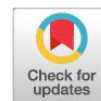


Research Article



First Comprehensive Seroepidemiological Survey of Peste des Petits Ruminants Virus (PPRV) in Small Ruminants in District Quetta, Pakistan

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Abstract | Peste des petits ruminants (PPR) is a highly contagious viral disease affecting small ruminants, causing significant economic losses in endemic regions like Pakistan. This study aimed to evaluate the seroprevalence of PPR virus (PPRV) in small ruminants in district Quetta, Pakistan, and assess associated risk factors and farmer awareness levels. A total of 200 blood samples were collected from goats and sheep and analyzed using competitive ELISA (cELISA) to detect PPRV-specific antibodies. The results revealed an overall seroprevalence of 36%, with goats showing a higher ($P > 0.05$) prevalence (37.5%; 45/120) compared to sheep (33.75%; 27/80). Key risk factors included larger herd sizes (>100 animals), free-grazing practices, and frequent animal movement, which significantly ($P < 0.05$) increased the likelihood of PPRV exposure. Farmer awareness of PPR was limited, with only 60% of farmers aware of the disease and a mere 25% regularly vaccinating their animals. The study also identified gaps in vaccination coverage and highlighted the role of shared grazing areas and animal movement in disease transmission. The statistical analysis revealed that there were a statistically significant association ($P < 0.05$) for awareness, vaccination practices, movement of animals and sharing of grazing areas. These findings underscore the urgent need for targeted interventions, including enhanced vaccination programs, farmer education, and improved herd management practices, to control PPR in the region. Strengthening diagnostic capacity and fostering collaboration between stakeholders are also critical for effective disease surveillance and control. This study provides valuable insights into the epidemiology of PPR in district Quetta and offers evidence-based recommendations for mitigating the disease's impact on small ruminant production and farmer livelihoods.

Keywords | Prevalence, Sheep, Goat, PPR, Farmer awareness

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INTRODUCTION

The livestock sector, particularly small ruminants such as sheep and goats, serves as a cornerstone of Pakistan's economy. These animals are vital to rural livelihoods, offering income, nutrition, and financial

resilience to smallholder farmers. However, their productivity is severely undermined by parasites and infectious diseases (Alani *et al.*, 2025; Fidèle *et al.*, 2024; Ismail *et al.*, 2025), particularly peste des petits ruminants (PPR), which threatens both economic stability and food security (Rahman *et al.*, 2020).

PPR is an acute, highly contagious viral disease caused by the PPR virus (PPRV), a member of the *Morbillivirus* genus within the *Paramyxoviridae* family (Balamurugan *et al.*, 2014). The disease is characterized by high morbidity (up to 90%) and mortality rates (40–80% in naïve populations). PPR manifests through fever, oral necrosis, diarrhea, and pneumonia, often culminating in death within 10 days of infection (FAO and OIE, 2016). The disease is classified as a transboundary animal disease by the Food and Agriculture Organization (FAO) and World Organisation for Animal Health (WOAH; formerly called OIE), with endemicity reported across Africa, the Middle East, and Asia. In endemic regions, mortality rates decline to 10–20% due to partial herd immunity, but recurrent outbreaks perpetuate cycles of poverty among small-scale farmers (Balamurugan *et al.*, 2014). Transmission occurs through direct contact with infected animals' secretions or excretions, facilitating rapid spread in densely populated herds and during seasonal migrations.

In Pakistan, PPR was first laboratory-confirmed in 1994 through polymerase chain reaction (PCR) testing, with subsequent outbreaks validated via antigen detection using immunocapture ELISA (IcELISA) (Hussain *et al.*, 2002). The country's geographical positioning bordering Afghanistan, Iran, India, and China creates a high risk of transboundary disease transmission. Shared grazing lands, water sources, and mixed farming systems involving small and large ruminants amplify cross-border viral exchange (Zafar *et al.*, 2024). Economic losses attributed to PPR in Pakistan are estimated at PKR 20.5 billion (USD 0.24 billion), driven by mortality, reduced milk and meat yields, abortion storms, and trade restrictions (Abubakar *et al.*, 2023). Despite these staggering losses, PPR control remains suboptimal due to fragmented vaccination policies, insufficient cold-chain infrastructure, and weak coordination between farmers, veterinary authorities, and policymakers (Munibullah *et al.*, 2024).

The challenges are particularly acute in Balochistan province, where District Quetta a critical hub for small ruminant production and cross-border trade exemplifies the interplay of risk factors driving PPR persistence. Balochistan's arid climate and vast rangelands support pastoralist communities, with livestock contributing nearly 40% of the province's agricultural output (Khan *et al.*, 2023). However, nomadic herding practices, limited veterinary infrastructure, and climate-induced resource scarcity create ideal conditions for disease transmission. Recent reports indicate an 18% surge in PPR outbreaks since 2020, correlating with increased cross-border livestock trade with Afghanistan and Iran (Rehman *et al.*, 2025). Compounding this issue, only 32% of small ruminants in Balochistan receive routine vaccinations, attributed to cold-chain failures, logistical barriers in

remote areas, and pastoralist mistrust of government-led campaigns (Abubakar *et al.*, 2017).

Globally, the FAO-OIE Global Control and Eradication Strategy (GCES) aims to eliminate PPR by 2030 through mass vaccination, surveillance, and capacity building (FAO, 2023). However, Pakistan's progress lags due to systemic gaps. Nomadic herders, who constitute 25% of Balochistan's livestock producers, frequently bypass vaccination programs due to mobility and cultural barriers, perpetuating viral reservoirs (Sadiq *et al.*, 2024). Furthermore, antigenic diversity in circulating PPRV strains complicates control efforts (Rizwan *et al.*, 2024).

District Quetta, a major transit point for regional livestock movement, lacks recent seroprevalence data, hindering evidence-based control measures. Therefore, this study was designed to evaluate the seroprevalence of PPR virus (PPRV) in small ruminants in district Quetta. This study addresses these gaps by providing the first comprehensive assessment of PPRV seroprevalence and associated risk factors in sheep and goats in district Quetta. By analyzing variables such as herd size, vaccination history, cross-border mobility, and farming practices, the findings will inform targeted vaccination strategies, enhance biosecurity protocols, and strengthen cross-border collaboration. As Pakistan aligns with the FAO-OIE 2030 eradication agenda, this research underscores the urgency of integrating localized epidemiological insights with global frameworks to safeguard livelihoods and achieve sustainable PPR control.

MATERIAL AND METHODS

STUDY AREA

This study was carried out in line with international ethical standards and approved by the Sindh Agriculture University Tandojam. The study was conducted in District Quetta, Balochistan, Pakistan, along with adjoining regions (Pishin, Kuchlak, Hanna Valley, and surrounding villages) as shown in Figure 1. These areas were selected due to their high small ruminant population density (goats and sheep), frequent reports of PPR outbreaks from local veterinary offices, and their proximity to the Afghanistan-Pakistan border, where transboundary animal movement increases disease transmission risk.

SAMPLING STRATEGY

A total of 200 blood samples (120 goats and 80 sheep) were collected from clinically healthy, small ruminants across 40 different herds to ensure representativeness. The inclusion criteria required animals to be older than six months (to avoid maternal antibody interference), with no recent PPR vaccination history (within the last six months), and no

visible clinical signs of PPR at the time of sampling. Convenient sampling approach was used to collect the samples.

later stored at -20°C in a deep freezer. Verbal informed consent was obtained before sampling and interviews. Farmer identities were anonymized to ensure privacy.



Figure 1: Map of the study area.

COLLECTION OF SAMPLES

For the blood collection OIE guidelines were followed to minimize stress. Animals were humanely restrained in a standing position, and the jugular vein area was disinfected with 70% ethanol. Using a sterile 5 mL disposable syringe and 21G needle, 5 mL of blood was drawn per animal and immediately transferred into labeled serum separation tubes (SST). The tubes were kept at a 45° angle for 12 hours at room temperature (25°C) to allow clotting, followed by centrifugation at 3000 rpm for 10 minutes to separate serum. The clear serum was aliquoted into pre-labeled 2 mL cryovials and stored at -20°C until further processing. For transportation to the Centre of Advanced Studies in Vaccinology and Biotechnology (CASVAB) Laboratory (University of Balochistan), samples were placed in insulated vaccine carriers with ice packs ($2-8^{\circ}\text{C}$) and

COMPETITIVE ELISA (cELISA) FOR PPRV ANTIBODY DETECTION

The ID Screen® PPR Competition ELISA Kit (ID Vet, France) was used to detect PPRV antibodies. The kit included pre-coated 96-well plates (PPRV nucleoprotein antigen), positive and negative controls, HRP-conjugated monoclonal anti-PPRV antibody, TMB substrate, and 1N H_2SO_4 stop solution. Laboratory equipment included a BioTek ELx800 microplate reader (USA), Eppendorf Multipette® pipettes (Germany), a 37°C incubator ($\pm 1^{\circ}\text{C}$), and an automated/manual plate washer.

The procedure was adopted as per the instructions of kit manufacturer. The serum samples were first diluted at a ratio of 1:5 using the provided sample diluent. Following this, 100 μL of each diluted sample was loaded in duplicate into wells of the ELISA plate, alongside positive and negative controls. The plates were then incubated for 45 minutes at 37°C to facilitate antigen-antibody binding. After incubation, the wells were washed three times with 300 μL of PBS-Tween 20 to remove unbound substances. Next, 100 μL of HRP-conjugated monoclonal antibody was added to each well, followed by a 30-minute incubation at 37°C . This was again followed by three washing steps using PBS-Tween 20. Subsequently, 100 μL of TMB substrate was added to initiate the enzyme-substrate reaction, and the plates were incubated for 15 minutes in the dark. The reaction was stopped by adding 50 μL of 1N sulfuric acid (H_2SO_4), and finally, the optical density (OD) was measured at 450 nm using a microplate reader.

The Sample-to-Negative (S/N%) ratio was calculated using the formula: $\text{S/N\%} = (\text{OD}_{\text{sample}} / \text{OD}_{\text{negative control}}) \times 100$. Based on this ratio, the results were interpreted as follows: samples with an S/N% of $\leq 50\%$ were considered positive, indicating the presence of PPRV antibodies; samples with an S/N% between 50% and 60% were classified as inconclusive, and a retest was recommended; samples with an S/N% $> 60\%$ were deemed negative, indicating no detectable antibodies against PPRV.

DATA COLLECTION ON RISK FACTORS AND FARMER AWARENESS

A structured questionnaire survey was administered to 200 small ruminant farmers to gather comprehensive information on various aspects related to PPR control. The survey covered five key areas: demographics, including location, herd size, and primary livestock species; management practices, such as the type of grazing system used (free-range or stall-fed) and whether herds were

mixed with other animals; disease history and vaccination status, focusing on any previous PPR outbreaks and the frequency of vaccination (regular, irregular, or never); biosecurity and animal movement, including the frequency of animal trade or market visits and the use of shared grazing lands or water sources; and farmer awareness of PPR, assessing their knowledge of the disease’s symptoms, modes of transmission, and prevention measures.

STATISTICAL ANALYSIS

Data analysis was performed using SPSS v26.0 (IBM, USA). Descriptive statistics were used to calculate prevalence rates with 95% confidence intervals (CI). The Chi-square (χ^2) test was used to assess the association between seropositivity and potential risk factors (species, age, herd size, and management). Predictors with $P < 0.05$ were considered statistically significant indicators of PPRV exposure.

RESULTS

SEROPREVALENCE OF PPRV ANTIBODIES

The data presented in Figure 2 show the seroprevalence of PPRV in small ruminants from District Quetta. Out of 200 samples tested, 72 (36%) were positive for PPRV-specific antibodies, indicating prior exposure to the virus, while 82 (41%) were negative and 46 (23%) were initially classified as inconclusive. However, according to the standardized protocol, inconclusive samples were retested and subsequently confirmed as negative; therefore, they were excluded from the risk factor analysis.

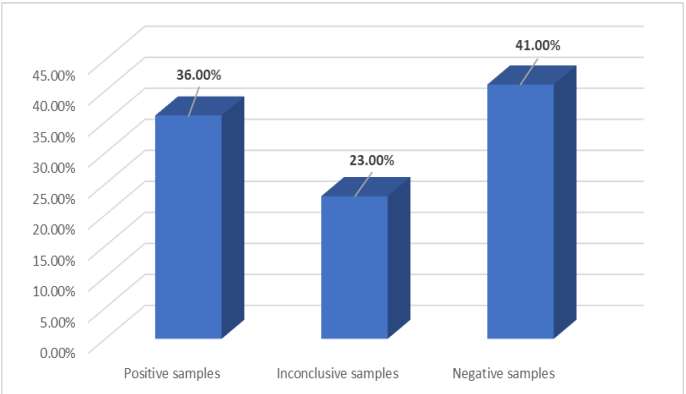


Figure 2: The percentage of positive, inconclusive and negative sera samples determined based on cELISA.

RISK FACTOR ANALYSIS

Table 1 analyzes the association of various risk factors with the seroprevalence of PPRV in small ruminants. The table categorizes risk factors such as species, age, sex, herd size, and management practices, along with the number of samples, percentage of positive cases, and p-values. Goats showed a slightly higher seroprevalence (37.5%) compared to sheep (33.75%), though the difference was

not statistically significant ($P > 0.05$). Animals aged 1-3 years had the highest seroprevalence (40%), followed by those over 3 years (36%) and less than 1 year (30%). Males and females exhibited equal seroprevalence (36%). Larger herds (>100 animals) had a higher seroprevalence (42%) compared to smaller herds (<50 animals, 30%). Free-grazing animals showed significantly ($P < 0.05$) higher seroprevalence (41.54%) than stall-fed animals (25.71%). The statistical analysis further revealed that there were a significant association ($P < 0.05$) between seroprevalence and age, and herd size of small ruminants studied. These findings highlight the influence of risk factors on PPR seroprevalence and underscore the need for targeted interventions to reduce disease transmission.

Table 1: Association of risk factors with PPR seroprevalence.

Risk factor	Category	No. of samples	Positive (%)	P value
Species	Goats	120	45 (37.5%)	0.08
	Sheep	80	27 (33.75%)	
Age	<1 year	60	18 (30%)	0.034
	1-3 years	90	36 (40%)	
	>3 years	50	18 (36%)	
Sex	Male	100	36 (36%)	0.512
	Female	100	36 (36%)	
Herd size	Small (<50)	80	24 (30%)	0.023
	Medium (50-100)	70	27 (38.57%)	
	Large (>100)	50	21 (42%)	
Management	Free-grazing	130	54 (41.54%)	0.012
	Stall-fed	70	18 (25.71%)	

PPR AWARENESS AMONG FARMERS

Table 2 summarizes the awareness and risk factors related to Peste des Petits Ruminants among small ruminant farmers in the study area. The table categorizes parameters such as awareness of PPR, vaccination practices, animal movement, and sharing of grazing areas, along with the number of farmers, percentages, and p-values. Approximately 60% of farmers were aware of PPR, while 40% lacked awareness. Vaccination practices varied, with only 25% of farmers vaccinating their animals regularly, 35% vaccinating irregularly, and 40% never vaccinating. Frequent movement of animals was reported by 45% of farmers, while 30% moved animals occasionally and 25% rarely. A significant majority (65%) shared grazing areas with other herds, increasing the risk of disease transmission. The statistical analysis revealed that there were a statistically significant association ($P < 0.05$) for awareness, vaccination practices, movement of animals and sharing of grazing areas.

DISCUSSION

The findings of this study provide a comprehensive

understanding of the seroprevalence of PPRV in small ruminants in district Quetta, Pakistan, along with the associated risk factors and farmer awareness levels. The results highlight the widespread exposure to PPRV in the region and underscore the urgent need for targeted interventions to control and mitigate the disease’s impact.

Table 2: Awareness and risk factors among farmers.

Parameter	Category	No. of farmers	Percentage (%)	p value
Awareness of PPR	Yes	120	60%	0.023
	No	80	40%	
Vaccination practices	Regular	50	25%	0.034
	Irregular	70	35%	
	Never	80	40%	
Movement of animals	Frequent	90	45%	0.019
	Occasional	60	30%	
	Rare	50	25%	
Sharing grazing areas	Yes	130	65%	0.012
	No	70	35%	

The seroprevalence results indicate that 36% of the tested samples were positive for PPRV-specific antibodies, suggesting significant exposure to the virus in the study area. This finding aligns with previous studies reporting high seroprevalence rates in small ruminants in Pakistan and other endemic regions (Abubakar *et al.*, 2017; Nkamwesiga *et al.*, 2023; Selim *et al.*, 2025; Zafar *et al.*, 2024). The presence of a substantial proportion of inconclusive results (23%) further emphasizes the need for confirmatory diagnostic tests and highlights the challenges in accurately assessing PPRV exposure. The statistically significant differences in seroprevalence ($p < 0.05$) across samples underscore the variability in virus exposure and the importance of region-specific control strategies.

Recent studies have also highlighted the increasing prevalence of PPR in South Asia, particularly in regions with high livestock density and poor vaccination coverage (Munibullah *et al.*, 2024; Rehman *et al.*, 2025). The high seroprevalence observed in this study is consistent with these trends, emphasizing the need for enhanced surveillance and vaccination programs in endemic areas. Similarly, Munibullah *et al.* (2024) reported a seroprevalence of 40.2% in northern Pakistan, further corroborating the high prevalence of PPRV in the region.

The variability in seroprevalence across different regions can be attributed to factors such as differences in vaccination coverage, animal husbandry practices, and the movement of animals. For example, regions with higher vaccination rates tend to have lower seroprevalence, while areas with poor vaccination coverage and frequent animal movement

exhibit higher seroprevalence (Khalafalla *et al.*, 2021). These findings highlight the importance of implementing region-specific control measures to address the unique challenges posed by PPR in different areas.

The analysis of risk factors reveals several key associations with PPR seroprevalence. Goats exhibited a slightly higher seroprevalence (37.5%) compared to sheep (33.75%), consistent with previous findings that goats are more susceptible to PPRV infection (Balamurugan *et al.*, 2012). This higher susceptibility may be due to differences in immune responses between goats and sheep, as well as variations in management practices. For instance, goats are often kept in larger herds and are more likely to be free-grazing, which increases their exposure to the virus (Kabir *et al.*, 2019).

Age was also a significant factor, with animals aged 1-3 years showing the highest seroprevalence (40%), likely due to increased exposure during grazing and movement. Younger animals (<1 year) had a lower seroprevalence (30%), which may be attributed to maternal immunity acquired through colostrum. However, this immunity wanes over time, making older animals more susceptible to infection (Munir *et al.*, 2020). Animals over 3 years had a seroprevalence of 36%, suggesting that repeated exposure to the virus may contribute to the development of immunity in older animals.

Larger herd sizes (>100 animals) and free-grazing management practices were associated with higher seroprevalence rates (42% and 41.54%, respectively), reflecting the role of high-density populations and shared grazing areas in facilitating virus transmission (Kabir *et al.*, 2019). These findings are consistent with previous studies that have identified herd size and management practices as significant risk factors for PPR transmission (Khalafalla *et al.*, 2021; Munibullah *et al.*, 2024). For example, a study by Khalafalla *et al.* (2021) in Sudan found that herds with more than 100 animals had a significantly higher risk of PPRV infection compared to smaller herds. Similarly, free-grazing animals were found to be at a higher risk of infection due to increased contact with infected animals and contaminated environments.

The findings of this study highlight the need for improved herd management practices, including controlled grazing, reduced herd density, and restricted animal movement, to minimize the risk of PPRV spread. Additionally, targeted vaccination programs should be implemented in high-risk areas to reduce virus transmission and build herd immunity.

The assessment of farmer awareness and practices reveals critical gaps in knowledge and vaccination coverage. Only 60% of farmers were aware of PPR, and a mere

25% reported regular vaccination of their animals. This low vaccination rate is concerning, given the high seroprevalence of PPRV in the region. Frequent animal movement and shared grazing areas were reported by 45% and 65% of farmers, respectively, further exacerbating the risk of disease transmission (Khalafalla *et al.*, 2021). These findings underscore the urgent need for educational campaigns to raise awareness about PPR and its control, as well as improved access to vaccination programs.

Recent studies have highlighted the importance of farmer education and community engagement in controlling PPR. For example, Rehman *et al.* (2025) found that targeted awareness campaigns significantly improved vaccination rates and reduced disease incidence in endemic regions. The findings of this study align with these recommendations, highlighting the need for comprehensive educational and vaccination initiatives in district Quetta and surrounding areas.

The low vaccination coverage observed in this study is consistent with previous reports from Pakistan and other endemic regions. For instance, Abubakar *et al.* (2017) reported that only 20–30% of small ruminants in Pakistan are vaccinated against PPR, despite the availability of effective vaccines. This low coverage can be attributed to factors such as limited access to veterinary services, lack of awareness about the disease, and inadequate vaccine distribution networks. Addressing these challenges will require a coordinated effort involving government agencies, veterinary authorities, and local communities (Chukwudi *et al.*, 2020).

The results of this study have important implications for PPR control in Pakistan and similar endemic regions. The high seroprevalence of PPRV, combined with the identified risk factors, underscores the urgent need for a multifaceted approach to disease management. Key strategies should include enhanced vaccination programs, ensuring regular and widespread immunization of small ruminants, especially in high-risk areas, to reduce virus transmission and establish herd immunity. Farmer education is also critical, and targeted awareness campaigns should be conducted to inform farmers about PPR, its modes of transmission, and the importance of vaccination and biosecurity practices. Additionally, improved herd management through practices such as controlled grazing, reduced herd density, and restricted animal movement can significantly lower the risk of PPRV spread. Finally, strengthened diagnostic capacity is essential, and increasing access to reliable diagnostic tools, such as competitive ELISA (cELISA), will support accurate disease surveillance and timely intervention (Njeumi *et al.*, 2025; Imanbayeva *et al.*, 2025).

This study has certain limitations that should be acknowledged. The cross-sectional design provides a snapshot of PPRV seroprevalence at a single point in time, which limits the ability to assess temporal trends or causality. Additionally, the use of a convenient sampling strategy may introduce selection bias and limit the generalizability of the findings to the broader population of small ruminants in the region.

CONCLUSIONS

The study revealed a seroprevalence of 36%, with goats showing higher exposure than sheep. Key risk factors included larger herd sizes, free-grazing practices, and frequent animal movement. Farmer awareness regarding the PPR was limited, with only 25% regularly vaccinating their animals. The study exhibited that PPR is a significant threat to small ruminant production in district Quetta and surrounding areas, with high seroprevalence rates and multiple risk factors contributing to disease transmission. The lack of farmer awareness and low vaccination coverage further exacerbate the problem. The findings highlight the widespread exposure to PPRV in the region and the urgent need for improved control measures. Addressing these challenges requires a multifaceted approach, including enhanced vaccination programs, farmer education, improved herd management practices, and strengthened diagnostic capacity. Effective control of PPR is essential to safeguard the livelihoods of small ruminant farmers and ensure food security in the region.

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NOVELTY STATEMENT

To the best of authors knowledge, this is first comprehensive study from the Quetta, the capital of Balochistan province of Pakistan, that have reported the PPR infection in sheep and goat population. The study also reported the PPR-associated risk factors and farmers' awareness in the study area.

AUTHOR'S CONTRIBUTION

SZ performed the experiments for data collection; AAK and MS hypothesized the study design and supervised the project; ZSA, SK and SZK helped in sample collection, analysis and interpretation of results; AAK and RAL wrote the paper and FS helped in editing.

GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

During the preparation of this manuscript, the author(s) used ChatGPT to paraphrase the initial draft in order to reduce the similarity index and enhance the language and readability of the text. Following the use of this tool, the author(s) thoroughly reviewed and edited the content as necessary and take full responsibility for the final version of the manuscript.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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