



Study on Seroprevalence of Contagious Caprine Pleuropneumonia in Goats Marketed in Kathmandu Valley

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ABSTRACT

Contagious caprine pleuropneumonia (CCPP) is a highly contagious respiratory disease of small ruminants that is caused by gram-negative, pleomorphic, facultative anaerobic bacterium *Mycoplasma capricolum*, sub-sp. *Capripneumoniae*. It is a severe respiratory disease of goats characterized by high morbidity and mortality, as well as the potential to spread across borders. A study was conducted to determine the seroprevalence of CCPP in the marketed goats of Kathmandu Valley of Nepal from June to August, 2024. The study was conducted in goats entering the Kathmandu valley through a Quarantine check post of Nagdhunga, Kathmandu to assess the prevalence of CCPP. A total of 168 goat serum samples were tested using a competitive enzyme-linked immunosorbent assay (c-ELISA) (IDEXX CCPP Ab test kit, United States) for antibodies against *Mycoplasma capricolum capripneumoniae*. Out of the total serum samples tested, 15 (8.9%) samples were seropositive for CCPP. Significantly higher seroprevalence was observed among goats of Rukum-Paschim 3 (50%), and Kailali 2 (22.2%) followed by Baitadi 1 (14.3%), Bardiya 3 (14.3%), Parasi 1 (14.3%), Kanchanpur 2 (12.5%), Rolpa 1 (7.7%), Dang 1 (2.4%) and Kathmandu 1 (2.8%). The result was negative in Salyan and Makwanpur districts. This study revealed the presence of CCPP in the goat population of Nepal and the potential circulation of the pathogen inside the country. Therefore, appropriate preventive measures such as maintaining effective biosecurity, minimizing stress in goats, and conducting regular research and investigations should be implemented to prevent potential disease outbreaks.

Keywords: Caprine, CCPP, ELISA, mycoplasma, pathogen

INTRODUCTION

Nepal is primarily an agricultural country, with around 66% of its population engaged in this sector and the livestock sector contributes about 24.01% of AGDP in Nepal



(DLS, 2020). Goats are very important to the rural economy, especially in hilly and mountainous areas. Goats, which are frequently called "poor man's cows," are an important source of food and revenue in rural Nepal since they are tough and can provide meat, milk, and manure (Bhattarai *et al.*, 2019). Goat meat, or "chevon," is important to both culture and the economy. This is especially true in cities like Kathmandu, where demand for it rises during festivals and other special events (Das *et al.*, 2019). With a population of 14.54 million, goats rank third in national meat production after chicken and buffalo (Krishi Diary, 2079/80). However, goat farming encounters a lot of challenges, especially with contagious caprine pleuropneumonia (CCPP), a highly contagious respiratory disease caused by the gram-negative, anaerobic bacterium *Mycoplasma capricolum* subsp. *capripneumoniae* (Ahaduzzaman, 2020). CCPP has been widely recorded in Asia, Africa, and the Middle East. It is a serious respiratory disease in goats that can spread across borders and can cause high morbidity and mortality (Iqbal *et al.*, 2019; Parray *et al.*, 2019).

CCPP is transmitted primarily through direct contact via aerosols from infected goats, making it especially difficult to contain during animal movement. Clinical signs include high fever (41–44 °C), coughing, nasal discharge, dyspnea, chest pain, depression, weight loss, and death, with mortality rates reaching up to 100% in acute cases (MacOwan & Minette, 1976; Radostits *et al.*, 2000; Ahaduzzaman, 2020). Common risk variables connected to increased CCPP seroprevalence are age, sedentary farming methods, species, location, season, the introduction of new animals from markets, and the lack of therapeutic intervention (Parray *et al.*, 2019). Diagnosing CCPP involves a combination of clinical observation and laboratory techniques such as ELISA, PCR, and serological tests. For the epidemiological research of CCPP, the competitive ELISA kit, based on a Mccp-specific monoclonal antibody, is a suitable instrument due to its high specificity (99.8–100%) and suitability for mass-scale testing (Asmare *et al.*, 2016).

The disease thrives in environments with high goat density and poor biosecurity practices (Kyotos *et al.*, 2022). Twenty-six (26) animal diseases are regarded as notifiable in Nepal per Animal Health and Livestock Service Act 2055 and regulation 2056. CCPP has been included in the list of notifiable diseases of the World Organization for Animal Health (WOAH) and Nepal (Bascunana *et al.*, 1994; Manso-Silvan *et al.*, 2011).

Kathmandu, is a major hub where goats are consumed, goats from all over the country are brought here, which raises the chance of CCPP spreading. Poor veterinary services



and lack of organized vaccination strategies further increases the problem, particularly in urban and peri-urban areas (Upreti *et al.*, 2012). Seasonal weather changes and varied topography make disease dynamics even more complicated and hard to control (Kyotos *et al.*, 2022). Though Nepal has a rising goat population and is self-sufficient in goat output, the prevalence of diseases such as CCPP makes it difficult for Nepal to reach regional and worldwide markets (Neupane *et al.*, 2018). Despite being a notifiable disease, no national immunization program against CCPP has yet been implemented. This study contributes updated seroprevalence data from goats entering Kathmandu Valley, which is important for tracking the spread of disease and making better disease management measures, especially at transit points and market hubs.

MATERIALS AND METHODS

Site and duration of study

Kathmandu is a major market for the goat population throughout the country. Each day around 1300-1500 goats are slaughtered in Kathmandu. The majority of goats are brought from all districts to Kathmandu (DLS 2020). The major entry point of the goat is Nagdhunga. So, the study was carried out to collect samples from the checkpoint. The trader brought a veterinary certificate which included the contact number of the owner, and address, and the study was then a proxy indicator of disease in that area. The cross-sectional study was carried out from June to August of 2024.

Sample size

According to the literature review, the prevalence of CCPP in Chitwan was found to be 4.71 % (Regmi *et al.*, 2023). As Chitwan is very close to our study area, its prevalence was used as a reference, and the desired sample size was calculated with an expected precision of 5% at a 95% confidence level (Thrusfield *et al.*, 2017). The calculated minimum sample size required for the study was 145.

In the study, a total of 168 samples were collected randomly from the vehicles entering through the Nagdhunga checkpoint.

$$n = \frac{1.96^2 \times P_{exp}(1 - P_{exp})}{d^2}$$

n = required sample size



d = Desired absolute precision (0.05)

P exp = Expected prevalence (0.05)

Sample collection

The study was carried out by sampling goats brought to Kathmandu Valley through animal quarantine check post-Nagdhunga. Goats are either brought in big trucks or in small vehicles. Simple random sampling was undertaken selecting every alternate vehicle bringing the animals. At most 10% of the animals were sampled from each vehicle. Selected animals were restrained by two people and the site was prepared around the mid-jugular vein by using 70% ethanol. Using a sterile disposable syringe and needle, blood was taken from the jugular vein aseptically. The collected blood samples were labelled. An icebox containing ice packs was used to transfer the sample to the Central Veterinary Laboratory (CVL) in Tripureswor, Kathmandu. The serum was separated and separated serum samples were transferred into sterile cryovial tubes and stored in a deep freezer at 5°C in the Central Veterinary Laboratory (CVL) in Tripureswor, Kathmandu, until serological analysis.

Laboratory examination

The serum samples were examined for the detection of specific antibodies against Mccp by using the C-ELISA test kit (IDEXX CCPP Ab test kit, US). The entire test was performed according to the manufacturer's guidelines. The result was interpreted by a percentage of inhibition (PI) = $((OD \text{ Mab} - \text{test serum}) / (OD \text{ Mab} - OD \text{ conjugate})) \times 100$ and Serum with a $PI \geq 55\%$ was judged positive for Mccp infection, whereas a $PI < 55\%$ were considered negative.

Data analysis: Data were analyzed using Microsoft Excel, SPSS, and R Studio. The chi-square test was utilized as a statistical test in which the confidence level was set at 95%, and the significance was fixed at $p < 0.05$. The value less than or equal to 0.05 were considered to be statistically significant.

Ethical statement

Ethical approval of the study was obtained from the internship advisory committee, Himalayan College of Agricultural Sciences and Technology (HICAST) and CVL, Tripureswor during the proposal seminar. Verbal consent was obtained from the



respondents by explaining the objectives of the study. Blood samples were collected with minimal pain to the animals.

RESULTS AND DISCUSSION

In this study, out of 168 samples collected, 15 (8.9%) samples were found to be seropositive.

District wise prevalence of disease

Among the district covered in this study, Bardiya and Rukum-paschim revealed highest number of positive cases as shown in figure no 2. below.

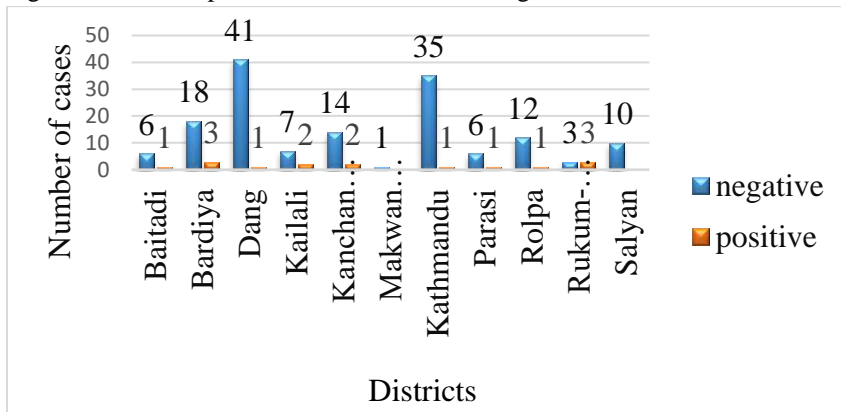


Figure 2. District wise results of CCPP according to the number of samples

The chi-square statistic of 20.88, coupled with a p-value of 0.0219, indicated that the differences observed in the distribution of seropositive and seronegative cases were statistically significant, with a less than 2.2% probability that these differences occurred by chance. This finding suggested that the prevalence of CCPP was not uniformly distributed across the country, with certain districts exhibiting either significantly higher (Rukum-Paschim) or lower rates of infection (Makwanpur, Salyan).

This study found that the total seroprevalence of CCPP among marketed goats in Kathmandu was 8.9% (15 out of 168 goats), which was higher than the findings of a study in Chitwan district at 4.71% (Regmi et al., 2023) and in Rupendehi and Palpa districts of Nepal at 3.90% (Adhikari et al., 2022). It is also higher than the study



conducted by CVL, Tripureswor in Dolakha, Rukum-West, and Chitwan which is 3.4 % (14 out of 402 goats) (Annual Technical Report of CVL, 2021/2022).

Table 1. Association of seropositivity within districts

Districts	Total no. of sample	Positive cases (%)
Bardiya	21	3 (14.3%)
Baitadi	7	1 (14.3%)
Dang	42	1 (2.4%)
Kailali	9	2 (22.2%)
Kanchanpur	16	2 (12.5%)
Makwanpur	1	0%
Parasi	7	1 (14.3%)
Rolpa	13	1 (7.7%)
Rukum-Paschim	6	3 (50%)
Salyan	10	0%
Kathmandu	36	1 (2.8%)
Total	168	15 (8.9%)

There is significant association at 5% significance level between districts and disease (CCPP) prevalence (chi square=20.882, df=10, p=0.022). In this study, a relatively high seroprevalence was seen in Rukum-Paschim(50%) followed by Kailali(22.2%) and Bardiya(14.3%), Baitadi(14.3%), Parasi(14.3%), Kanchanpur (12.5%), Rolpa (7.7%), Dang (2.4%) and Kathmandu (2.8%). The result was negative in Salyan and Makwanpur. This study showed that the goat populations in the districts of Kailali, Baitadi, Bardiya, Parasi (which borders India), and Rukum-Paschim are at high risk of acquiring CCPP infection. The higher prevalence observed in marketed goats of Kathmandu could be due to concentration of goats from various regions of the country, potentially creating ideal conditions for the transmission of CCPP.

In this study, the overall seroprevalence of CCPP in marketed goats in Kathmandu was found less than serological studies carried out in various regions of South Asia, where prevalence rates ranged from 32.5 to 45.7% in Pakistan (Awan *et al.*, 2010), in a different state of India like 33.67 % in Nagpur (Sivakumar *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). There are many possible reasons for the differences in seroprevalence, such as the small sample size, goat management and production systems, geographic area,



the research locations, the population density, the risk of exposure, the availability of veterinary care, and the methods used to measure seropositivity.

India has had a lot of cases of CCPP, especially in the states of Kerala and Kashmir. Using molecular typing, the disease was confirmed, and *Mycoplasma capricolum* subsp. *capripneumoniae* was found to be the cause. There is also a vaccination program for CCPP in India (Farooq *et al.*, 2018). In 2020/2021, Nepal imported 13,827 live goats from India in 2020/2021 (Euro-meat news.com, 2024). The Krishnanagar border crossing in western Nepal has been identified as the largest entry point for these animals. The high CCPP seroprevalence in Rukum-Paschim and Indian bordering districts like Bardiya and Kailali may be a sign of cross-border disease transmission.

Similar findings in Ethiopia's Guji and Borana zones demonstrated that the primary source of CCPP transmission was cross-regional movement of animals, highlighting the importance of restricting livestock movement to reduce the disease's spread (Bekele *et al.*, 2011). This study found no seropositive cases in goats from Salyan and Makwanpur, possibly due to smaller sample sizes or effective local control measures. Another factor contributing to the greater transmission rates may be the movement of goats across the nation without appropriate quarantine protocols. The increased risk of disease transmission may arise from traveling goats frequently interacting with animals from different regions.

Long-term travel stress may also impair the animals' immune systems, making them more susceptible to disease-like CCPP. These results align with research conducted in Ethiopia, where the movement of livestock was identified as a significant factor in the spread of CCPP (Hadush *et al.*, 2009). A study analyzing outbreaks of CCPP found that poor housing and stressful transportation conditions greatly enhanced the probability of disease transmission among goats. Stress-related suppression of immune functions facilitates the rapid colonization of respiratory tissues by *M. capricolum*, leading to severe symptoms such as pleuropneumonia and higher morbidity and mortality rates in goat populations (Abraham *et al.*, 2015).

CONCLUSION

In this study, the overall seroprevalence of CCPP among marketed goats in Kathmandu was found to be 8.9 % (15 out of 168 goats). As there is no CCPP vaccination program in Nepal, seropositivity results could be the result of the causative agent's spontaneous infection. This will serve as a foundation for future surveillance



and monitoring. In order to comprehend the epidemiology of CCPP in Nepal, our study offers basic data on the disease among goats in that country. An understanding of the disease's prevalence in Nepal can be greatly enhanced by doing additional epidemiological research using suitable sample sizes across the nation.

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