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RESEARCH ARTICLE

Seroprevalence and Risk Factor Analysis of Peste des Petits Ruminants (PPR) in Small Ruminants of South-Western Balochistan, Pakistan

Zubaria Shahid Amin[1*], Muhammad Shafee[1*], Asadullah[1], Zeeshan Iqbal[2], Shafiq Ahmad[3,] Mohsin Raza[2], Babar Hilal Ahmad Abbassi[1], and Abid Hussain Mastoi[3]

[1]CASVAB, University of Balochistan, Brewery Road Quetta, Pakistan;[2] KBCMA College of Veterinary & Animal Sciences, Narowal Sub-Campus, Pakistan; [3]Balochistan Agriculture Research & Development Center, (BARDC), Brewery Road, Quetta Pakistan
*Corresponding author: shafeedr73@gmail.com; zubaria.qta@gmail.com

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ABSTRACT

Peste des Petits Ruminants (PPR) is a highly contagious viral disease of small ruminants, responsible for significant economic losses in livestock-dependent communities worldwide. This study investigated the seroprevalence of PPR virus (PPRV) antibodies in sheep and goats from Mastung, Sikandar Abad (Surab), and Kech (Turbat) districts of Balochistan, Pakistan, while also identifying risk factors associated with disease transmission. A cross-sectional survey was conducted using 450 serum samples (270 goats, 180 sheep) collected from 30 herds across the three districts. Samples were analyzed through competitive enzyme-linked immunosorbent assay (c-ELISA) to detect PPRV-specific antibodies. Additionally, a structured questionnaire gathered data on herd size, species, age, tick infestation, and grazing practices. Logistic regression was applied to determine associations between these factors and seropositivity. The overall seroprevalence was 46% (206/450), with goats showing higher prevalence (50.3%; 136/270) compared to sheep (39%; 70/180). District-wise prevalence was 41% in Mastung, 45% in Sikandar Abad, and 52% in Kech. Risk factor analysis revealed significant associations with species ($P=0.002$), tick infestation ($P=0.018$), age ≥1 year ($P<0.001$), and larger herd size (≥50 animals; $P<0.0001$). Grazing style (open vs. stall-fed) was not statistically significant ($P=0.074$). These findings highlight moderate to high circulation of PPRV in southwestern Balochistan, with notable variation across districts and management practices. The study provides essential baseline data to guide evidence-based interventions, including targeted vaccination, improved husbandry, and strategic planning for progressive control and eventual eradication of PPR in the region.

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INTRODUCTION

Peste des Petits Ruminants (PPR) is a highly contagious and economically important transboundary viral disease of small ruminants recognized by the World Organisation for Animal Health (WOAH) (Kabir *et al.*, 2025; Alamerew *et al.,* 2025). The disease primarily affects sheep and goats and is characterized by severe clinical manifestations, high morbidity, and considerable mortality, resulting in substantial economic losses to livestock-dependent communities worldwide (Rahman *et al.,* 2022). PPR has emerged as a major constraint to small ruminant production, particularly in developing countries where livestock plays a critical role in food security, rural livelihoods, and poverty alleviation (Tella *et al.,* 2022).

The causative agent, Peste des Petits Ruminants virus (PPRV), is an enveloped, single-stranded negative-sense RNA virus belonging to the genus *Morbillivirus* within the family *Paramyxoviridae* (Kabir *et al*., 2020; Wassif *et al*.,2024). Based on genetic characterization of the fusion (F) gene, PPRV is classified into four distinct genetic lineages (I–IV). Among these, Lineage IV has become the most widespread and dominant lineage globally, circulating extensively across Asia, the Middle East, and large parts of Africa (Tounkara *et al.,* 2019; Clemons *et al.,* 2021). In Pakistan, Lineage IV is predominantly responsible for outbreaks across all provinces (Kabir *et al*., 2025). Phylogenetic studies further indicate that Pakistani isolates cluster into two subgroups, IVa and IVb, suggesting continuous viral evolution and regional circulation patterns (Zahur *et al.,* 2008; Mehmood *et al.,* 2009; Kamal *et al.,* 2024).

Transmission of PPRV occurs mainly through direct contact between infected and susceptible animals via respiratory secretions, saliva, nasal discharge, ocular exudates, and contaminated materials, including urine and feces (Kutumbetov *et al*., 2025). The incubation period typically ranges from 2 to 8 days, followed by the onset of fever, depression, nasal and ocular discharge, anorexia, erosive stomatitis, pneumonia, and severe diarrhea (Ahmed *et al.,* 2021). The severity and outcome of infection are influenced by several host and environmental factors, including animal age, breed susceptibility, immune status, climatic conditions, humidity, and vector burden (Esonu *et al.,* 2022). These interacting factors contribute to variations in disease expression and epidemiological patterns across different geographical regions.

The economic consequences of PPR are substantial. In Pakistan alone, annual economic losses attributed to the disease are estimated to exceed 20 billion PKR due to mortality, reduced milk and meat production, treatment costs, and restrictions on livestock trade (Abubakar *et al.,* 2023). Recent nationwide investigations have reported an overall seroprevalence of approximately 51%, with regional variations including Sindh (61%), Khyber Pakhtunkhwa (44%), Punjab (54%), Gilgit Baltistan (51%), and Balochistan (53%) (Zafar *et al.,* 2024). These findings highlight the endemic nature of PPR and emphasize the need for region-specific surveillance and control strategies. (Imanbayeva *et al.,* 2025).

Recognizing the global importance of the disease, the Food and Agriculture Organization (FAO) and the World Organisation for Animal Health (WOAH) have jointly launched a global eradication program targeting the elimination of PPR by 2030 (Benfield *et al*., 2023). The program follows a progressive four-stage approach consisting of assessment, control, eradication, and post-eradication monitoring (Fathelrahman *et al.,* 2021). Despite these coordinated efforts, several regions, particularly those with extensive animal movement and limited veterinary infrastructure, continue to experience persistent viral circulation. Previous studies in Pakistan have indicated comparatively higher prevalence rates in Balochistan compared with other provinces (Ahmad *et al.,* 2005; Khan *et al.,* 2007).

Balochistan is the largest province of Pakistan, covering approximately 44% of the country's total land area. The province contributes significantly to the national livestock sector, harboring nearly 48% of the country's sheep population and 22% of goats. Livestock production forms the backbone of rural livelihoods, contributing nearly 70% to the rural economy of the province. However, the extensive pastoral production system, nomadic animal movement, and limited access to veterinary services increase vulnerability to transboundary animal diseases such as PPR.

The selected districts Mastung, Sikandar Abad (Surab), and Kech (Turbat) represent diverse ecological conditions and livestock production systems within Balochistan. Mastung and Surab experience relatively colder climates that support mixed sheep and goat rearing, whereas Kech district faces extreme temperatures reaching up to 50°C, favoring goat production adapted to arid environments. Additionally, Kech shares an international border with Iran, facilitating frequent cross-border animal movement that may enhance the risk of transboundary disease transmission. Although PPR prevalence has been widely reported across Pakistan, epidemiological data from Balochistan remain limited despite its large and small ruminant population and strategic geographic importance. Previous studies indicate prevalence exceeding 50%, yet comprehensive risk factor–based investigations in south-western Balochistan remain scarce. Therefore, this study aimed to determine PPRV seroprevalence and associated risk factors to generate baseline epidemiological evidence supporting surveillance, vaccination planning, and effective disease control strategies.

MATERIALS AND METHODS

Study Area: The present cross-sectional study was conducted in three districts of south-western Balochistan, Pakistan, namely Mastung (29.80°N, 66.85°E), Sikandar Abad (Surab; 29.18°N, 67.05°E), and Kech (Turbat; 26.00°N, 63.05°E). These districts were selected due to their high density of small ruminant populations and proximity to the Pakistan–Iran border, which facilitates transboundary animal movement and may increase the risk of disease transmission. Mastung and Sikandar Abad are characterized by relatively colder climatic conditions favoring sheep rearing, whereas, Kech district experiences extremely high temperatures (up to 50°C during summer) and predominantly supports goat production systems than sheep population. The geographical locations of the study areas are illustrated in Fig. 1.

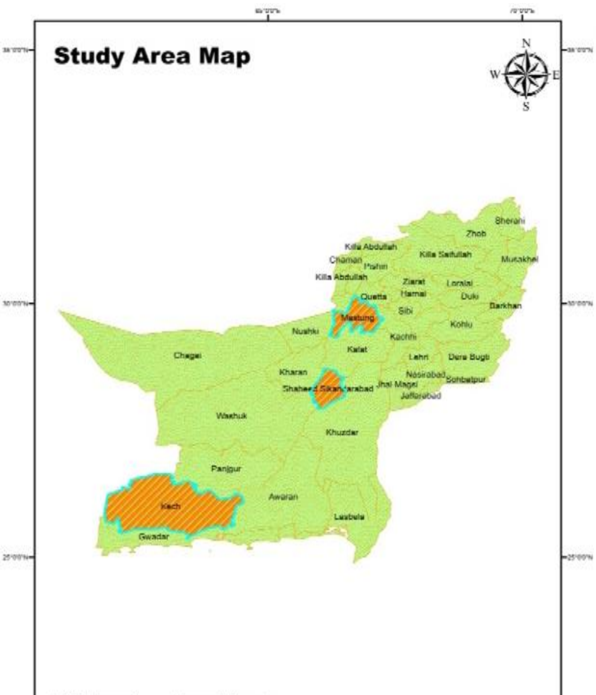

Fig. 1: Map of Balochistan showing the study area of Mastung, Sikandar abad (Surab), and Kech (Turbat).

Study design and sample collection: A cross-sectional study design was adopted. A total of 450 blood samples were collected from apparently healthy small ruminants, including goats (n=270) and sheep (n=180), from 30 different herds across the three districts. Each district contributed 150 samples (Table 1). Animals older than six months of age with no recent history of vaccination against PPR were included in the study. Blood samples were aseptically collected from the jugular vein using sterile vacutainer tubes. Serum was separated by centrifugation at 3000 rpm for 10 minutes and transported under cold chain conditions to the Pathobiology Laboratory, Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, for further analysis (Fig. 2).

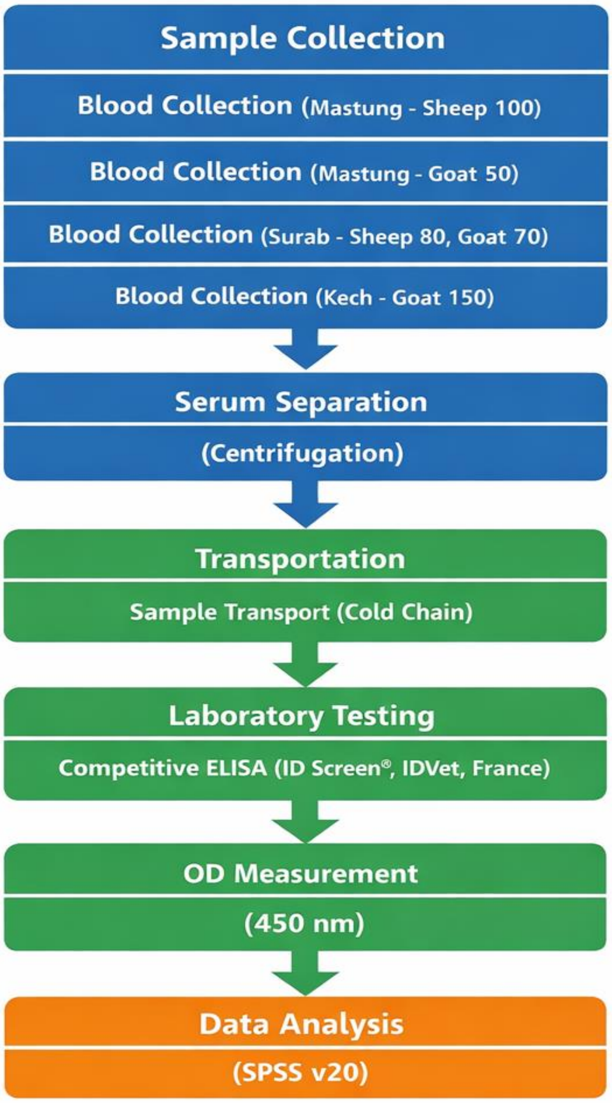

Fig. 2: Sample collection and laboratory workflow for PPR antibody detection in small ruminants.

Table 1: Distribution of blood samples collected from sheep and goats across three districts of Balochistan, Pakistan

District	Sheep (n)	Goat (n)	Total (n)
Mastung	100	50	150
Sikandar Abad (Surab)	80	70	150
Kech (Turbat)	0	150	150
Total	180	270	450

All animals were clinically healthy and >6 months of age with no history of PPR vaccination.

Serological analysis using competitive ELISA (c-ELISA): Detection of antibodies against PPR virus was performed using a commercially available competitive enzyme-linked immunosorbent assay (c-ELISA) kit (ID Screen® PPR Competition ELISA, IDvet, France) following the manufacturer's instructions.

Briefly, serum samples were diluted at a ratio of 1:5 using the supplied sample diluent. Approximately 100µL of diluted serum samples were added to antigen-coated 96-well microplates along with positive and negative controls. Plates were incubated at 37°C for 45 minutes and subsequently washed three times with phosphate-buffered saline containing Tween-20 (PBS-T). Thereafter, 100µL of horseradish peroxidase (HRP)-conjugated monoclonal antibody was added and incubated at 37°C for 30 minutes.

Following washing steps, 100µL of tetramethylbenzidine (TMB) substrate was added and incubated for 15 minutes at room temperature in the dark. The reaction was stopped by adding 50µL of 1N sulfuric acid (H_2SO_4), and optical density (OD) values were measured at 450nm using a microplate ELISA reader (BioTek Instruments, USA).

The Sample-to-Negative (S/N%) ratio was calculated using the formula:

S/N% = (OD sample / OD negative control) × 100: Samples with S/N% ≤ 50% were considered positive for PPRV antibodies, whereas samples with S/N% > 60% were considered negative. Samples with S/N% between 50 and 60% were considered doubtful and retested.

Assessment of risk factors: Information regarding potential risk factors associated with PPR infection was collected using a pre-designed structured questionnaire administered to livestock farmers. Variables included animal species (sheep vs. goat), age (<1 year vs. ≥1 year), herd size (<50 vs. ≥50 animals), grazing management system (open grazing vs. stall-fed), tick infestation status (present vs. absent), vaccination history (vaccinated vs. unvaccinated), farmer awareness regarding PPR (aware vs. unaware), animal health status (healthy vs. previously sick), and access to veterinary healthcare services (accessible vs. not accessible).

Ethical approval: All procedures involving animals were conducted following approval from the Ethical Review Committee of the Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB), University of Balochistan, Pakistan.

Statistical analysis: Data were entered and analyzed using Statistical Package for Social Sciences (SPSS) version 20 (IBM Corp., Armonk, NY, USA). Seroprevalence was calculated as the proportion of seropositive animals among tested samples with 95% confidence intervals. The Chi-square (χ^2) test was applied to evaluate associations between seropositivity and investigated risk factors. Variables with P<0.25 in univariable analysis were included in the multivariable logistic regression model to identify independent risk factors. Odds ratios (OR) and 95% confidence intervals were calculated, and differences were considered statistically significant at P<0.05.

RESULTS

Seroprevalence of PPR antibodies in study areas: A total of 450 serum samples collected from small ruminants (goats=270; sheep=180) across three districts of south-western Balochistan were analyzed using competitive ELISA for detection of antibodies against PPRV. Overall, 206 out of 450 samples tested positive, indicating an overall seroprevalence of 45.8% (95% CI: 41.2-50.4%).

District-wise seroprevalence revealed variation among study areas (Table 2). The highest seroprevalence was recorded in Kech (Turbat) district (52.0%, 78/150), followed by Sikandar Abad (Surab) (44.7%, 67/150), while the lowest prevalence was observed in Mastung (40.7%, 61/150).

Table 2: District-wise seroprevalence of PPR antibodies in small ruminants of Balochistan, Pakistan

District	Total Samples	Positive (n)	Seroprevalence (%)	95% CI
Mastung	150	61	40.7	32.9-48.9
Sikandar Abad (Surab)	150	67	44.7	36.7-52.9
Kech (Turbat)	150	78	52.0	43.9-60.0
Total	450	206	45.8	41.2-50.4

Species-wise seroprevalence: Species-specific analysis demonstrated variation in antibody prevalence between goats and sheep (Table 3). Goats showed comparatively higher seropositivity (50.4%, 136/270) than sheep (38.9%, 70/180). The findings indicate greater exposure of goats to PPRV infection across the studied regions.

Table 3: Species-wise seroprevalence of PPR antibodies in small ruminants

Species	Total Samples	Positive (n)	Seroprevalence (%)	95% CI
Goats	270	136	50.4	44.3-56.4
Sheep	180	70	38.9	31.9-46.3
Total	450	206	45.8	41.2-50.4

District-wise distribution of seropositive animals: In Mastung district, 61 out of 150 samples were seropositive, with higher prevalence observed in goats (52.0%, 26/50) compared to sheep (35.0%, 35/100). Similarly, in Sikandar Abad (Surab), overall seroprevalence reached 44.7%, where goats (45.7%, 32/70) and sheep (43.8%, 35/80) exhibited nearly comparable infection levels. In Kech district, only goats were sampled, showing a seroprevalence of 52.0% (78/150). Spatial distribution of seropositivity across districts is illustrated using a heat-wave model (Fig. 3), whereas network visualization of sampling sites and positive cases (Fig. 4).

Association of risk factors with PPR seropositivity: The association between investigated risk factors and PPR seropositivity is summarized in Table 4. Species showed a significant association with disease prevalence (P=0.0168), with goats demonstrating higher seropositivity compared to sheep.

Herd size was significantly associated with PPR occurrence (P=0.000047), where herds containing more than 50 animals exhibited higher prevalence compared to smaller herds. Age of animals also showed significant association (P=0.022), indicating higher seropositivity among animals older than one year.

Table 4: Univariable analysis of risk factors associated with PPR seropositivity in small ruminants of Balochistan, Pakistan

Risk Factor	Category	Total Samples	Positive (n)	Positive (%)	P-value	Inter-pretation
Species	Goat	270	136	50.4	0.0168	Significant
	Sheep	180	70	38.9		
Herd Size	1–50 animals	300	110	36.7	0.000047	Significant
	>50 animals	150	96	64.0		
Age	≤1 year	150	50	33.3	0.022	Significant
	>1 year	300	156	52.0		
Tick Infestation	Present	48	30	62.5	0.018	Significant
	Absent	402	176	43.8		
Grazing Style	Free grazing	400	185	46.3	0.074	Non-significant
	Stall feeding	50	16	32.0		

Similarly, tick infestation was significantly associated with PPR antibody detection (P=0.018). In contrast, grazing management system (free grazing versus stall feeding) did not show a statistically significant association with seropositivity (P=0.074).

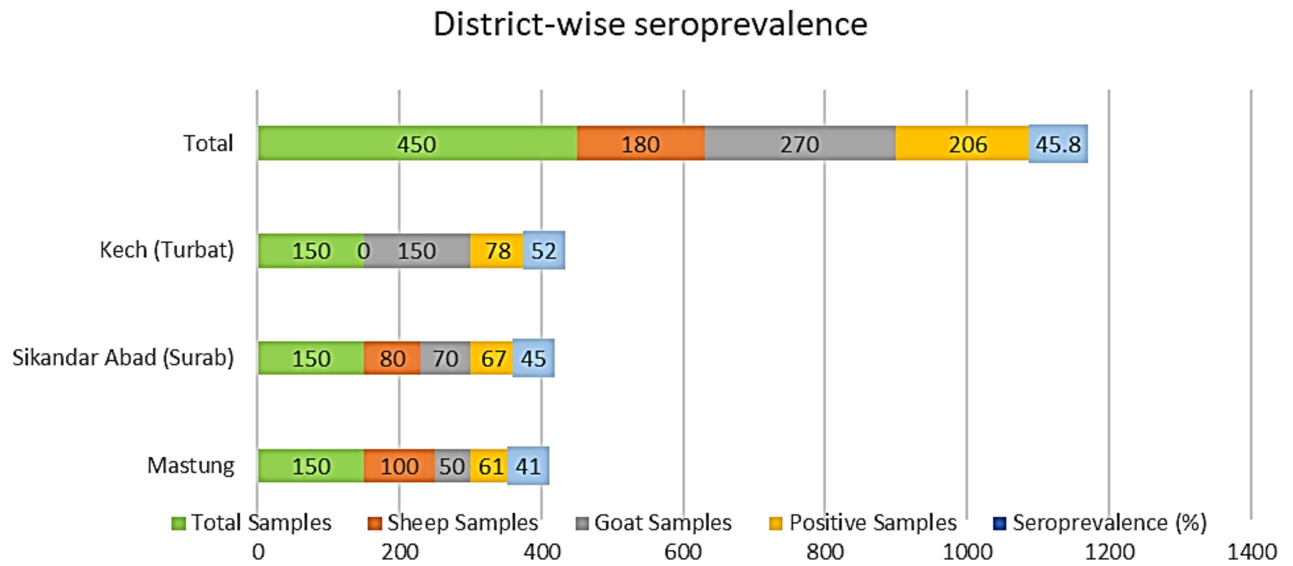

Fig. 3: District-wise seroprevalence of PPR antibodies in small ruminants of Mastung, Sikandar Abad (Surab) and Kech districts of Balochistan, Pakistan.

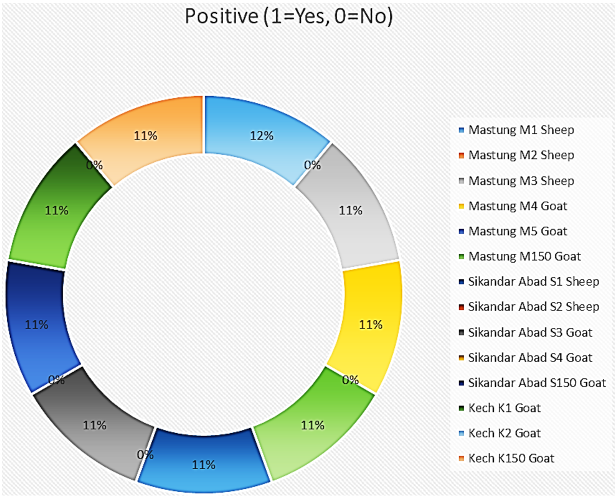

Fig. 4: Distribution of seropositive and negative small ruminant samples against PPR virus across Mastung, Sikandar Abad (Surab), and Kech districts of Balochistan, Pakistan using Sunken-model representation.

Multivariable analysis of independent risk factors for PPR seropositivity: Multivariable logistic regression analysis was performed to identify independent risk factors for PPR seropositivity after adjusting for potential confounders (Table 5). The results confirmed that herd size >50 animals remained the strongest independent risk factor (AOR = 3.12; 95% CI: 2.01–4.85; P<0.001), followed by age >1 year (AOR = 2.18; 95% CI: 1.42–3.34; P<0.001), tick infestation (AOR = 2.10; 95% CI: 1.12–3.94; P=0.018) and goat species (AOR = 1.62; 95% CI: 1.09–2.41; P=0.016). Grazing style remained non-significant in the multivariable model (AOR = 1.78; 95% CI: 0.94–3.36; P=0.074).

Table 5: Multivariable logistic regression analysis of independent risk factors for PPR seropositivity in small ruminants of Balochistan, Pakistan

Risk Factor	Category	Adjusted Odds Ratio (AOR)	95% Confidence Interval	P-value	Significance
Species	Goat (vs. Sheep)	1.62	1.09–2.41	0.016	Significant
Herd Size	>50 animals (vs. 1–50)	3.12	2.01–4.85	<0.001	Significant
Age	>1 year (vs. ≤1 year)	2.18	1.42–3.34	<0.001	Significant
Tick Infestation	Present (vs. Absent)	2.10	1.12–3.94	0.018	Significant
Grazing Style	Free grazing (vs. Stall feeding)	1.78	0.94–3.36	0.074	Non-significant

Note: Hosmer-Lemeshow goodness-of-fit test p-value = 0.324, indicating good model fit

DISCUSSION

The present study provides a comprehensive analysis of the seroprevalence of PPR and associated risk factors in small ruminants across three ecologically distinct districts of Balochistan, Pakistan: Mastung, Sikandar Abad (Surab), and Kech (Turbat). The findings demonstrate substantial viral circulation with an overall seroprevalence of 45.8%, confirming that PPR remains highly endemic in southwestern Balochistan and poses a significant threat to livestock productivity, rural livelihoods, and food security in the region. These results have important implications for the design and implementation of targeted control strategies aligned with the Global PPR Eradication Programme (GEP) target of 2030 (Kabir *et al*., 2025).

The observed overall seroprevalence of 45.8% (206/450) is consistent with previous estimates from Balochistan and other regions of Pakistan. Zafar *et al*. (2024), in a comprehensive meta-analysis synthesizing data from 2004 to 2023, reported a pooled provincial prevalence of 53% for Balochistan, closely matching our findings. Similarly, Zain *et al*. (2026) documented 36% seroprevalence in small ruminants from Quetta district, while Abubakar *et al*. (2017) reported 38.2% seroprevalence in Balochistan based on a large-scale national serosurvey. The slightly lower prevalence in some earlier studies may reflect differences in sampling periods, study locations, diagnostic methods, or temporal variation in disease transmission dynamics. The consistent detection of moderate-to-high seroprevalence across multiple studies spanning nearly two decades confirms that PPR is firmly entrenched in Balochistan's small ruminant populations.

Comparisons with other provinces reveal that Balochistan's seroprevalence (45.8%) is comparable to Punjab (54%), slightly lower than Sindh (61%), but higher than Khyber Pakhtunkhwa (44%) and Gilgit Baltistan (51%) (Naseem *et al*., 2022; Zafar *et al*., 2024). This geographic variation underscores the heterogeneous distribution of PPR risk across Pakistan and highlights the need for province-specific and even district-specific control strategies rather than a uniform national approach.

District-wise variation in seroprevalence. The distinct variation in seroprevalence across the three study districts—ranging from 40.7% in Mastung to 52.0% in Kech—warrants careful interpretation. The highest seroprevalence in Kech district (52.0%) is particularly noteworthy given its geographical location. Kech shares an approximately 900-kilometer border with Iran, facilitating uncontrolled cross-border animal movements, informal trade, and potential introduction of infected animals from neighboring regions (Medley *et al*., 2021; Munibullah *et al*., 2024). Similar border-associated risks have been documented in other settings; for instance, studies along the Pakistan-Afghanistan border have reported elevated PPR seroprevalence attributed to transboundary animal movement (Kabir *et al*., 2024; Munibullah *et al*., 2024). The porous nature of these borders, combined with limited veterinary surveillance and control infrastructure, created conditions conducive to sustained virus circulation and periodic reintroduction.

The extreme arid climate of Kech, with summer temperatures exceeding 50°C, and higher goat population (0.68 million) than sheep (0.086 Million) in the area may also influence disease dynamics. While high temperatures can reduce environmental viral survival, the concentration of animals around limited water and grazing resources may increase contact rates and facilitate transmission. Additionally, Kech's predominant goat population (100% of sampled animals) may contribute to the higher observed prevalence, given the species susceptibility.

In contrast, the lower seroprevalence in Mastung (40.7%) and Sikandar Abad (44.7%) may reflect different production systems, cooler climates, lower animal densities, or potentially better vaccination coverage, although vaccination history was reportedly absent in sampled animals. These districts' mixed sheep-goat populations and relatively less intensive transboundary movement may reduce introduction risk.

Species susceptibility: Goats at higher risk. The significantly higher seroprevalence in goats (50.4%) compared to sheep (38.9%) (P=0.0168) aligns with a substantial body of evidence demonstrating that goats are more susceptible to PPRV infection and clinical disease. This species difference has been consistently reported across diverse geographical settings, including Pakistan (Abubakar *et al*., 2017; Zafar *et al*., 2024), India (Balamurugan *et al*., 2012), Sudan (Khalafalla *et al*., 2021) and Nigeria (Esonu *et al*., 2022).

Several biological and management factors may explain this differential susceptibility. First, goats may have inherent genetic or immunological differences that render them more permissive to PPRV infection and replication. Experimental infection studies have demonstrated that goats develop more severe clinical signs, higher viral loads, and prolonged shedding compared to sheep (Kumar *et al*., 2014; Enchery *et al*., 2019; Esonu *et al*., 2022). Second, behavioral differences, including goats' greater curiosity, exploratory behavior, and tendency to roam, may increase their contact-probability with infected animals or contaminated environments. Third, management practices in the study region often involve goats being herded separately or allowed to browse more extensively, potentially exposing them to diverse sources of infection. Fourth, cross-species protection from other morbillivirus infections, such as subclinical infection with related viruses in sheep, has been hypothesized but remains unproven.

The practical implication of this finding is clear: PPR control programs in Balochistan and similar ecological settings must prioritize goat populations for vaccination and surveillance. However, given that sheep can also maintain virus transmission, comprehensive vaccination of both species remains essential for herd immunity and eventual eradication.

Age as a determinant of seropositivity. The significant association between age ≥1 year and higher seroprevalence (P=0.022) reflects cumulative exposure to PPRV over time and is consistent with previous studies (Jones *et al*., 2016; Munibullah *et al*., 2024). Young animals (<1 year) may be protected by maternally derived antibodies during the first few months of life, which can persist for up to 3-6 months depending on colostrum intake and maternal antibody titers (Haq *et al*., 2016; Herzog *et al*., 2020). As maternal immunity wanes, young animals become susceptible to infection, and with continued environmental exposure, seropositivity accumulates with age.

The age-prevalence relationship has important implications for vaccination strategy. In endemic areas with continuous virus circulation, young animals should be targeted for vaccination after the decline of maternal immunity, typically around 4-6 months of age. Repeated vaccination campaigns at appropriate intervals are necessary to maintain population immunity and interrupt transmission cycles.

Herd size: A critical risk factor. The strong association between larger herd size (≥50 animals) and increased seroprevalence (P=0.000047) is one of the most striking findings of this study. Larger herds had nearly double the seroprevalence (64.0%) compared to smaller herds (36.7%). In multivariable analysis, herd size >50 animals remained the strongest independent risk factor (AOR = 3.12; 95% CI: 2.01–4.85; P<0.001). This finding aligns with studies from Pakistan (Kabir *et al*., 2019; Munibullah *et al*., 2024; Alvi *et al*., 2026), Sudan (Khalafalla *et al*., 2021) and Nigeria (Ullah *et al*., 2022), all reporting herd size as a consistent risk factor for PPR.

The biological plausibility is straightforward: larger herds have higher animal density, greater contact rates, more frequent introductions of new animals, and greater difficulty in implementing biosecurity measures and vaccination. Additionally, larger herds are more likely to be managed under extensive production systems with shared grazing lands, watering points, and marketing channels, all of which facilitate virus introduction and spread.

From a control perspective, this finding suggests that interventions should prioritize large herds, which serve as amplification hubs for virus transmission. Targeted vaccination campaigns focusing on large flocks could yield disproportionately high benefits by reducing overall viral load in the population and protecting surrounding smaller herds through herd immunity effects.

Tick infestation: An unexpected association. The significant association between tick infestation and PPR seropositivity (P=0.018) is intriguing and merits careful interpretation. Multivariable analysis confirmed tick infestation as an independent risk factor (AOR = 2.10; 95% CI: 1.12–3.94; P=0.018). Ticks are not biological vectors for PPRV, and mechanical transmission through tick mouthparts is theoretically possible but unlikely to be epidemiologically significant. Therefore, the observed association likely reflects confounding rather than causation.

Tick infestation may serve as a proxy for several underlying risk factors: (1) poor management practices and limited veterinary care, which also increase PPR risk; (2) extensive grazing systems where animals are exposed both to ticks and to infected animals from other herds; (3) compromised health and nutritional status, potentially increasing susceptibility to PPRV infection; and (4) lack of acaricide treatment, which may correlate with lack of vaccination.

Nevertheless, the possibility of mechanical transmission cannot be completely dismissed and warrants further investigation. Regardless of the mechanism, the association highlights that flocks with tick problems should be prioritized for integrated health interventions, including both vector control and vaccination.

Grazing practices: A non-significant finding. The lack of significant association between grazing style (open grazing vs. stall-feeding) and PPR seropositivity (P=0.074) was unexpected, given that open grazing is often assumed to increase contact with infected animals. Multivariable analysis confirmed that grazing style remained non-significant (AOR = 1.78; 95% CI: 0.94–3.36; P=0.074). Several explanations may account for this finding. First, the relatively small number of stall-fed animals in our sample (n=50) may have limited statistical power to detect a difference. Second, in the study region, even stall-fed animals may have contact with grazing animals during communal watering, marketing, or through introduction of new animals, blurring the distinction between management systems. Third, other factors such as herd size and animal movement may override the effect of grazing practice.

It is important not to interpret this non-significant finding as evidence that grazing management is irrelevant for PPR control. In other settings, controlled grazing and separation of herds have been shown to reduce disease transmission (Kock *et al.*, 2025). Rather, our results suggest that in the context of southwestern Balochistan, other risk factors may be more immediately actionable targets for intervention.

Implications for PPR control in Balochistan. The findings of this study have several important implications for PPR control strategy in Balochistan and similar endemic settings:

Targeted vaccination: The high overall seroprevalence confirms that PPR is endemic, and vaccination remains the cornerstone of control. Priority should be given to large herds (≥50 animals), goat populations, and districts with highest prevalence, particularly border areas like Kech. Vaccination campaigns should be conducted at optimal times, such as before the high-risk transmission seasons associated with animal movement and gathering.

Age-based strategies: Given higher seroprevalence in older animals and susceptibility of young animals after maternal immunity wanes, vaccination campaigns should ensure inclusion of replacement stock and young animals. Repeated campaigns at 6-12 months intervals may be necessary to achieve and maintain herd immunity.

Border surveillance: The elevated prevalence in Kech district underscores the need for enhanced surveillance along the Pakistan-Iran border. Cross-border coordination with Iranian veterinary services, establishment of buffer zones, and regulation of animal movement could help reduce introduction risk.

Integrated herd health: The association with tick infestation suggests that integrated approaches combining vaccination with parasite control, improved nutrition, and biosecurity measures may be more effective than vaccination alone.

Risk communication: Farmers should be educated about the importance of herd size management, avoiding mixing of herds, reporting suspicious cases, and participating in vaccination campaigns.

Contribution to global eradication goals. This study contributes essential baseline data to Pakistan's progressive control pathway toward the global PPR eradication target by 2030 (Roger *et al.*, 2021). The GEP's four-stage approach assessment, control, eradication and post-eradication—requires comprehensive epidemiological data at national and subnational levels. Our findings from previously understudied districts of Balochistan strengthen the assessment phase by identifying high-risk populations and areas requiring intensified control efforts. Continued surveillance, molecular characterization of circulating strains, and monitoring of vaccine coverage and effectiveness will be necessary as Pakistan progresses through subsequent stages toward eradication.

While this study provides valuable insights, several limitations should be acknowledged. First, the cross-sectional design captures seroprevalence at a single time point and cannot distinguish between recent and past infection or vaccination. Second, the absence of vaccination history, while reportedly negative, relied on farmer recall, which may be subject to recall bias. Third, the relatively small sample size within each district limited our ability to detect associations for some risk factors. Fourth, we did not perform virus isolation or molecular characterization to identify circulating lineages, which would inform vaccine matching and track transboundary movement.

Future studies should address these limitations through: (1) longitudinal designs to monitor seroconversion and antibody persistence; (2) larger sample sizes covering additional districts and ecological zones of Balochistan; (3) molecular characterization of PPRV strains to understand viral diversity and movement patterns; (4) evaluation of vaccine coverage and post-vaccination sero-monitoring and (5) investigation of small ruminant movement patterns, particularly across the Iran-Pakistan border, to inform risk-based surveillance.

Conclusions: This study demonstrates high seroprevalence (45.8%) of PPR in small ruminants across three districts of southwestern Balochistan, with significant variation by district, species, age, herd size, and tick infestation. The highest prevalence in Kech district highlights the importance of transboundary animal movements in PPR epidemiology. Goats, older animals, tick-infested flocks, and larger herds are at increased risk and should be prioritized for vaccination and improved management interventions. These findings provide essential baseline data to support Pakistan's progressive control pathway toward the global goal of PPR eradication by 2030. Targeted, evidence-based interventions informed by local epidemiological data are urgently needed to reduce disease burden, protect livelihoods, and contribute to regional and global eradication efforts.

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Authors Contribution: ZSA, MS conceived and designed the study; SA and AHM executed the field work and collected samples; ZSA, AS, BHAA and MS performed laboratory analyses and data compilation; ZI and MR analyzed the data and performed statistical analysis; All authors interpreted the data, critically revised the manuscript for important intellectual contents, and approved the final version.

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