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Dynamics of Seroprevalence and Seroconversion Patterns of Peste des Petits Ruminants Virus Following Vaccination in Goats and Sheep of Azad Jammu and Kashmir

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BS conceptualized the idea; manuscript writing and supervised the study. MB contributed to the sample collection, experimentation and performed statistical analysis. MIK involved in methodology settings and final approval of the version to be published.

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PPRV, Seroconversion, Dynamics, Goats, Sheep, AJK

ABSTRACT

Peste des petits ruminants (PPR) is a highly contagious and economically significant infection, of domestic small ruminants bringing morbidity and mortality up to 90%. This study was conducted from 2022-25 to evaluate the seroprevalence of PPRV in goats and sheep across all ten districts of Azad Jammu and Kashmir (AJK), Pakistan. A total of 2,018 blood samples (947 pre-vaccination and 1,071 post-vaccinations) were collected from sheep and goats and analyzed using competitive ELISA to detect PPRV-specific antibodies. Blood samples were collected from apparently healthy as well as suspected goats and sheep with common symptoms of PPR. The study aimed to assess regional differences in seropositivity before and after vaccination, along with evaluating species-, sex-, and age-wise variations. The overall seroprevalence increased significantly from 40.02% pre-vaccination to 62.09% post-vaccination, indicating a 22.07% rise in seroconversion($P<0.0001$). District-wise, the highest pre-vaccination seroprevalence was recorded in Mirpur (47.76%), while Haveli had the lowest (19.23%; $P<0.05$). Post-vaccination, Bhimber (77.88%), Muzaffarabad (74.50%), and Haveli (73.85%) showed the highest antibody levels. Substantial increases were observed in Haveli (+54.62%), Neelum (+37.41%), and Bhimber (+34.40%), while Poonch experienced a decline in post-vaccination seroprevalence (-10.99%), likely due to logistical constraints. Species-wise, sheep (48.71%) showed higher seroprevalence than goats (28.78%) in both phases. Females (33.22%) and older animals consistently exhibited higher antibody levels ($P<0.0001$). These findings underscore the need for district-specific strategies, continuous surveillance, and enhanced vaccination efforts to achieve PPR control and eventual eradication in AJK.

INTRODUCTION

Small ruminants, particularly goats and sheep, are integral to the rural economy of Azad Jammu and Kashmir (AJK), with the 2022 population census estimating their numbers at approximately 1.945 million and 0.249 million, respectively (AJK Bureau of Statistics, 2022). These animals serve as essential sources of livelihood, food security, and income for a large segment of the population. However, the rising outbreaks of infectious diseases pose a serious threat to this sector, particularly Peste des Petits Ruminants (PPR), a transboundary animal disease being one of the most serious viral diseases affecting economically goats and sheep herders. It is acute, highly contagious with symptoms of fever, respiratory distress, nasal and ocular discharges, oral lesions, and diarrhea. Morbidity and mortality rates can reach up to 100% and 90% respectively (Baron *et al*., 2016; Almeshay *et al*., 2017). PPR is endemic in many regions across Africa, the Middle East, and Asia, including Pakistan. It imposes major economic losses in areas heavily dependent on livestock production (Donduashvili *et al*., 2018; Omani *et al*., 2019).

The global efforts are underway to eradicate PPR by 2030, led by the Food and Agriculture Organization (FAO) and the World Organization for Animal Health (WOAH) through their Global PPR Eradication Program (FAO and WOAH, 2015). The regular vaccination is considered

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the one of the most effective available control strategies, particularly in areas where regulation of livestock movement is not robust to maintain herd immunity (Jones *et al*., 2016). Despite of fact that this disease is a severe threat to the livelihood generation of the local farming community, no prior vaccination campaigns or comprehensive surveillance studies have been conducted in AJK. Many outbreaks remain undocumented due to the absence of post-vaccination sero-surveillance and a weak disease reporting system. Therefore, the present study has been conducted to evaluate the baseline seroprevalence of PPRV in small ruminants (sheep and goats) across all ten districts of AJK, both pre- and post-vaccination, using competitive ELISA. The primary aim of this research was to assess the immunological response to the PPR PEST VAC K vaccine (Nigeria 75/1 strain) and determine seroconversion trends over time in different species, age groups, and sexes.

This study also seeks to provide district-wise sero-epidemiological data to support evidence-based decision-making in regional vaccination programs. This study represents the first comprehensive, large-scale assessment of PPRV antibody dynamics in AJK, offering novel insights into the geographic and demographic variability in seroconversion rates and vaccine coverage. The findings are expected to inform future vaccination strategies and contribute meaningfully to national and international efforts to eradicate PPR.

MATERIALS AND METHODS

Sampling area

This study was carried out in all ten administrative districts of Azad Jammu and Kashmir (AJK), Pakistan. These included Neelum: 34.59°N 73.91°E, Muzaffarabad: 34°21'34.8768"N 73° 28'15.7944"E, Jhelum Valley (Hattian Bala): 34°10'09"N 74°44'36"E, Bagh: 33.9735°N 73.7918°E, Haveli: 33°56′N 74°06′E, Poonch:33°83'69"N 73°88'89"E, Sudhnoti: 33°70'60"N 73°72'42"E, Kotli: 33°50'08"N 73°90'07"E, Mirpur: 33.2137'N 73.7949'E, and Bhimber: 32°58'32.45"N 74°04'45.34"E. these were selected based on their geographical location, ecology, the higher number of sheep population, husbandry practices, and increased animal movement through the areas (Fig. 1).

Sample collection

A total of 2,018 blood samples were collected from small ruminants, comprising 947 samples prior to vaccination and 1,071 samples post-vaccination. Blood was drawn aseptically from the jugular vein using sterile syringes. For each sampled animal, data including species, age, sex, and district of origin were recorded using a standardized questionnaire. The sample size for this study was calculated based on the standard Cochran's formula used for estimating prevalence in a large population:

$$n = \frac{Z^2 . P . (1-P)}{d^2}$$

Where; n is required sample size, Z is Z-value for the desired confidence level (1.96 for 95% confidence), P is expected prevalence (assumed 50% = 0.5 for maximum variability), and d is desired precision or margin of error (5% = 0.05).

$$n = \frac{(1.96)^2 . 0.5 . (1-0.5)}{(0.05)^2} = 384.16$$

Thus, a minimum of 384 samples would be required to estimate prevalence with 95% confidence and 5% precision in a large population. However, considering population heterogeneity (across 10 districts, species, age, sex), the design effect (due to clustering in field conditions), separate analysis for pre- and post-vaccination phases, and potential non-response or invalid samples, we increased the sample size proportionally. This ensured sufficient statistical power and representativeness for subgroup analyses and district-level comparisons.

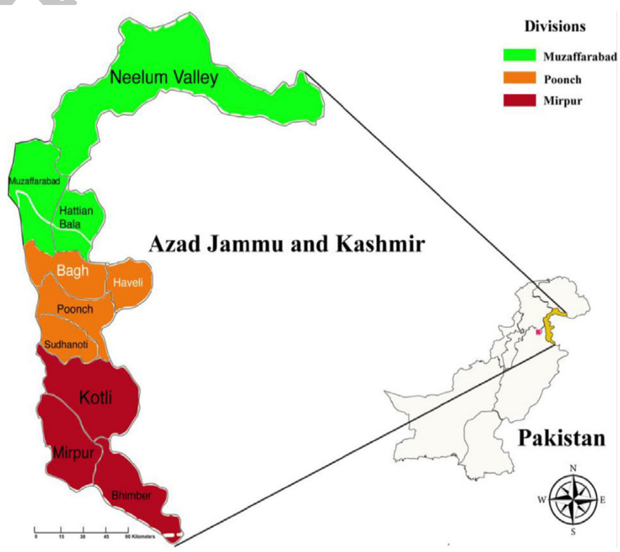

Fig. 1. Map of Azad Jammu and Kashmir indication ten districts.

Serum preparation

Approximately 5 mL of blood was collected per animal and allowed to clot overnight in a slanted position under refrigerated conditions. Samples were then transported in a cold chain to the Central Laboratory at the Livestock and Dairy Development Complex, Muzaffarabad. The separated serum was decanted into 2 mL labeled cryovials and stored at -20°C until laboratory analysis.

Serological testing: Competitive ELISA

To detect antibodies specific to Peste des Petits Ruminants Virus (PPRV), a competitive enzyme-linked immunosorbent assay (cELISA) was performed using the ID Vet PPR Competition ELISA Kit (ID Vet, 310 Rue Louis Pasteur, 34790 Grabels, France). The assay is based on competition between serum antibodies and a monoclonal antibody targeting the PPRV hemagglutinin (H) protein. Reduced color development in the reaction indicates the presence of PPRV-specific antibodies (Libeau *et al*., 1994).

All reagents were brought to room temperature (21±5°C) and mixed by inversion or vortexing. Phosphate-buffered saline (dilution buffer 13) was added to each well, followed by 25 µL of positive control in wells A1 and B1, negative control in C1 and D1, and serum samples in the remaining wells. Plates were shaken gently for 2 min and incubated at 37°C for 45 min. After incubation, the plates were washed three times using a wash buffer containing Tween 20 or Triton X-100. Subsequently, 10 µL of HRP-conjugated anti-mouse antibody was added to each well and incubated at 21±5°C for 30 min. Following another washing step, 100 µL of substrate solution was added and incubated for 15 min in the dark to allow colour development. To halt the enzymatic reaction, 100 µL of stop solution was added to each well. In the absence of specific antibodies, the substrate remains unreacted, and no color change is observed. If specific antibodies are present, the enzymatic reaction leads to a blue color development, which turns yellow upon the addition of the stop solution, indicating a positive result (Fig. 2).

The optical density (OD) was read at 450 nm using a Thermo Scientific Multiskan FC ELISA reader. Results were interpreted based on the Sample-to-Negative control (S/N) ratio using the formula:

$$S/N\% = (\text{OD sample}/\text{OD}Nc) \div 100$$

Samples presenting S/N %: 50% or less were considered positive; >50% and ≤60% were considered doubtful; >60% were considered negative.

Statistical analysis

Data from field sampling and laboratory testing were compiled using Microsoft Excel and analyzed using GraphPad Prism (version 9). Descriptive statistics were used to summarize the seroprevalence data. Chi-square (χ^2) tests were applied to assess statistical associations between seroprevalence and categorical variables such as district, species, age group, and sex. A P-value of less than 0.05 was considered statistically significant with a 95% confidence interval.

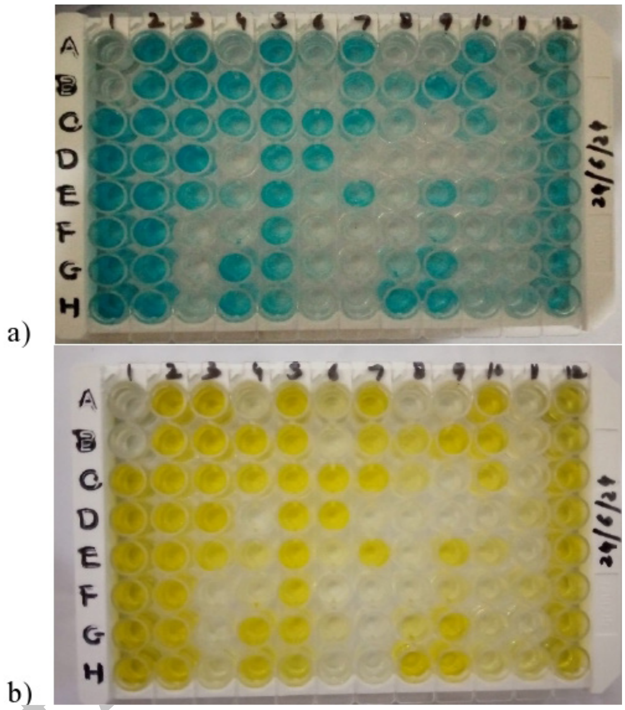

Fig. 2. Colorimetric indication of PPRV antibodies: Blue coloration prior to stopping the reaction, transitioning to yellow upon addition of stop solution.

RESULTS AND DISCUSSION

District-wise seroprevalence of PPRV in AJK

The comparative seroprevalence of Peste des Petits Ruminants Virus (PPRV) among goats and sheep in the ten districts of Azad Jammu and Kashmir (AJK), before and after vaccination, is summarized in Table I.

Pre-vaccination

The serological assessment conducted before vaccination across all ten districts of AJK (40.02%) revealed the presence of PPRV antibodies, indicating widespread exposure among small ruminants. District-wise analysis showed significant variability in seropositivity. The highest pre-vaccination seropositivity was recorded in Mirpur (47.76%) and Muzaffarabad (47.48%), while the lowest levels were observed in Haveli (19.23%) and Neelum (27.41%). A significant difference in seropreare common routes districts was observed statistically (P=0.011; χ^2=0.914 for Haveli and P=0.00015; χ^2=0.949 for Neelum), which could be attributed to differences in ecological conditions and livestock movement specially during the summer months as herders move for raising their animals on pastures at high altitude and Muzaffarabad district is a common route.

Table I. Comparative district-level seroprevalence of PPR in goats and sheep in AJK pre- and post-vaccination.

Districts	Goat			Sheep			Overall small ruminants		
	Samples screened	Samples positive	Sero-positivity %	Samples screened	Samples positive	Sero-positivity %	Samples screened	Samples positive	Sero-positivity %
Pre-vaccination									
Neelum[a]	58	13	22.41^{b**ch*}	77	24	31.17^{e**i***}	135	37	27.41^{h*}
Muzaffarabad[b]	186	93	50.00	52	20	38.46^{c**i**}	238	113	47.48
Jhelum valley[c]	34	15	44.12	10	3	30.00	44	18	40.91
Bagh	49	15	30.61^{b*}	9	3	33.33	58	18	31.03^{b*}
Haveli[d]	9	0	0^{bcfgh*}	17	5	29.41^{h*}	26	5	19.23^{b**h*}
Poonch[e]	62	16	25.81^{b**h*}	38	28	73.68	100	44	44.00
Sudhnoti[f]	79	28	35.44^{b*}	10	6	60.00	89	34	38.20
Kotli[g]	80	26	32.50^{b*}	18	12	66.67	98	38	38.78
Mirpur[h]	58	25	43.10	9	7	77.78	67	32	47.76
Bhimber[i]	61	16	26.23^{b*}	31	24	77.42	92	40	43.48
Grand total	676	247	36.54	271	132	48.71	947	379	40.02
	P-value=0.0002**; χ^2=0.98			P-value<0.0001; χ^2=0.997***			P-value<0.001**; χ^2=0.990		
Post vaccination									
Neelum[a]	32	16	50.0^{bi*}	22	19	86.36	54	35	64.81
Muzaffarabad[b]	209	153	73.21	89	69	77.53	298	222	74.50
Jhelum valley[c]	66	26	39.39^{dgh*i***}	22	20	90.91	88	46	52.27^{b***d*}
Bagh	9	5	55.56^{i*}	7	2	28.57^{abci**}	16	7	43.75^{d*}
Haveli[d]	20	14	70.00	45	34	75.56^{i*}	65	48	73.85
Poonch[e]	81	25	30.86^{bhi***df*}	22	9	40.91^{ci**}	103	34	33.01^{bdi***ah**}
Sudhnoti[f]	54	27	50.00^{b*}	16	4	25.00^{bci***}	70	31	44.29^{b***i*}
Kotli[g]	181	101	55.80^{b**i*}	12	9	75.00	193	110	56.99^{b***d*}
Mirpur[h]	60	40	66.67	11	4	36.36^{ci**}	71	44	61.97
Bhimber[i]	100	75	75.00	13	13	100.00	113	88	77.88^{b*}
Grand total	812	482	59.36	259	183	70.66	1071	665	62.09
	P<0.0001***; $\chi^{2=}$1.000			P<0.0001***; χ^2=0.995			P<0.0001***; χ^2=1.00		

*, P<0.05; **, P<0.01; ***, P<0.0001; a, Neelum vs other Districts; b, Muzaffarabad vs other Districts; c, Jhelum Valley vs other Districts; d, Haveli vs other Districts; e, Poonch vs other Districts; f, Sudhnoti vs other Districts; g, Kotli vs other Districts; h, Mirpur vs other Districts; i, Bhimber vs other Districts.

Our findings agree with prior studies reporting moderate to high seroprevalence in Azad Jammu and Kashmir. For instance, Zahur *et al.* (2014) and Abubakar *et al.* (2009) observed seroprevalence levels ranging from 50% to 65% in selected AJK districts. Similarly, Manzoor *et al.* (2020) recorded 50% seroprevalence in district Kotli, linked with localized outbreaks. In contrast, in another nationwide study by Abubakar *et al.* (2017) assessed a lower overall PPR prevalence of 9.93% in AJK, though district-level variation was not fully differentiated. These differences across studies may be attributed to differences in sampling periods and size, diagnostic methodologies, animal health infrastructure, and the incidence of outbreaks at the time of data gathering.

Likewise, Baloch *et al.* (2021) observed a higher PPR prevalence in Pakistan's semi-arid districts like Tharparkar (35.38%) and Umerkot (28.64%) compared to more humid areas such as Hyderabad (18.27%) and Thatta (25.71%). Globally, a minimal pre-vaccination prevalence (3.3%) was reported in Tanzania by Mdetele *et al.* (2015), while Njue *et al.* (2018) noticed a 62% occurrence in Somalia. In India and Bangladesh, studies by Tajpara *et al.* (2022) and Rahman *et al.* (2023) have similarly emphasized district-level alteration affected by ecological and management conditions.

The higher seropositivity; in districts Mirpur and Bhimber may be attributed to factors such as proximity to the areas of Punjab where animals movement is difficult to control and repeated outbreaks, while in Muzaffarabad it may be due to a massive upward movement of herders from Punjab and Khyber Pakhtunkhwa during the summer months in search of green pastures. On the other hand, the low baseline immunity in Haveli and Neelum suggests either reduced virus circulation or insufficient observation and reporting. These outcomes highlight the need for region-specific control measures and the importance of targeted vaccination coverage to address local vulnerabilities.

Post-vaccination

After a massive vaccination campaign, overall, 62.09% immune reaction was observed that ensure the efficacy of vaccine. The highest seroprevalence i.e., 77.88% was observed in Bhimber district followed by Muzaffarabad (74.50%) and Haveli (73.85%). Whereas the lowest post-vaccination seroprevalence (33.01%) was observed in Poonch district, suggesting possible deficiencies in cold chain maintenance and vaccine administration.

The findings of present investigations are aligned with findings from others. Tajpara *et al*. (2022) reported a post-vaccination seroprevalence of 72.34% in the Indian state of Gujrat. Similarly, Mdetele *et al*. (2015) observed a significant rise to 71.1% post-vaccination in the Tarime district of Tanzania. Njue *et al*. (2018) also indicated a significant ($P<0.05$) increase from 62% to 76% post-vaccination of small ruminants in Somalia. The immune response achieved post-vaccination in each district of AJK highlights the importance of continuous and massive vaccination programs to achieve optimal herd immunity and control PPR outbreaks.

Seroconversion

District-wise seroconversion rates of PPRV antibodies following vaccination are summarized in Table II. Overall, the seroprevalence increased from 40.02% pre-vaccination to 62.09% post-vaccination, indicating a substantial seroconversion rate of 22.07% (χ^2=98.04; P=0.0001). This improvement in seroprevalence showed the success of the vaccination campaign in boosting herd immunity among sheep and goat in the region of Azad Jammu and Kashmir. The remarkable improvements (P=0.0001) seen in Haveli (seroprevalence increased from 19.23% to 73.85%, reflecting a 54.62% increase) followed by Neelum (up from 27.41% to 64.81%, a 37.41% increase), and Bhimber (growth from 43.48% to 77.88%, a 34.40% rise) highlights successful execution of vaccination strategies in these areas, possibly owing to farmers engagement, and strict compliance with vaccination manufacturers guidelines. Districts like Muzaffarabad and Kotli also showed significant seroconversion rates of 27.02% (χ^2=41.21; P=0.0001) and 18.22% (χ^2=8.63; P=0.003), respectively, reflecting successful vaccination campaigns. The decline in Poonch's seroprevalence post-vaccination (decrease from 44.00% to 33.01%, indicating a -10.99% change) is concerning and may point to challenges such as inadequate vaccine coverage, cold chain issues, or logistical constraints. Similarly, the marginal improvement in Sudhnoti (6.08% rise) suggests the need for more robust vaccination efforts. These findings align with global observations where effective vaccination programs lead to substantial increases in seroprevalence, contributing to the control and eventual eradication of PPR.

Table II. District-wise seroconversion rates of PPRV antibodies in domestic small ruminants of AJK following vaccination.

District	Seroconversion rates (%)			Chi Sq	P value
	Pre-vaccination (%)	Post-vaccination	Change		
Neelum	27.41	64.81	37.41	22.89	0.0001
Muzaffarabad	47.48	74.50	27.02	41.21	0.0001
Jhelum Valley	40.91	52.27	11.36	1.51	0.21
Bagh	(31.03)	43.75	12.72	0.9	0.34
Haveli	19.23	73.85	54.62	22.78	0.0001
Poonch	44.00	33.01	-10.99	2.59	0.10
Sudhnoti	38.20	44.29	6.08	0.6	0.43
Kotli	38.78	56.99	18.22	8.63	0.003
Mirpur	47.76	61.97	14.21	2.81	0.09
Bhimber	43.48	77.88	34.40	25.58	0.0001
Grand total	40.02	62.09	22.07	98.04	0.0001

Khan *et al*. (2009) reported that 100% of the sheep and 90% goats had seroconverted 45 days post-vaccination in Punjab, Pakistan. Kabir *et al*. (2016) reported a seroprevalence of 20.31% and 75% in pre- and post-vaccination respectively in goats in Bangladesh. Similarly, in Somalia a rise in seroprevalence from 62% to 76% pre- and post-vaccination in small ruminants was observed (Njue *et al*. 2018). In Nepal Regmi *et al*. (2019) observed a seroprevalence of 75.2% to 25.8% pre- and post-vaccination respectively in goats. Balamurugan *et al*. (2024) studied an increase in seroprevalence from 61.1% to 73.4% pre- and post-vaccination respectively in Karnataka, India. In Ethiopia, Mandefro *et al*. (2024) noted a significant rise from 3.61% to 76.66% following vaccination.

The variations of seroprevalence among different district and demographic groups highlights the need for a robust vaccination campaign in target areas to curb the disease. Poonch district that showed a minimal post-vaccination seroprevalence need to be focused for a strong vaccination campaign and strict monitoring measures. However, the overall vaccine response is encouraging, need further improvement for ongoing surveillance, focused interventions, and flexible vaccination approaches to effectively control and eventually free AJK from this disease.

Species-specific pre-vaccination seroprevalence

Table III summarizes the species-specific seroprevalence of PPR in pre-vaccinated small ruminants of AJK utilizing c-ELISA. A serological study performed on 947 unvaccinated small ruminants indicated a total PPRV seroprevalence of 40.02%, with 379 animals showing positive antibody results. Analysis by specie revealed a significantly higher seroprevalence in sheep (48.7%) in contrast to goats (36.54%) ($P=0.00051$; $\chi^2=0.981$).

These findings are associated with several other studies in Pakistan. For example, Khan *et al*. (2009) documented significantly higher ($P=0.002$) seroprevalence in sheep (51.29%) than goats (39.02%). Nizamani *et al.* (2015) observed a seroprevalence rate of 69.3% with higher rates in sheep compared to goats. Kabir *et al.* (2020) found higher PPRV prevalence in sheep (61.82%) compared to goats (55.88%). Saeed *et al*. (2018) in Sudan and Gelana *et al*. (2020) in Ethiopia observed the similar trends. Balamurugan *et al*. (2024) in India observed a higher pre-vaccination seroprevalence (64%) in sheep compared to goats (58%). Conversely, only a few studies like that of Tajpara *et al*. (2022) and Ejigu *et al*. (2023) indicated greater seroprevalence in goats than sheep.

Species-specific post-vaccination seroprevalence

Out of the overall 1,071 samples, 665 animals (62.09%) showed positive results for PPRV antibodies. Sheep again exhibited a higher seroprevalence (70.66%) compared to goats (59.36%; $P=0.001$; $\chi^2=0.973$) (Table III). The post-vaccination seroprevalence ratios recorded in our study are align with findings from other studies. Balamurugan *et al*. (2020) recorded an overall seroprevalence of 65.39%, with a greater percentage in sheep (79.39%) than in goats (59.29%) in India. Notably, another study in India reported a higher seroprevalence in goats (75.6%) than in sheep (72%), though this marginal difference was not statistically significant (Balamurugan *et al*., 2024). The consistently elevated seroprevalence in sheep indicates potential species-related variations in immune response or exposure levels. Management practices could also play a role, as sheep are typically kept longer for wool production, which may lead to increased exposure to the virus, while goats, primarily reared for meat, have shorter lifespans and quicker turnover.

Table III. Species-specific seroprevalence of PPR in pre- and post-vaccinated small ruminants of AJK using c-ELISA.

Species	Total samples screened	Seropositive (n)	Seropositive (%)	P value	χ^2
Pre-vaccinated					
Goat	676	247	36.54%	0.00051	0.981***
Sheep	271	132	48.71%		
Total	947	379	40.02%		
Post-vaccinated					
Goat	812	482	59.36%	0.001	0.973*
Sheep	259	183	70.66%		
Total	1,071	665	62.09%		

Additionally, factors such as sample size, breed, husbandry practices, environmental conditions (e.g., humidity and season), and local epidemiological dynamics may influence the observed seroprevalence patterns (Singh *et al*., 2004). These results highlight the importance of species-targeted vaccination strategies and ongoing serological surveillance to effectively control and ultimately eradicate PPR in the region.

Sex-wise seroprevalence of PPR

Pre-vaccination findings

The pre-vaccination seroprevalence data revealed a higher prevalence of PPRV antibodies in female small ruminants compared to males (Table IV). Specifically, 41.87% of females tested positive, whereas the seropositivity in males was 30.52%. This difference was statistically significant ($P=0.015$; $\chi^2=0.926$). When analyzed by species, female goats exhibited a seroprevalence of 37.79%, while male goats showed 30.43%. In sheep, females had a seroprevalence of 51.72%, compared to 30.77% in males.

These findings are consistent with previous studies. A higher seroprevalence in female goats (69.96%) than male goats (63.92%) were recorded in Gilgit Baltistan, Pakistan (Rasheed *et al*., 2020). Similarly in another study conducted in Sindh female goats had a prevalence of 25.00% compared to 5.00% in males, and female sheep had 7.00% seropositivity versus 3.00% in males (Rind *et al*., 2023). Additionally, Marma *et al*. (2025) noted a similar pattern in Bangladesh, with 63% seroprevalence in females compared to 37% in males.

Table IV. Sex-wise pre- and post-vaccine seroprevalence of PPR in domestic small ruminants of AJK.

Sex	Goat			Sheep			Overall small ruminants		
	Samples screened	Samples positive	Sero-positive %	Samples screened	Samples positive	Sero-positive %	Samples screened	Samples positive	Sero-positive %
Pre-vaccination									
Female	561	212	37.79	232	120	51.72	793	332	41.87
Male	115	35	30.43	39	12	30.77	154	47	30.52
Grand total	676	247	36.54	271	132	48.71	947	379	40.02
	P=0.13; χ^2=0.71			P=0.015*; χ^2=0.90			P=0.015*; χ^2=0.926		
Post-vaccination									
Female	682	414	60.70	237	169	71.31	919	583	63.44
Male	130	68	52.31	22	14	63.64	152	82	53.95
Grand total	812	482	59.36	259	183	70.66	1071	665	62.09
	P=0.074; χ^2=0.78			P=0.44; χ^2=0.50			P=0.025*; χ^2=0.87		

Post-vaccination findings

Post-vaccination data showed an overall increase in seroprevalence across both sexes, with females maintaining a higher seropositivity rate. Overall, 63.44% of females were seropositive compared to 53.95% of males, a statistically significant difference (P=0.025; χ^2=0.87). In goats, females showed a seroprevalence of 60.70%, while males had 52.31%. In sheep, females exhibited a seroprevalence of 71.31%, compared to 63.64% in males (Table IV). The findings of present study are consistent with results of Tajpara *et al*. (2022) in India. The sustained higher seroprevalence in females may be attributed to their longer retention in herds for breeding purposes, which increases their likelihood of being vaccinated and exposed to the virus, potentially leading to stronger immune responses. Additionally, the physiological demands of reproduction, such as pregnancy and lactation, may modulate immune function, influencing vaccine efficacy and antibody production.

While most studies report higher seroprevalence in females, some have observed the opposite. For instance, Tajpara *et al*. (2022) observed higher seropositivity in male sheep compared to female. These differences could be attributed to different sample sizes, regional epidemiology, or exposure risk and management. In certain regions, male animals might have higher exposure to PPRV due to their frequent movement because of their preference for sacrifices in Muslim community on occasion of Eid ul Adha, trade and breeding purposes.

Age-wise seroprevalence

Pre-vaccination

A significant difference was observed in pre-vaccination seroprevalence among different age group (Table V). Animals older than five years exhibited the highest seroprevalence, goats at 80.77% and sheep at 72.06%, achieving an overall seroprevalence of 74.46%. This higher seroprevalence with increase in age may be attributed to cumulative exposure to the PPR virus over time, leading to the development of antibodies. Conversely, animals below 5 years and above 6 months showed the lowest seroprevalence rates, with goats at 23.15% and sheep at 29.93%, culminating in an overall seroprevalence of 25.22%. This lower seroprevalence could be attributed to the decline of maternal antibodies and less exposure to the virus. Animals under 6 months showed intermediate seroprevalence of 45.43% in goats and 63.64% in sheep, with an overall seroprevalence of 48.39%. This may be because of passive immunity (maternal antibodies) that offer partial protection.

The highly significant (P<0.0001; χ^2=1.00) age-based differences, indicate a strong correlation between age and PPRV seroprevalence. Similar relationships have been observed by Rasheed *et al*. (2020), Rind *et al*. (2023), and Balamurugan *et al*. (2024).

Post-vaccination

There was a notable increase in seroprevalence post-vaccination across all age groups. Animals aged above 5 years maintained high seroprevalence rates, with goats at 78.86% and sheep at 82.29%, exhibiting an overall seroprevalence of 76.24%. This indicated that vaccination efforts were very effective in boosting immunity in older animals. Interestingly, animals aged less than 6 months showed the highest post-vaccination seroprevalence (goats73.80% and sheep at 81.25%), achieving an overall seroprevalence of 77.49%. This boost may be due to the administration of vaccines at an early age, improving

Table V. Age-wise pre- and post-vaccine seroprevalence of PPR in domestic small ruminants of AJK.

Age	Goat			Sheep			Overall small ruminants		
	Samples screened	**Samples positive**	**Sero-positive %**	**Samples screened**	**Samples positive**	**Sero-positive %**	**Samples screened**	**Samples positive**	**Sero-positive %**
Pre-vaccination									
[a]< 6 Months	339	154	45.43	66	42	63.64	405	196	48.39
[b]> 6 Months- 5 Year	311	72	23.15[a***b**]	137	41	29.93[a***]	448	113	25.22[ab***]
> 5 Year	26	21	80.77[b***]	68	49	72.06[b***]	94	70	74.47[a***]
Grand total	676	247	36.54	271	132	48.71	947	379	40.02
	$P<0.0001$; $\chi^2=1.00$			$P<0.0001$; $\chi^2=1.00$			$P<0.0001$; $\chi^2=1.00$		
Post-Vaccination									
[a]< 6 Months	313	231	73.80	80	65	81.25	431	334	77.49
[b]> 6 Months- 5 Year	376	154	40.96[a***]	83	39	46.99[a***]	459	193	42.05[a***]
> 5 Year	123	97	78.86[ab***]	96	79	82.29[b***]	181	138	76.24[b***]
Grand total	812	482	59.36	259	183	70.66	1071	665	62.09
	$P<0.0001$; $\chi^2=1.00$			$P<0.0001$; $\chi^2=1.00$			$P<0.0001$; $\chi^2=1.00$		

*, P<0.05; **, P<0.01; ***, P<0.0001; a, < 6 Months vs other age groups; b, > 6 Months- 5 Year vs >5 Year.

immunity before the decreasing of maternal antibodies. However, more than 6 months to 5 years age group continued to show comparatively lower seroprevalence rates post-vaccination, (goats at 40.96% and sheep at 46.99%), capping in an overall seroprevalence of 42.05%. This persistent lower seroprevalence highlights potential gaps and need of intervention in vaccination coverage or response in this age group, emphasizing targeted interventions (Table V).

The statistical significance of these age-related differences remained strong post-vaccination ($P<0.0001$; $\chi^2=1.00$). Abubakar *et al.* (2009) reported that most seropositive animals were over 2 years old. Awa *et al.* (2000) observed maternal antibodies waning below protective levels by 3.5–4.5 months in lambs and kids, aligning with our finding of lower immunity in the >6 months to 5 years group. Nevertheless, young animals under 6 months appeared to benefit from vaccination via a booster effect, while older animals also responded well due to cumulative exposure and immune memory. These findings suggest that age-specific vaccination strategies are essential to improve immunity, especially in the vulnerable 6-month to 5-year age group.

Demographic trends in PPRV seroprevalence pre- and post-vaccination

Figure 3 illustrates the change in seroprevalence of PPRV antibodies among various demographic groups sex, species, and age in small ruminants before and after vaccination in AJK. Across all demographic categories, a marked increase in seroprevalence was observed post-vaccination, demonstrating a significant seroconversion and indicating the efficacy of the PPR vaccine.

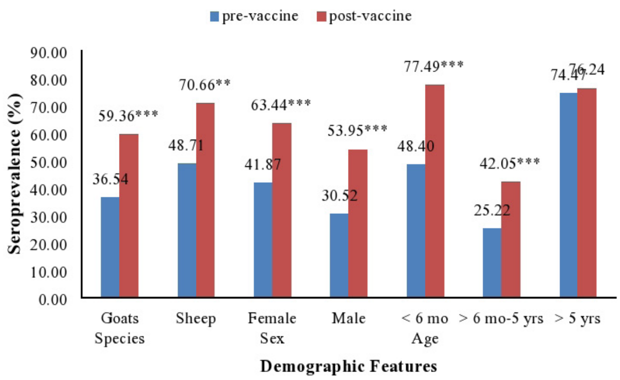

Fig. 3. Seroconversion of PPRV antibodies among various demographic factors of pre- and post-vaccinated small ruminants of AJK. (**P<0.01, ***P<0.0001).

Species-wise response

Among species, sheep showed a higher post-vaccination seroprevalence (70.66%) compared to goats (59.36%). Pre-vaccination levels were also higher in sheep (48.71%) than in goats (36.54%), suggesting that sheep may either have greater exposure or a more robust immune response to vaccination.

Sex-wise response

Regarding sex, females demonstrated higher seroprevalence both pre- (41.87%) and post-vaccination

(63.44%) than males (30.52% pre-vaccination, 53.95% post-vaccination). This difference may be attributed to longer retention of females in herds for breeding purposes, thus increasing their exposure to the vaccination programs and natural virus exposure. These findings are supported by previous studies, including Rasheed *et al*. (2020) and Alemu *et al*. (2019).

Age-wise response

In terms of age, animals aged 6 months to 5 years showed the greatest increase in seroprevalence from 25.22% pre-vaccination to 77.49% post-vaccination highlighting strong immune responsiveness in this group. Young animals (<6 months) also showed a significant rise from 48.40% to 76.24%, indicating successful immunization despite potential interference from maternal antibodies. Interestingly, animals older than 5 years already exhibited high pre-vaccination seroprevalence (74.47%), which only slightly increased post-vaccination (76.24%), likely due to prior natural exposure and antibody saturation.

The statistical significance levels ($P<0.01$ to $P<0.0001$) confirm that these differences are not due to chance and reinforce the positive impact of vaccination across all groups. These results support the effectiveness of targeted mass vaccination programs in AJK and suggest that demographic factors should be considered in optimizing disease control strategies.

Seroprevalence trends over time post-vaccination

Figure 4 shows the fluctuation in PPRV antibody seroprevalence in small ruminants at various intervals from day 30 to day 510 post-vaccination. Initially, a sharp increase in seroprevalence was observed from 22% on day 30 to a peak of 94% on day 90 indicating a strong immunological response within the first three months post-vaccination. However, by day 120, seroprevalence declined to 54%, suggesting waning immunity. A secondary increase was noted on day 150 (80%) and day 270 (76%), potentially due to natural virus exposure acting as a booster. Despite this, seroprevalence declined to 22% by day 300 but then recovered moderately to 60% on day 360. At 510 days, seroprevalence settled at 54%, indicating long-term antibody persistence, though at reduced levels.

These results suggest that while initial vaccine response is strong, immunity diminishes over time. Multiple factors could influence this trend, including host immunity, environmental exposure, vaccine handling, and administration protocols. The drop after day 90 underscores the need for sero-monitoring and timely booster vaccinations, especially beyond six months post-vaccination. Comparable results were found in earlier studies. Khan *et al*. (2009) reported 100% seroconversion in sheep and 90% in goats by day 45. Zahur *et al*. (2014) reported PI values over 90% by day 30, with significant antibody titres persisting for up to three years. Kardjadj *et al*. (2015) found that all vaccinated sheep and goats in Algeria showed 100% seroconversion by day 28. Awa *et al*. (2000) reported that protective antibody titres were maintained up to 12 months post-vaccination in both sheep and goats. On the contrary, Turan *et al*. (2012) observed lower seropositivity in sheep compared to goats eight months post-vaccination in Turkey. Variations in antibody titres across intervals may be due to environmental and management differences. For instance, animals in open grazing systems may encounter natural virus boosts, unlike station-reared animals with lower exposure risks.

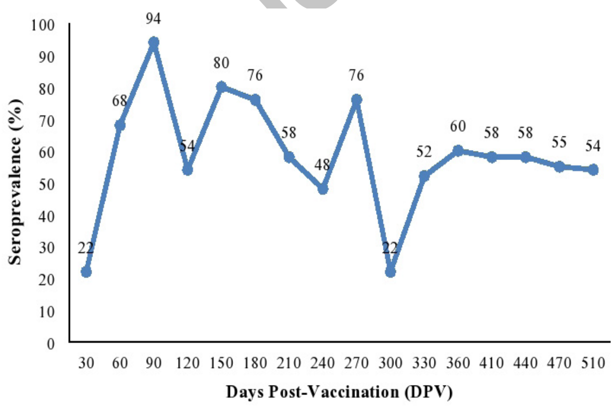

Fig. 4. Seroprevalence of PPRV antibodies in small ruminants at different intervals post-vaccination in AJK.

CONCLUSIONS

The study confirms the effectiveness of PPR vaccination in enhancing herd immunity among small ruminants in AJK. The results support district-focused and age-targeted immunization strategies, coupled with continuous surveillance, to eliminate PPR from the region.

DECLARATIONS

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IRB approval

This study was approved by the Board of Advanced Studies and Research at the University of Azad Jammu and Kashmir, Muzaffarabad, under approval number F-BASR/(85th M)/18-29/3288-89/2022.

Ethical statement

The ethics committees of the authors' institutions have approved this study which is in accordance with the rules and regulations under notification number F-BAS&R/(101st M)/28-56/2844-46/2024.

Generative AI and AI-assisted technology statement

The authors declare that they have not used generative AI or AI-assisted technologies in the writing or editing of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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