Short Communication

Seroprevalence of Contagious Caprine Pleuropneumonia (CCPP) in Rupandehi and Palpa Districts of Nepal

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Abstract | A cross-sectional study was conducted to determine the seroprevalence and risk factors of Contagious Caprine Pleuropneumonia (CCPP) in the Rupandehi and Palpa districts of Nepal. A total of 89 goat serum samples were tested by using a competitive enzyme-linked immunosorbent assay (c-ELISA) for the presence of antibodies against *Mycoplasma capricolum capripneumoniae*. Out of the total serum sample tested, 3 were seropositive for CCPP giving an overall apparent seroprevalence of 3.37% and true seroprevalence of 3.4%. Significantly higher seroprevalence ($p<0.05$) was observed among goats with a history of respiratory symptoms. This study revealed the goat population in Rupandehi (district bordering India) and Palpa districts are at high risk of acquiring CCPP infection. Therefore, appropriate preventive measures including regular research and investigation, vaccination and regulatory policies on transboundary animal movements should be implemented to prevent the potential outbreak of the disease. This is the first-ever report of sero-detection of CCPP antibodies in the goat population of Nepal. This article confirms the presence of CCPP in Nepal and the potential circulation of the pathogen to other parts of the country warranting the concerned authorities and farmers to be vigilant for keeping this disease at bay.

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Introduction

Contagious caprine pleuropneumonia (CCPP) is a rapidly spreading, highly infectious, mycoplasmal disease of small ruminant characterized by respiratory distress, high morbidity and mortality (Arif *et al*., 2007; Iqbal *et al*., 2019). CCPP in goats and sheep is caused by *Mycoplasma capricolum* subspecies *capripneumoniae* commonly known as Mccp and previously known as *Mycoplasma*

biotype F38 (Iqbal *et al*., 2019). The major source of contamination is direct contact with the infected animals via inhalation of infected aerosols (Thiaucourt *et al*., 1996). CCPP usually has per-acute, acute and chronic forms. In the per-acute form, the animal dies within 1-3 days with minimal clinical manifestation while signs of high fever (41-43˚C), lethargy, anorexia, coughing and laboured breathing are seen in acute forms of the disease (OIE, 2009). Debility, chronic cough and nasal discharges are the major clinical signs in the chronic form of the disease (OIE, 2009). The pathogenicity of CCPP is usually restricted in the lungs and pleura with characteristic fibrinous pleuropneumonia (Ahaduzzaman, 2020). Consolidation of lungs (100%), pleural adhesion (72.72%) and alveolar exudation (90.9%) are the major gross findings and septal peribronchiolar fibrosis (81.8%), fibrinous pleuritis (63.63%) and peribronchiolar cuffing of mononuclear cells (54.54%) are the typical microscopic findings of the disease (Hussain *et al*., 2012).

CCPP is an important transboundary disease especially in low and middle-income countries and causes significant trade and economic disturbances in various parts of the world (Ahaduzzaman, 2020; Jores *et al*., 2020). Nepal has not reported any outbreaks of CCPP to OIE but the clinical descriptions of CCPP has been reported in Nepal (IDRC, 2016). Different studies show the prevalence and incidences of CCPP in India (Iqbal *et al*., 2019). Seroprevalence of CCCP was 33.67 % in Nagpur. (Ingle *et al*., 2008), 10.65 % in Jabalpur (Gupta *et al*., 2016), 9.93% among Himalayan pashmina goats of Ladakh (Parray *et al*., 2019), 16.05 % in goats and 20.24 % in sheep population of Maharashtra (Suryawanshi *et al*., 2015) and 8.11% in Uttar Pradesh of India (Jain, 2015). The presence of CCPP in India, Tibet of China (Chu *et al*., 2011; Yu *et al*., 2013), and Pakistan (Awan *et al*., 2010; Wazir *et al*., 2016) can increase the risk of an outbreak of CCPP in Nepal.

In the fiscal year 2019/20, Nepal imported 327,897 live goats worth Rs 1.93 billion. Porous Nepalese borders with India, weak disease surveillance, and poor quarantine facilities increase the risk of CCPP in Nepal. Widespread movement of livestock between India and Nepal might favor a spread of Mccp in small ruminants of Nepal (Parajuli and Acharya, 2019). The objectives of our study were to evaluate the seroprevalence of Mccp in goats of Rupandehi and Palpa districts of Nepal and identify the risk factors associated with Mccp seropositivity.

Materials and Methods

Study design, area, and population

The cross-sectional study was conducted for 3 months between January 2020 and March 2020 to detect the presence of antibodies against *Mycoplasma capricolum capripneumoniae*. The study was conducted in the selected Rohini rural municipality of Rupandehi and the Tinau rural municipality of Palpa (Figure 1). Rohini rural municipality has a total area of 64.62 sq. km. It is located in the eastern south part of Rupandehi district at 27˚51'21" N latitude, 83˚54'97"E longitude, and at an altitude of 92.1 m. This municipality shares its southern boundary with the Uttar Pradesh state of India. Tinau rural municipality of Palpa district has a total area of 202 sq. km. It is located in the southern part of the district on 27˚68'13" N to 27˚46' 53 "N latitude, 84˚40'32"E to 83˚30'57 "E longitude and at an altitude between 300 m to 1220 m.

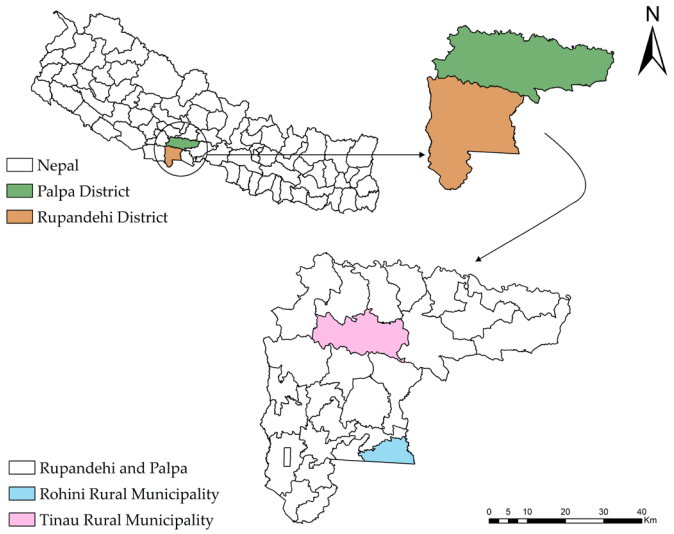

Figure 1: *Map of Nepal highlighting the study area.*

The study population was goats of both local and exotic breeds in the study area. Local breeds include Terai in the lower plains of Rohini rural municipality, Rupandehi district, and Khari in mid-hills of Tinau rural municipality, Palpa district. The housing system of the goat includes the intensive and semi-intensive type of housing system.

Sample size calculation

The sample size necessary for the calculation of the prevalence of CCPP was be calculated by EpiTools Epidemiological Calculators by Ausvet (Sergeant,

2018). According to the literature review, the prevalence of CCPP in neighboring state i.e. Uttar Pradesh of India was found to be 10.5 % (Jain *et al*., 2015). As Uttar Pradesh is very close to our study area, its prevalence was taken as a reference and the desired sample size was calculated with expected precision of 5 % at a confidence level of 95%. The calculated minimum sample size required for the study was 145 based on the total goat population (392,791) of the two districts i.e., Rupandehi (185,332) and Palpa (207,459) as per the livestock statistics of Nepal, 2017 (MOLD, 2017). Only 101 samples were collected due to the limited time frame, COVID-19 crisis and financial constraints. Despite collecting 101 serum samples only 89 samples were included in the study. The remaining serum samples were discarded due to haemolysis. Out of 89 serum samples, 30 were from Rohini rural municipality of Rupandehi district and 59 were from the Tinau rural municipality of Palpa district.

Questionnaire administration, sample collection, and laboratory analysis

A semi-structured questionnaire was administered to collect data from the goat farmers by the purposive sampling method. The close and open-ended questionnaire was pretested to ensure its validity and verbal consent was obtained from the respondent by explaining the objectives of the survey.

No specific criteria were set for sample collection. Five millilitres (5ml) of blood samples were collected from the jugular vein of each properly restrained selected goat with the help of a 5 ml syringe and 21-gauge needle using plain vacutainer tubes. The collected blood samples were labelled and kept in a slanted position overnight to allow serum separation. The separated serum samples were transferred into sterile 1.8 ml cryovial tubes and stored in a deep freezer at -20°C in the Laboratory of Animal Health Research Division, Nepal Agricultural Research Council, Khumaltar, Lalitpur, until serological analysis. The serum samples were examined for the detection of specific antibodies against Mccp by using the C-ELISA test kit manufactured by IDEXX (CIRAD-Montpellier, France) containing the monoclonal anti-Mccp antibody Mab 4.52 in pre-coated plates with ready-made reagents. The assay was conducted according to the test protocol supplied by the kit.

Data management and analysis

The collected data in the field along with serological results obtained from laboratory analysis were entered in a Microsoft Office Excel spreadsheet. The entered data in Microsoft Office Excel spreadsheet were imported into SPSS V 20.0 to perform statistical analysis. The collected data were calculated by using descriptive statistics and inferential statistics. The overall seroprevalence was calculated by dividing the number of seropositive goats by the total number of goats tested. Chi-square test and fisher's exact test were used to detect the association between seropositivity and questionnaire variables. For the analysis, a confidence level of 95% and a p-value of < 0.05 were considered to determine statistical significance.

Ethical statement

Ethical approval of the study was sought from the internship advisory committee, Veterinary Teaching Hospital, Paklihawa Campus, Institute of Agriculture and Animal Science and Animal Health Research Division, Nepal Agricultural Research Council (NARC) during the proposal seminar. Verbal consent was obtained from the respondent by explaining the objectives of the survey. Blood was collected by a technical expert with minimal pain to the animals.

Results and Discussion

Among the total 89 serum samples tested by cELISA, 3 serum samples were found seropositive for specific antibodies against *Mycoplasma capricolum capripneumoniae* with an overall apparent seroprevalence of 3.37% (1.15±9.45). Assuming the sensitivity and specificity of the ELISA kit to be 1 and 0.99, the true prevalence of the study was calculated via Epitools- Epidemiological calculator by Ausvet (Matope *et al*., 2011; Peyraud *et al*., 2014). The true prevalence of the study was 3.4% (0.93±9.49). Among the two rural municipalities, the seropositivity for CCPP was found slightly high in Tinau than in Rohini.

The association of factors with seropositivity of CCPP was identified by using the chi-square test and fisher's exact test whenever necessary to find out the p-value (Table 1). The results obtained after analysis showed that host-related factors such as age and sex of the goats were not significantly associated with CCPP seroprevalence ($p > 0.05$). However, the seropositivity was slightly higher in young (4.76

%) and male goats (4.00%) compared to adult (2.94%) and female goats (3.13%), respectively. The environment-related factors; altitude and districts, were also analysed but the seroprevalence was not significantly associated with the altitude and district. Similarly, the seroprevalence was not significantly associated with farm type, breed, and pregnancy of animals (Table 1). The seroprevalence of CCPP was significantly associated with 'history of respiratory symptoms' (p = 0.01).

Table 1: *Association of seropositivity with questionnaire variables in Rupandehi and Palpa districts of Nepal.*

Sample categories		Positive (%)	Negative (%)	Total	P value
Districts	Rupandehi	1 (3.33%)	29(96.67%)	30	0.71
	Palpa	2 (3.39%)	57(96.61%)	59	
Husbandry practices	Intensive	3 (6.38%)	44(93.62%)	47	0.14
	Semi intensive	0 (0.00%)	42(100%)	42	
Age group	Young	1 (4.76%)	20(95.24%)	21	0.55
	Adult	2 (2.94%)	66(97.06%)	68	
Sex	Male	1 (4.00%)	24(96.00%)	25	0.63
	Female	2 (3.13%)	62(96.88%)	64	
Breed	Khari (local breed)	2 (6.67%)	28(9.33%)	30	0.26
	Others	1 (1.69%)	58(98.31%)	59	
Pregnant	Yes	1 (3.85%)	25(96.15%)	26	0.65
	No	2 (3.17%)	61(96.83%)	63	
Respiratory signs	Present	3(15.00%)	17(85.00%)	20	0.01*
	Absent	0 (0.00%)	69(100%)	69	

To the best of our knowledge, this is the first study conducted to determine the seroprevalence of CCPP and associated risk factors in Nepal. The overall apparent prevalence was found to be 3.37% which is lower in comparison to the findings of (Udit and Chand, 2008) i.e., 10.5% in Uttar Pradesh state of India. Although Nepal is considered a CCPP free country, this study portrait the high risk of CCPP infection among sheep and goats in Nepal. This study also hints the presence of CCPP among goats and sheep in other parts of Nepal, which may have gone undiagnosed due to lack of diagnostic facilities, high cost, and difficulty in isolation, culture, and identification of the organism. The seropositivity of CCPP in our study may be due to the porous nature of the international borders of the country. Hence there is a need for cross-border harmonization of disease control programs including regular investigation, vaccination, strict quarantine, and transboundary animal movement regulation (Kipronoh *et al.*, 2016; Parajuli and Acharya, 2019).

In contrast to this study, higher seroprevalences were reported in a different state of India like 33.67 % in Nagpur (Ingle *et al.*, 2008), 10.65 % in Jabalpur, Madhya Pradesh (Gupta *et al.*, 2016), 9.93% in Ladakh (Parray *et al.*, 2019), 16.05 %, and 20.24 % in sheep and goats respectively in Maharashtra (Suryawanshi *et al.*, 2015). Very low seroprevalence in our study could be due to the fact that Nepal is CCPP free country. Also, the difference in prevalence might be due to spatial and geographical variations and differences in total population, sample size, sampling techniques, and testing procedure and criteria. Our study suggests that age and sex have no role in CCPP epidemiology. In contrast to our findings, a study in Tanzania showed the seroprevalence of CCPP to be significantly ($P < 0.05$) higher in adults and male goats ($P<0.05$) (Nyanja *et al.*, 2013). In another study in the Afar region of Ethiopia, age and sex were significantly associated with the prevalence of CCPP (Regassa *et al.*, 2010). A study in Egypt also found that age >4 years of a goat is an important risk factor of CCPP (Selim *et al.*, 2021). Another study in Himalayan pashmina goats in Ladakh, India reported a significant association ($P\leq0.05$) of seropositivity with age (Parray *et al.*, 2019). Significant association of CCPP with adult goats may be due to fact that older animals are at risk of exposure for a long period. All seropositive goats were raised in an intensive housing system but the housing system was not significant for seropositivity.

Severe respiratory distress, sero-mucoid nasal discharge, dyspnoea, coughing, and pyrexia are characteristic symptoms of CCPP (Iqbal *et al.*, 2019). The history of respiratory symptoms in goats was significantly associated with the seropositivity of CCPP in our study. This suggests that goats might have been infected with CCPP at some stage of their life. The government of Nepal, regional and local laboratories, animal health workers, and related stakeholders should actively participate in the establishment of diagnostic (molecular and culture) facilities throughout the country. An increase in the number of prevalence studies with a large sample size, awareness of the disease with its economic importance among goat farmers, and effective governmental strategies to identify and manage the disease are

urgently needed to prevent the potential economic loss.

Conclusions and Recommendations

Although Nepal is considered a CCPP free country, this study indicated the presence of CCPP in the goats of Rupandehi and Palpa districts of Nepal. The history of respiratory symptoms among the goats was significantly associated with seropositivity of CCPP which provides an additional clue about the incidence of disease in Nepal. Our study provides preliminary data on CCPP among goats in Nepal which can be the baseline for understanding the epidemiology of CCPP in Nepal. Further epidemiological studies with appropriate sample size covering different parts of the country can provide crucial insights into the prevalence of the disease in Nepal.

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Novelty Statement

This is the first-ever report of serological detection of CCPP antibodies in the goat population of Nepal.

Author's Contribution

Conceptualization: Basanta Kumar Adhikari, Chet Narayan Kharel, Krishna Kaphle and Doj Raj Khanal.
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Project administration: Basanta Kumar Adhikari, Deepak Subedi and Sumit Jyoti.
Writing original draft: Basanta Kumar Adhikari, Deepak Subedi and Sumit Jyoti.
Writing review editing: Deepak Subedi, Krishna Kaphle, Chet Narayan Kharel and Doj Raj Khanal.

Conflict of interest
The authors have declared no conflict of interest.

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