Seroprevalence of PPR in Goats of Buffer Zone of Chitwan National Park

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Abstract

A study conducted from September to December 2019 aimed to assess the seroprevalence of Peste des Petits Ruminants (PPR) in goats residing in the buffer zone of Chitwan National Park. A total of 221 serum samples were collected from unvaccinated goat flocks across the buffer zone regions (48 from Bhojad, 64 from Bhandara, 66 from Padampur, 25 from Gondrang, and 18 from Kathar) using random sampling techniques. Competitive Enzyme-Linked Immunosorbent Assay (ELISA) was employed to detect antibodies against the PPR virus (PPRV). The study revealed an overall seroprevalence rate of 28.96%. The highest seroprevalence rate of 39.58% was observed in goats from Bhojad, while the lowest rate of 15.63% was found in goats from Bhandara. The findings emphasize the need for regular vaccination of small ruminants in Bhojad, Padampur, and Kathar to enhance immunity and prevent PPR infection. Additionally, such measures can help mitigate the transmission of PPR between goats and wild animals as they share common grazing lands.

Keywords: Seroprevalence, PPR, Goats, Buffer Zone

Introduction

Peste des petits ruminants (PPR), also known as 'goat plague,' is a viral disease that significantly impacts the economy and health of small ruminants. The World Organization for Animal Health (WOAH) describes it as an acute condition primarily affecting goats and sheep, presenting symptoms: sudden initiation of high fever, severe depression, diminished appetite, clear nasal discharge, mouth sores, diarrhea, pneumonia, and severe cases, death (Couacy-Hymann et al., 2023). The virus, belonging to the morbillivirus family, is a single-stranded, non-segmented RNA virus and shares genetic characteristics with well-known diseases such as rinderpest, measles, and

canine distemper (Amarasinghe et al., 2017; Benfield et al., 2023). Though PPR can experimentally affect cattle and some wild ruminants, it predominantly infects goats and sheep (Abdollahi et al., 2023). The virus is known to be present in various bodily fluids, such as tears, nasal discharge, cough secretions, and feces (Ettman et al., 2020; Parida et al., 2019). Transmission occurs mainly through close contact, especially when animals inhale airborne droplets from coughs or sneezes. The contamination of water sources, feed troughs, and bedding materials can also spread the infection. (Herzog et al., 2020). This disease is critical because infected animals can begin shedding the virus before symptoms appear, enabling the silent spread through asymptomatic carriers (Couacy-Hymann et al., 2009).

The disease typically has an incubation period of 3-6 days (N. Kumar et al., 2014). As the condition worsens, the nasal discharge may thicken and turn yellowish, forming crusts that block the nostrils and cause respiratory distress. Oral ulcers can develop across various mouth parts, including the gums, dental pad, hard palate, cheeks, and tongue. In some cases, severe diarrhea leads to dehydration and significant weight loss (Altan et al., 2019). Pneumonia often develops in the later stages of the disease, and infected pregnant animals may experience miscarriages (P. Kumar et al., 2018). The prognosis for affected animals is generally poor, with many dying within five to ten days after fever onset. Younger animals, particularly goats, are usually more severely affected, and the disease can sometimes lead to sudden death. However, PPR can also occur in a milder or subclinical form, which allows it to persist in a population without noticeable symptoms until it reaches susceptible hosts (Cebra & Cebra, 2012; Ishag et al., 2023).

International health organizations estimate that the global economic impact of Peste des petits ruminants (PPR) ranges from US$1.4 billion to US$2.1 billion annually. This substantial economic burden includes direct costs related to disease management, livestock mortality, decreased production, and expenses for ongoing PPR treatments. Nearly two-thirds of the world's domestic small ruminants are susceptible to this virus, illustrating its extensive reach (Jones et al., 2016). The significant economic disruptions caused by PPR have led to its prioritization within global veterinary emergency programs and listed under the WOAH Terrestrial Animal Health Code. This status requires nations to report outbreaks promptly by following specific guidelines. By 2030, the Food and Agricultural Organization (FAO) and WOAH have selected this disease to eradicate globally (Benfield et al., 2023). To achieve the global eradication of PPR, it is essential to

thoroughly understand the prevalence of the virus in various regions of the country. With the help of a prevalence study, various targeted programs and campaigns can be prepared. Additionally, planning can be done to allocate resources such as PPR vaccines, veterinary supplies, and veterinary personnel specifically to targeted locations. Lastly, the data can also add up to establish a robust surveillance system in that region and prepare various animal health policies to control and prevent diseases in that region.

In Nepal, goats account for about 48.31% of the nation's total livestock farming (*Ministry of Agriculture and Livestock Development*, 2023). The first recorded outbreak of PPR was seen in 1995 and impacted several districts, and since then, the disease has spread across various ecological zones and developmental regions of the country (K. P. Acharya et al., 2020). The introduction of PPR pathogens into Nepal is facilitated by various vectors, including wildlife from neighboring countries, insects, migratory birds, international cargo, and human travel. The practice of transporting carrier animals to crowded marketplaces and exhibitions significantly increases the risk of disease transmission (Prajapati et al., 2021). Most of these activities usually occur in the buffer zone of various national parks of Nepal, which is directly connected to the open border of India. Moreover, the interaction between domestic and wild animals, through direct contact or by means of contaminated forage, is usually observed more in the buffer zone of such national parks connected to international borders, making this area more prone to PPR.

Research conducted at the Regional Agricultural Research Station in Bandipur has documented high mortality rates associated with PPR (N. Acharya et al., 2018). Further studies in districts like Syangja and Kaski have shown an antibody seroprevalence rate exceeding 80%, demonstrating the virus's widespread nature (Prajapati et al., 2021). Comprehensive studies determining the seroprevalence of PPR in different locations within Nepal are still scarce. Hence, this study aims to find the seroprevalence of PPR in the buffer zone of Chitwan National Park.

Materials and methods

Study location and design

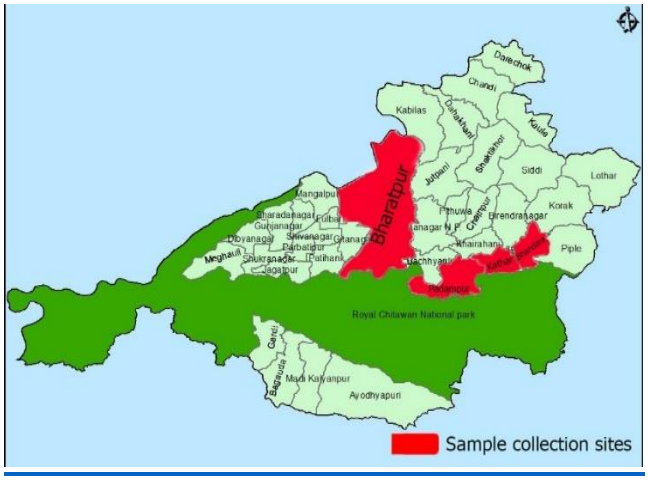

Figure 1. Map of Chitwan district showing the study area. (In this map, the area covered with red colour indicates the study area from where the samples from goats were taken. The area covered with dark green indicates the location of Chitwan National Park.)

For this study, Bhojad, Bhandara, Kathar, Padampur, and Gondrang areas of the Chitwan district were selected. The purpose of selecting this area was that this area is connected to Chitwan National Park and the probability of high incidence of PPR can be suspected in this region. The study was designed as a cross-sectional analysis, employing a hybrid sampling approach that combined purposive and convenience sampling techniques. The study was conducted between September and December 2019 across five locations in the Chitwan district, specifically targeting readily accessible, unvaccinated goat populations. Within this district, which is home to approximately 256,993 goats (*Ministry of Agriculture and Livestock Development*, 2023), a total of 221 serum samples were collected: 48 from Bhojad, 64 from Bhandara, 66 from Padampur, 25 from Gondrang, and 18 from Kathar. The goats sampled included various genders and age groups from a mix of breeds, chosen based on factors such as accessibility, local farmers' cooperation, and the density of the animal population in these areas.

Sampling

Each goat was restrained for blood sampling using a technique where the animal was positioned between the handler's legs. This method involved the handler placing their legs around the goat's

body to hold it gently but securely in place, minimizing movement. The handler also supported the goat's head and neck, positioning the head to extend slightly, which better exposes the jugular vein for sampling. The targeted site for blood collection was the jugular vein. The site was first cleaned with an alcohol swab and using an 18–20-gauge needle connected to a blood vacutainer, the vein was punctured. Once the needle was correctly placed in the vein, blood was drawn into the blood vacutainer. About 5 ml of blood was collected from each goat. After the blood was drawn, the needle was carefully removed, and pressure was applied to the puncture site to stop any bleeding. The sample was then immediately stored in an ice box to maintain its integrity and transported to a laboratory where it was kept at -20°C until serum extraction and. The process of serum extraction occurred on the same day as the collection, and the collected sample was stored at -20°C for further analysis.

Screening of Sera Samples by c-ELISA

Serum specimens were examined using a competitive enzyme-linked immunosorbent assay (c-ELISA) kit called ID Screen© PPR Competition, produced by ID.vet Innovative Diagnostics, located at 310 Louis Pasteur Street, Grabels, France. This diagnostic kit was created to identify antibodies targeting the nucleoprotein (NP) of the Peste des Petits Ruminants Virus (PPRV).

Each well was coated with purified recombinant PPR nucleoprotein, and then 25µl of a dilution buffer was added along with 25µl of the samples to be tested and the controls. This mixture was incubated at 37°C for 45 minutes. If anti-NP antibodies were present, they formed an antibody-antigen complex that covered the NP epitopes. Subsequently, each well was washed three times with 300µl of a wash solution. Next, 100µl of anti-NP-peroxidase (HRP) conjugate was added to each well and incubated at 21°C for 30 minutes. This conjugate is attached to the remaining free NP epitopes, creating an antigen-conjugate-HRP complex. The wells were washed thrice with the wash solution to remove excess conjugate. Following this, 100µl of substrate solution (TMB) was added and incubated at 21°C for 15 minutes. After 15 minutes of incubation, 100µl of stop solution was introduced. The resulting coloration was indicative of the quantity of specific antibodies in the tested sample. When no antibodies were present, the solution appeared blue, but it turned yellow upon the addition of the stop solution. Conversely, in the presence of antibodies, no coloration change occurred. Subsequently, the microplate was analyzed at a wavelength of 450 nm

using an ELISA reader. To assess the results quantitatively, the S/N% for each sample was calculated using the following formula:

$$S/N\% = \frac{OD_{sample}}{\mathrm{OD_{NC}}} x100$$

Result	Status
S/N% ≤ 50%	POSITIVE
50% < S/N% ≤ 60%	DOUBTFUL
S/N% > 60%	NEGATIVE

Test Validation

For 1st lot test of ELISA

The mean OD_{NC} must be more than 0.7, which is found to be 1.118, and the mean ODPC, which is 0.1341, must be less than 30% of the mean ODNC, which is 0.3354. Hence the test is valid.

For 2nd lot test of ELISA

The mean OD_{NC} must be more than 0.7, which is found to be 1.0674, and the mean ODPC, which is 0.1256, must be less than 30% of the mean ODNC, which is 0.3202. Hence the test is valid.

Statistical analysis

The chi-square test was employed to assess the association between geographic areas and the prevalence of Peste des Petits Ruminants (PPR). The null hypothesis asserted that PPR prevalence was consistent across all regions. Due to multiple comparisons, the Bonferroni correction was applied to control for Type I errors, setting the significance threshold at a p-value less than 0.005, adjusted from the standard 0.05 to account for 10 pairwise comparisons.

Results and discussion

Out of the 221 serum samples processed using competitive enzyme-linked immunosorbent assay (c-ELISA) for the detection of Peste des Petits Ruminants Virus (PPRV) antibodies, 64 tested positive. The overall seroprevalence rate was determined to be 28.96%, with variable prevalence across different locations. The highest seroprevalence was recorded in Bhojad (39.58%) and

Padampur (39.39%), while the lowest was observed in Bhandara (15.63%). The prevalence in Gondrang and Kathar was 20% and 22.22%, respectively, as shown in Table 1.

Overall, the initial chi-square analysis revealed significant differences in seroprevalence rates between the areas, with a p-value of 0.011. This indicates a notable variation in the impact of the disease across the studied regions. However, when applying the Bonferroni correction to account for multiple comparisons, a statistically significant difference in seroprevalence was observed only between Bhandara and Padampur. No significant differences in prevalence rates were detected in comparisons involving other areas.

Table 1. Seroprevalence of PPRV infection across different buffer zones of Chitwan National Park.

Place	**Total**	**Positive**	**p-value vs**			
			Bhojad	**Bhandara**	**Padampur**	**Gondrang**
Bhojad	48	19 (39.58%)	-	0.0081	1.0000	0.1534
Bhandara	64	10 (15.63%)	0.0081	-	0.0046	0.8567
Padampur	66	26 (39.39%)	1.0000	0.0046	-	0.1350
Gondrang	25	5 (20%)	0.1534	0.8567	0.1350	-
Kathar	18	4 (22.22%)	0.3038	0.7622	0.2845	1.0000
Total	221	64 (28.95%)	**Overall p Value**: 0.011			

This study observed an overall seroprevalence of 28.96%, which is consistent with findings from similar research conducted on goat populations in Pakistan which reported a seroprevalence rate of 27.53% (Abubakar et al., 2017). In contrast, research across five states in India indicated a higher rate of 34.54% (Balamurugan et al., 2014). In contrast, a study in Bangladesh found a much lower seroprevalence of 8.70% (Islam et al., 2016), and research in Tibet documented a rate of 34.5% (Wang et al., 2009). These discrepancies may be attributed to geographical and agroclimatic differences that influence disease transmission dynamics, including variations in domestic and wild animal population densities, which share common grazing and watering areas (Salih et al., 2014). Moreover, seroprevalence of PPR in goats was found to be high compared to the study done in Cattle in Chitwan district (4.22%) (Prajapati et al., 2021). It could have happened due to the inherent biological predisposition of goats to the PPR virus, coupled with intensive intermingling

and less stringent management practices in goat herds compared to cattle (Fakri et al., 2017; Zheng et al., 2020).

The high seropositivity rates in Bhojad and Padampur could be due to frequent movement within buyer and seller networks, contributing to the spread of infection among susceptible animals. Additionally, the transmission of PPRV may also occur through interactions between grazing domestic animals and infected wild counterparts, a phenomenon supported by findings from Gurung et al. (2012). Prevalence rates were notably higher in areas like Syanja and Kaski (82.60%), likely due to the extensive use of forest resources for goat rearing despite the challenging geographical conditions that predispose animals to PPR (N. Acharya et al., 2018). The persistence of PPR in these regions highlights the critical role of environmental factors and animal management practices in disease transmission.

Conclusion

The seroprevalence of Peste des Petits Ruminants (PPR) in goats within the buffer zone of Chitwan National Park was found to be 28.96%. This rate varies significantly across different areas within the zone, with the highest rates observed in Bhojad and Padampur. These variations suggest that local factors such as animal movement, interaction between domestic goats and wildlife, and specific farming practices may influence the spread of PPR. Understanding these dynamics is crucial for implementing effective disease control measures, which could include targeted vaccination programs and restrictions on animal movement to mitigate the spread of PPR in the region.

Control measures and recommendations

Considering the significant economic losses reported by farmers in the buffer zones of national parks, effective preventive measures are essential. These should include regular vaccination campaigns using thermostable vaccines, strict regulations on the movement of goats, especially in forested areas, and public awareness initiatives to educate farmers about PPR risks and mitigation strategies. If implemented effectively, such measures could significantly reduce the incidence and impact of PPR in endemic regions.

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