongly associated indicators of disease severity. These findings underscore the diagnostic value of pyrexia and nasal discharge as practical field markers for early suspicion of CCPP, particularly in resource-limited settings where laboratory confirmation may be delayed or unavailable. Other frequently observed signs, including cough and dyspnea,

demonstrated moderate associations with disease severity and reflected the progressive inflammatory involvement of the lower respiratory tract. In contrast, anorexia and generalized weakness, while common, showed weaker associations and appeared to reflect systemic compromise rather than disease-specific pathology. Similar clinical patterns have been described in previous investigations, where inflammatory mediators induced by mycoplasmal surface proteins were linked to respiratory distress, while reduced feed intake and weakness were secondary consequences of prolonged illness and metabolic stress (18). Collectively, these findings support a clinical framework in which a combination of major and supportive signs improves diagnostic confidence in field conditions.

Antimicrobial susceptibility testing revealed substantial variability in the response of Mccp isolates to commonly used antibiotics, highlighting an important therapeutic challenge. Fluoroquinolones, particularly enrofloxacin and ciprofloxacin, demonstrated the highest in-vitro efficacy, followed by gentamicin, indicating strong inhibitory activity against the isolates. These results align with previous reports describing superior penetration and activity of fluoroquinolones against *Mycoplasma* species due to their intracellular action and favorable pharmacokinetic properties (19,20). In contrast, moderate responses observed with oxytetracycline, metronidazole, and tylosin suggest declining effectiveness of these agents, potentially reflecting widespread and prolonged use in the field. Earlier studies have documented the gradual emergence of resistance to tetracyclines and macrolides, which complicates treatment outcomes and necessitates careful antibiotic selection (21). The complete lack of activity observed with cloxacillin and ceftiofur sodium was biologically plausible given the absence of a cell wall in *Mycoplasma* species, and similar findings have been reported previously, although occasional contradictory results for ceftiofur sodium indicate the need for further clarification under standardized conditions (22).

The strengths of this study include its district-wide coverage across multiple agro-climatic zones, a relatively large sample size, and the integration of clinical, bacteriological, and antimicrobial susceptibility data generated from natural outbreaks. This comprehensive approach enhanced the external validity of the findings and provided region-specific evidence relevant to disease control strategies in Sindh. However, certain limitations should be acknowledged. The reliance on in-vitro antibiotic susceptibility testing limits direct extrapolation to clinical treatment outcomes, as in-vivo efficacy may be influenced by drug pharmacodynamics, host immunity, and disease stage. Molecular confirmation of Mccp and genetic characterization of resistance mechanisms were not performed, which may have provided deeper insight into strain diversity and resistance patterns. Additionally, the cross-sectional design restricted the ability to assess temporal dynamics of infection and long-term treatment responses. Future research should focus on longitudinal studies to evaluate in-vivo therapeutic efficacy and treatment outcomes, supported by molecular diagnostics and resistance profiling to guide evidence-based antimicrobial stewardship (23). Strengthening biosecurity measures, improving early clinical recognition, and rationalizing antibiotic use based on local susceptibility data remain critical for reducing the burden of CCPP. Overall, the present findings contribute to a clearer understanding of the clinical and therapeutic landscape of CCPP in endemic settings and underscore the importance of integrating clinical surveillance with laboratory-based evidence to inform effective disease management strategies.

CONCLUSION

The findings of this study highlight that fever and nasal discharge represent the most consistent and clinically useful indicators for early recognition of contagious caprine pleuropneumonia in small ruminants under field conditions. The results further demonstrate that fluoroquinolones and aminoglycosides, particularly enrofloxacin, ciprofloxacin, and gentamicin, show superior activity against *Mycoplasma capricolum* subsp. *capripneumoniae*, whereas commonly used β-lactam antibiotics such as cloxacillin and ceftiofur sodium are ineffective. These observations underscore the importance of informed clinical assessment combined with evidence-based antimicrobial selection. Adopting rational antibiotic use guided by local susceptibility patterns is essential to improve therapeutic outcomes, limit the spread of infection, and reduce the risk of antimicrobial resistance, thereby strengthening control strategies for CCPP in endemic small ruminant production systems.

AUTHOR CONTRIBUTIONS

Author	Contribution
Rameez Raja Kaleri	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Reema Bughio	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Habibullah Janyaro	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Ali Asghar Baloch	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Muhammad Ishaq	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Hiranand Lohana	Substantial Contribution to study design and Data Analysis Has given Final Approval of the version to be published
Muhammad Anees Memon	Contributed to study concept and Data collection Has given Final Approval of the version to be published

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