Seroprevalence of Contagious Caprine Pleuropneumonia (CCPP) in Bharatpur, Chitwan, Nepal

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ABSTRACT

A study was conducted to determine the seroprevalence of Contagious Caprine Pleuropneumonia (CCPP) in 106 goats of Bharatpur Municipality of Chitwan, Nepal from July to October 2021. A questionnaire was used to determine the potential risk factors. Collected samples were subjected to a competitive enzyme-linked immunosorbent assay (cELISA) for the detection of antibodies. The seroprevalence of Contagious Caprine Pleuropneumonia in goats was 4.71% (5/106). Although seroprevalence was high among females and goats below or equal to 5 years, there was no statistically significant. This study was carried out in the Chitwan district for the first time, applying the (Mccp) Antibody test technique, considering the need for control of this economically important disease for poor livestock farmers in Chitwan and it can play important role in the epidemiological study of CCPP in Nepal.

***Keywords**: Seroprevalence, Goats, CCPP, cELISA, Nepal*

INTRODUCTION

The goat population in Nepal is estimated at 12.28 million and goat meat ranks third in terms of national meat output (13.58 percent), behind chicken and buffalo meat (Krishi Diary, 2020). Small ruminants provide meat, milk, manure, leather, and packaging material to Nepalese rural households, contributing greatly to their livelihoods. Even though goats represent plenty of national resource in the country, their productivity is hampered by a variety of reasons, including a lack of feed, a lack of genetic potential, and infectious diseases (Boyazoglu et al., 2005; Ahuya et al., 2005).

Among the infectious diseases, Contagious Caprine Pleuropneumonia (CCPP) which is

caused by *Mycoplasma capricolum subspecies capripneumonia* is a highly contagious severe respiratory disease affecting goats in over 40 countries throughout the globe, particularly in Asia, Africa, and the Middle East (Iqbal et al., 2019; Parray et al., 2019). It is an OIE-listed notifiable devastating disease in goats (Bascunana et al., 1994; Manso-Silván et al., 2011). The major transmission route is direct contact between sick and healthy animals, with the predominant route of infection being the inhalation of infective droplets from the carrier or active animals (Shahzad et al., 2016). Anorexia, dyspnea, coughing, thoracic pain lying down, nasal discharge, depression, high temperature (41–44 °C), lagging, loss of body condition score, and substantial morbidity (up to 100%) and mortality leading to 80–100% are the symptoms of acute and chronic stage of CCPP (MacOwan & Minette, 1976; Radostis et al., 2006; Ahaduzzaman, 2021). Age, sedentary agricultural systems, species, location, season, the introduction of new animals from markets, and the absence of therapeutic intervention are all common risk factors linked to higher CCPP seroprevalence (Parray et al., 2019).

Biochemical, microbiological, serological, and gene-based identification are used to confirm the diagnosis following a clinical tentative diagnosis. The competitive ELISA kit which is based on Mccp-specific monoclonal antibody has a high specificity (99.8–100%) and its applicability for mass-scale testing is an appropriate tool for epidemiological analysis of CCPP (Thiaucourt et al., 1994; Asmare et al., 2016). This study was carried out to determine the seroprevalence of CCPP and related risk factors in Bharatpur, Chitwan, Nepal. This study will help in the epidemiological study of CCPP in Nepal.

MATERIALS AND METHODS

A cross-sectional study was carried out in Bharatpur Metropolitan (Ward Number: 4, 5, 12, 14, 15, and 17) (27.6706° N, 84.4385° E) of Chitwan, Nepal (Figure 1) from 15 July 2021 to 21 October 2021 through multistage random sampling techniques among goat population. A questionnaire survey was done to collect general descriptions, herd history, and individual animal history.

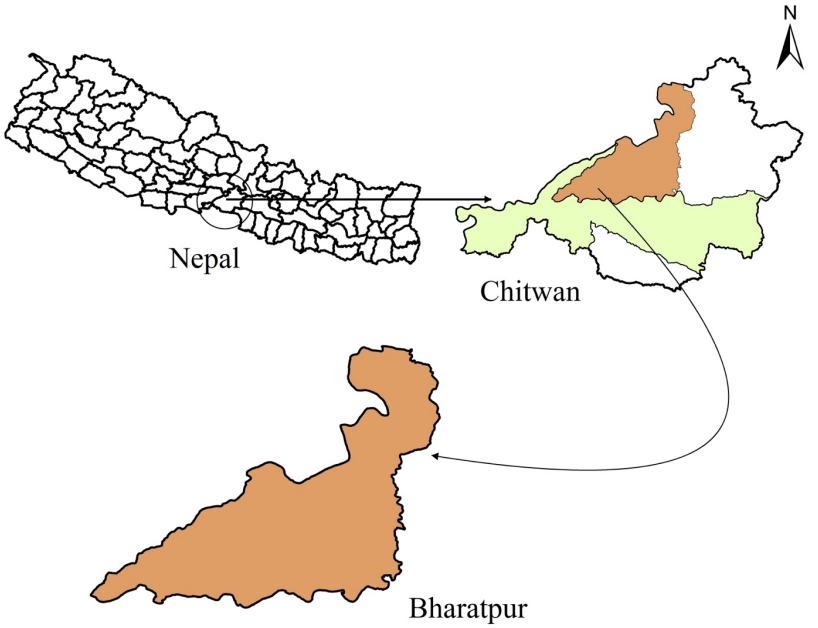

Figure1. Map of Nepal and Chitwan with study area i.e., Bharatpur

The total goat population in Bharatpur Metropolitan is 94,531 and in western Chitwan is 61,348 (Krishi Diary, 2020). There had been no previous reports on the prevalence of CCPP in Chitwan. 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% (Thrusfield et al., 2017). The calculated minimum sample size required for the study was 145. In the study 106 samples were taken due to time and budget constraints.

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

n = Required sample size,
d = Desired absolute precision (0.05)
P_{exp} = Expected prevalence (0.05)

A semi-structured questionnaire was administered to collect data from the goat farmers by the purposive sampling method. The closed 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.

One hundred six blood samples were taken from goats with a history of nasal discharge, and cough from the herd of healthy animals. Selected animals were restrained by two people and the site was prepared around the mid-jugular vein by using 70% ethanol.

Using a 10 ml sterile disposable syringe and needle, five ml of blood was taken from the jugular vein aseptically.

To prevent blood hemolysis and extract the serum fluid, the syringe with blood was left undisturbed at room temperature in a 45-degree position overnight. The serum was extracted, transferred to serum vials, and maintained in a cool box. An icebox containing ice packs was used to transfer the sample to the Central Veterinary Laboratory (CVL) in Tripureswor, Kathmandu.

Collected goat serum samples were examined by the competitive enzyme-linked immunosorbent assay (cELISA) (Thiaucourt et al., 1994) by IDEXX CCPP Ab Test which is based on the monoclonal antibody Mab 4.52 recommended by the International Organization for Animal Health and CIRAD -EMVT (France). The test was performed according to the manufacturer's guidelines. All reagents were allowed to come to 18-26°C before use and reagents were mixed by gentle inverting or swirling. Controls were dispensed anywhere on the microplate. The serum samples were diluted and mixed in an uncoated plate with a specific monoclonal anti-Mccp antibody (Mab 4.52), then the homogenized components of the uncoated plate were transferred to the Mccp antigen-coated microplates and incubated for 1 hour at 37°C with gentle agitation to avoid desiccation of the plates. After removing the solution, each well was washed twice with the washing solution. Each well was filled with a diluted Conjugate solution of Anti-mouse IgG horseradish peroxidase, and the microplate was covered at 37°C for 30 minutes with gentle agitation. The technique was repeated 3 times, after which TMB Substrate was added to each well and incubated at + 37 °C, for 20 minutes. Finally, a Stop Solution was added in each well, and the absorbance values (OD) of controls and samples were measured and recorded at 450 nm using an ELISA plate reader. The result was interpreted by a percentage of inhibition (PI)= ((OD Mab - test serum)/ (OD Mab – OD conjugate)) ×100 and Serum with a PI≥ 55% was judged positive for Mccp infection, whereas a PI < 55 % were considered negative.

The data entry of the serological test was done using Microsoft office excel and statical analysis was done by SPSS V 20.0. Chi-square was utilized as a statistical test. In all of the analyses, the confidence level was set at 95%, and the significance level was fixed at $p < 0.05$.

Ethical approval of the study was sought from the Ethical Approval Committee of Agriculture and Forestry University, Nepal. 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

In this study, the seroprevalence of CCPP in the goat population was found to be 4.71% (5 out of 106 goats). Among the tested population, 81 were females and 25 were males. The seroprevalence of CCPP was higher in the female (4.93%) than in male (4%) goats, although the relationship was not significant at a 5% level of significance (p=0.663). The goats below or equal to 5 months of age were found to have higher seroprevalence (7.69%) of CCPP than above 5 months of age (3.75%). The age-wise prevalence was not significant at a 5% level of significance (p=0.357) (Table 1).

Table 1: Sex and age-wise seroprevalence of CCPP in Bharatpur, Chitwan, Nepal.

SN	Risk Factors		Total	Positive (Percentage)	p-value
1	Sex	Male	25	1 (4%)	0.663
		Female	81	4 (4.93%)	
2	Age	Below or equal 5 months	26	2 (3.75%)	0.357
		Above 5 months	80	3 (7.69%)	

DISCUSSION

In this study, the seroprevalence of CCPP was found to be 4.71% (5 out of 106 goats) which is higher than a study conducted in the Rupandehi and Palpa districts of Nepal (3.40%) (Adhikari et al., 2022) and lower than serological research conducted in different parts of South Asia, where the prevalence rates of 32.5 to 45.7 % in Pakistan (Awan et al., 2010), 9.9 to 33.7 % in India (Suryawanshi et al., 2015), 8.52 % in Pakistan (Shahzad et al., 2016), and 10.65% in India (Gupta et al., 2016) were reported from the goats. The differences in seroprevalence might be due to the low sample size, goat management and production systems, agroecological systems, variances in research areas, population density, and the procedures employed to quantify seropositivity.

The seroprevalence of CCPP was found to be higher in female goats (4.93 percent) than in male goats (4%) in our investigation. Similarly, Abegunde (1981) discovered a greater frequency of CCPP in female goats (16.1%) than in male goats (10.7%). A study by Bekele et al., (2011), also found a higher prevalence in females (14%) than in males (11.6%). The female seroprevalence of CCPP was somewhat higher (10.67%) than the male seroprevalence (10.61%) reported by Gupta et al., (2016). However, Suryawanshi et al., (2015) reported a higher prevalence in males (35.71%) than in females (17.14%). This result could be attributed to a lack of CCPP data in the Nepalese context for comparison and a lack of CCPP tendency for either gender.

Furthermore, goats younger than or equal to 5 months of age had a higher seroprevalence of CCPP (7.69%) than goats older than or equal to 5 months of age (3.75%). However, the study of Bekele et al., (2011) found a significant seroprevalence of 9.2 percent, 11.0

percent, and 34.8 percent in young (2 years), adult (2-5 years), and old (>5 years) age groups. A study by Suryawanshi et al., (2015) discovered a higher frequency (23.08%) in goats aged 2-3 years. Also, Gupta et al., (2016) found the highest prevalence in goats over 12 months of age (10.67%) followed by 10.64% in goats under 6 months of age, and 10.59% in goats 6-12 months of age, although the result was insignificant with each other. Goats become more susceptible to illness as they age. They're also prone to being infected time and again. These results were in contrast to our findings, which were based on a separate comparative age group, with younger goats (<5 months) being more susceptible to CCPP infection than the goats (>5 months). This could be related to differences in sample size, past infections, predisposing factors, and due to the weak immune system of the kids.

CONCLUSION

Our results conclude a seroprevalence (4.71%) of contagious caprine pleuropneumonia in the goat of Bharatpur Metropolitan, Chitwan conducted in 106 goats. There was a variation in the seroprevalence level among the age and sex. Female goats were found to be more susceptible to CCPP than the male goats and young goats below or equal to 5 months were more susceptible to CCPP than the goats above 5 months in this study. Since there is no vaccination practice for CCPP in Nepal, seropositivity results might be from natural infection of the causative agent. As this is the first research conducted in Chitwan, Nepal for CCPP, it will provide a base for further surveillance and monitoring in the future.

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AUTHOR'S CONTRIBUTION

LBR: Conceptualized and designed the study, LBR, SP: collected data and samples, SP and DS: drafted the manuscript, DS and RA: Revised the manuscript, RA: Supervised the study. All authors read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Abegunde, T.A., 1981. A serological survey of Mycoplasma putrefaciens infection in goats. American J. Vet. Res., 42(10): 1798-1801.
2. Adhikari, B. K., Subedi, D., Jyoti, S., Kaphle, K., Kharel, C. N., & Khanal, D. R. (2022). Seroprevalence of contagious caprine pleuropneumonia (CCPP) in Rupandehi and Palpa Districts of Nepal. Veterinary Sciences: Research and Reviews, 8(1), 23-29. https://dx.doi.org/10.17582/journal.vsrr/2022.8.1.23.29
3. Ahaduzzaman, M.D., 2021. Contagious caprine pleuropneumonia (CCPP): A systematic review and meta-analysis of the prevalence in sheep and goats. Transbound Emerg Dis., 68(3): 1332-1344. https://doi.org/10.1111/tbed.13794
4. Ahuya, C.O., Okeyo, A.M., Mwangi-Njuru, Peacock, C., 2005. Developmental challenges and opportunities in the goat industry: The Kenyan experience. Small Rumin. Res., 60(1-2): 197-206. https://doi.org/10.1016/j.smallrumres.2005.06.013
5. Asmare, K., Abayneh, T., Mekuria, S., Ayelet, G., Sibhat, B., Skjerve, E., Szonyi, B., Wieland, B., 2016. A meta-analysis of contagious caprine pleuropneumonia (CCPP) in Ethiopia. Acta Tropica., 158: 231-239. https://doi.org/10.1016/j.actatropica.2016.02.023
6. Awan, M.A., Abbas, F., Yasinzai, M., Nicholas, R.A.J., Babar, S., Ayling, R.D., Attique, M. A., Ahmed, Z., Wadood, A., Khan, F. A., 2010. First report on the molecular prevalence of Mycoplasma capricolum subspecies capripneumoniae (Mccp) in goats the cause of contagious caprine pleuropneumonia (CCPP) in Balochistan province of Pakistan. Mol. Biol. Rep., 37(7): 3401–3406. https://doi.org/10.1007/s11033-009-9929-0
7. Bascunana, C.R., Mattsson, J.G., Bolske, G., Johansson, K.E., 1994. Characterization of the 16S rRNA genes from Mycoplasma sp. strain F38 and development of an identification system based on PCR. J. Bacteriol. Res., 176(9). https://doi.org/10.1128/jb.176.9.2577-2586.1994
8. Bekele, T., Asfaw, Y., Gebre-Egziabeher, B., Abebe, G., 2011. Seroprevalence of contagious caprine pleuropneumonia in Borana and Guji lowlands, Southern Ethiopia. Ethiop. vet. j., 15(2): 69-76. https://doi.org/10.4314/evj.v15i2.67695
9. Boyazoglu, J., Hatziminaoglou, I., Morand-Fehr, P., 2005. The role of the goat in society: Past, present and perspectives for the future. Small Rumin. Res, 60(1-2):13-23. https://doi.org/10.1016/j.smallrumres.2005.06.003
10. Gupta, D.K., Shukla, P.C., Tiwari, A., Baghel, R.P.S., Sharma, V., Shivhare, J., Gupta, N., 2016. Seroprevalence Study on Goat Contagious Caprine Pleuropneumonia in Jabalpur, Madhya Pradesh. J Anim Res., 6(4): 743-746. https://doi.org/10.5958/2277-940x.2016.00092.9
11. Iqbal Y.M., Raffiq P.O., Tauseef B.S., Muheet, A.B.R., Gopalakrishnan, A., Karthik, K., Dhama, K., Vir Singh, S., 2019. Contagious caprine pleuropneumonia–a comprehensive review. Vet Q., 39(1): 1-25. https://doi.org/10.1080/01652176.2019.1580826
12. Jain, U. D. I. T., Verma, A. K., & Pal, B. C. (2015). Occurrence of mycoplasma infection in Barbari goats of Uttar Pradesh, India. Haryana Vet, 54(1), 53-55.
13. MacOwan, K.J., Minette, J.E., 1976. A mycoplasma from acute contagious caprine pleuropneumonia in Kenya. Trop. Anim. Health Prod., 8(1). https://doi.org/10.1007/BF02383376

14. Manso-Silván, L., Dupuy, V., Chu, Y., Thiaucourt, F., 2011. Multi-locus sequence analysis of mycoplasma capricolum subsp. capripneumoniae for the molecular epidemiology of contagious caprine pleuropneumonia. Vet. Res., 42(1): 1-10. https://doi.org/10.1186/1297-9716-42-86
15. Parray, O.R., Yatoo, M.I., Bhat, R.A., Malik, H.U., Bashir, S.T., Magray, S.N., 2019. Seroepidemiology and risk factor analysis of contagious caprine pleuropneumonia in Himalayan Pashmina Goats. Small Rumin. Res., 171: 23-36. https://doi.org/10.1016/j.smallrumres.2018.12.004
16. Radostits, O. M., Gay, C. C., Blood, D. C., Hinchcliff, K.W., 2000. A textbook of the diseases of cattle, sheep, pigs, goats and horses. Vet. Med., 9: 603-700.
17. Shahzad, W., Yaqoob, T., Mukhtar, N., Munir, R., Ahmad, R., Khan, M.S., Hussain, A. 2016. Seroprevalence of mycoplasma capricolum subsp. Capripneumoniae in goats through celisa in different districts of Punjab, Pakistan. J Anim. Plant Sci., 26(4).
18. Suryawanshi, S.N., Tembhurne, P.A., Gohain, S., Kesharkar, J.A., Tumlam, U. M., Ingle, V.C., 2015. Seroprevalence of contagious caprine pleuropneumonia in small ruminants in Maharashtra. The Ind. J. Vet. Sci. Biot., 10(4).
19. Thiaucourt, F., Bölske, G., Libeau, G., Le Goff, C., Lefèvre, P.C., 1994. The use of monoclonal antibodies in the diagnosis of contagious caprine pleuropneumonia (CCPP). Vet. Micobioil., 41(3):191-203. https://doi.org/10.1016/0378-1135(94)90100-7
20. Thrusfield, M., Christley, R., Brown, H., Diggle, P.J., French, N., Howe, K., Kelly, L., O'Connor, A., Sargeant, J., Wood, H., 2017. Veterinary Epidemiology: Fourth Edition. https://doi.org/10.1002/9781118280249