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Seroprevalence and Confirmation of Peste des Petits Ruminants Infection in Prevaccinated Goats and Sheep of Azad Jammu and Kashmir

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

Key words
PPRV, Prevalence, Sheep, Goats, cELISA, AJK

ABSTRACT

Peste des petits ruminants (PPR) is a highly contagious and economically significant infection affecting both sheep and goats. This retrospective study, conducted from 2022-2023, assessed the incidence of PPR in Azad Jammu and Kashmir (AJK), where vaccination records were lacking. A total of 755 blood samples from suspected and healthy goats and sheep across ten districts were tested using competitive enzyme-linked immunosorbent assay (cELISA) kits. The findings identified 59 suspected outbreaks, predominantly in the Mirpur Division (39). Among the samples analyzed, a total of 51.67% from suspected cases and 28.98% from healthy animals were positive, yielding an overall seroprevalence of 32.58%. Specifically, 33.44% of goat samples and 28.78% of sheep samples were tested positive for PPR virus (PPRV) antibodies. The Mirpur District exhibited the highest seroprevalence at 43.10%, while District Haveli had the lowest at 18.18% (P=0.038). Goats showed higher susceptibility (33.44%) compared to sheep (28.78%; P=0.289), and females (33.22%) were more affected than males (29.79%; P=0.432). Adult goats over 5 years old showed significantly higher susceptibility (75%; P<0.0001) compared to kids aged under 6 months (16.36%) and young goats aged 6 months to 5 years (33.94%). In sheep, lambs (33.33%) were more susceptible than adults (15.79%). Risk factor analysis indicated a strong association of PPR occurrence with poor body condition (48.81% vs. 29.29%), season (summer 43.72% vs. winter 27.67%), and herd size (large 44.19% vs. medium 32.12% vs. small 27.91%). Antigen detection confirmed ongoing outbreaks, which were verified as PPRV through sandwich ELISA. The findings highlight the need for preventive measures to manage and eradicate PPR in AJK, safeguarding the livelihoods of local herders.

INTRODUCTION

In Pakistan, people are residing in rural areas and depend on livestock including buffalo, cattle, goats, and sheep, for their livelihood. However, due to the rising impact of different livestock diseases, this sector is under sustainibility.

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In the local vernacular, Peste des Petits Ruminants (PPR) is commonly called "Kata" among shepherds. PPRV, which is a member of the Paramyxoviridae family, is the source of this highly contagious disease (Fine *et al*., 2020). The PPR virus first appeared in the West African country of Cote d'Ivoire in 1942. This virus was renamed as small ruminant Morbillivirus by the International Committee on Taxonomy of Viruses in 2016 (Amarasinghe *et al*., 2017).

The history of PPRV in Pakistan commenced in 1991 when the viral infection was initially diagnosed by clinical signs and symptoms (Pervez *et al*., 1993), but afterwards higher-tech testing methods like enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) were applied (Abubakar *et al*., 2008; 2018).

Transmission of disease occurs by inhalation of tiny, infected droplets from sneezing and coughing of animals maintained in close contact. The infection can also be spread through physical contact of animals with contaminated water, bedding, and feed troughs (Abubakar *et al.*, 2015). The virus enters the animal body via respiratory and digestive tracts, resulting in symptoms like inflammation of the mouth, ulcerative sores, diarrhea, fever, and discharge from the eyes and nose (Abubakar and Irfan, 2014). The period of incubation in PPR virus is from day 4 to 6, however sometimes it could take up to 14 days to show the symptoms. The body temperature may rise to 41°C, which can persist for 3 to 5 days and is accompanied by dehydration, distress, malnutrition, mucus and pus from the nasal and tear glands, increased saliva production, erosive bruises that can undergo necrosis. In more advance cases the bruises may become severe due to the accumulation of fibrin on the tongue with additional symptoms of coughing, weight loss, and difficult breathing, which can ultimately lead to death of animal (Parida *et al.*, 2015).

Depending on the clinical indications, PPR may be tentatively diagnosed and confirmed in the laboratory by differential diagnosis with related disorders until laboratory confirmation is obtained (OIE, 2019). The death rate can exceed 90% and the morbidity rate may reach 100% with PPR (Baron *et al.*, 2016). Owing to PPR's catastrophic economic impact, the Food and Agriculture Organization (FAO) and the International Epizootics Organization (OIE) of the United Nations have launched a global eradication programme (OIE and FAO, 2015).

Pakistan's economy is centered on agriculture, according to economic survey of Pakistan 2023, Livestock contributes 14.36% in GDP with 62.68% in agriculture value added, with 3.78% growth in 2022-23 (Economic Survey of Pakistan, 2023).

The significance of sheep and goats has increased because of the need for halal meat. However, the biggest hindrance is the spread of infectious disease. According to an Animal and Plant Health Agency (APHA), England, an increased incidence of PPR virus is linked to high raising costs for sheep and goats (Banyard *et al.*, 2014) and a similar situation is being faced by farming community in AJK where a large number of sheep and goat herders are relying small ruminant for their livelihoods. Although the prevalence of this disease has been studied in other Provinces of Pakistan. However, there is currently no comprehensive study on seroprevalence of PPR available in Azad Jammu and Kashmir (AJK). Therefore, the purpose is to identify the seroprevalence of PPR disease of goats and sheep in AJK using a contemporary biological approach (e.g. ELISA).

MATERIALS AND METHODS

Sampling area

The research was conducted during the year 2022-2023 in AJK, a territory situated in the northern area of Pakistan. The topography of AJK is characterized by its unique geographical and socio-economic landscape ranging from valleys to mountains, with climate varies from temperate to alpine. The livelihood of rural people is largely supported by agriculture and traditional livestock keeping, with sheep and goat herding playing a pivotal role. Samples for this study were selected from sheep and goat herders across all three divisions of AJK (Muzaffarabad, Poonch and Mirpur) and their ten districts.

Sample collection

Blood and swab samples were collected from apparently healthy as well as suspected goats and sheep with common symptoms of PPR. The jugular vein of 755 goats and sheep was punctured to collect blood samples. A structured questionnaire was utilized to document the demographic distribution across different species, age groups, genders, breeds, and geographical locations.

Serum samples

Five milliliters of blood were extracted in sterile disposable syringes and labeled properly. Then samples were transferred to the central laboratory at Livestock and Dairy Development Complex Muzaffarabad by maintaining the cold chain and placed in slanting position overnight to separate the serum. The serum was decanted into 2 mL serum tubes and labeled accordingly and then placed in deep freezer at -20°C.

Swab samples

Using sterile cotton swabs sticks 34 samples (nasal/fecal/tissue) were collected from goats and sheep exhibiting flu like symptoms like nasal discharge, cough, high fever and small blister containing pus clinically suspected of PPR. These sticks were put in cryogenic vials containing phosphate buffer saline. The vials were labeled properly and put in zip-bags and were stored at -20°C till further processing (Akwongo *et al.*, 2022).

Outbreak investigation

Reports on suspected outbreaks of PPR were collected through laboratories and civil veterinary hospitals of livestock department from all districts of the state of AJK during the study period (2022-2023).

Antibody detection through competitive ELISA

The Livestock and Dairy Complex Muzaffarabad's

Microbiology Laboratory employed a competitive enzyme-linked immunosorbent assay (cELISA) using an ELISA kit to determine if the blood serum contains PPRV-specific antibodies. The ID Vet Innovative Diagnostic kit of Grables France was used. In an ELISA-based assay for PPR antigen binding, a particular immunoglobulin in blood samples competed with the anti-H protein of the PPRV monoclonal antibody (MAB) (Libeau *et al*., 1994). The reactivity of the monoclonal antibody is inhibited by the PPRV-specific antibodies present in the sera. This inhibition causes the expected colour change to be lessened after the chromogen solution and enzyme-labeled anti-mouse conjugate are added.

To each well, phosphate buffer saline, often known as dilution buffer 13 was transferred. Wells A1 and B1 received a solution of the positive control (25 µL), while C1 and D1 wells were subjected to a 25 µL addition of the negative control. The remaining wells received a 25 µL addition of serum sample. Plates were shaken for two min and then incubated at 37°C (±2°C) for 45 min, plus or minus five min. Wash buffer + Tween 20 or Tritin X-100 washed three times through each sample. Next, 10 µL of conjugate 1X (horseradish peroxidase or alkaline phosphatase) was transferred to each well, then it was incubated at 21°C (±5°C) for approximately 30 min, with a permissible variation of ±3 min at the same temperature after being diluted with dilution buffer 19. The wash solution was used in 300 µL increments to completely wash each well three times. Following the addition of the substrate solution (100 µL) to all wells, the mixture underwent incubation at 21°C (±5°C) for 15±2 min, shielded from light. To halt the reaction, stop solution (100 µL) was introduced to all wells.

OD values were measured in Thermo Scientific Multiskan FC ELISA reader at 450 nm. S/P% indicating the number of antibodies against PPRV. This is how the percentage of positive control was computed:

$$S/N\% = (OD\ sample/ODNc) \div 100$$

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

Antigen detection through sandwich ELISA

Sandwich ELISA would be performed for confirmation of PPRV antigen in swab samples from diseased animals with commercial PPR Sandwich ELISA kit for detection of antigens (ID Vet Grables France) produced conjointly by Biological Diagnostic Supplies (BDSL) and Flow Laboratories and CIRAD, EMVT, France already applied by Karim *et al*. (2016). OD values will be measured in Thermo Scientific Multiskan FC ELISA reader at 450nm.

The S/P% will be calculated as follows:

$$S/N\% = \frac{(OD\ sample - ODNc)}{(ODPc - ODNc)} \times 100$$

Samples presenting S/N %: < 20% were categorized as negative whereas, ≥ 20% were considered positive.

Statistical analysis

Spreadsheets made with Microsoft Excel were used to record the data collected from fieldwork and the subsequent laboratory analysis. Version 9 of the Graph Pad Prism program was used to analyze this data. Descriptive statistical methods were utilized to summarize the findings regarding the seroprevalence of PPR antibodies. Chi-square (χ^2) analysis test was performed to examine the relationship among demographic variables including different districts, species, sex, and age group. $P<0.05$ indicated statistical significance for the data, which had a 95% confidence interval.

RESULTS AND DISCUSSION

Given that the present research based on antibody as well as antigen detection methods for PPR from both healthy and diseased animals, its finding can be interpreted to indicate past as well as present exposure of sheep and goat utilized in this study to the PPRV. During the year 2022-2023, a total of 59 infectious disease outbreaks were reported in the Azad Jammu and Kashmir (AJK) region (Table I). Maximum outbreaks were recorded from district Mirpur (22) and minimum cases were reported from Bagh (01) and Poonch (01) whereas no outbreak was reported from Haveli and Sudhnoti.

Seroprevalence with respect to health status

This study highlighted the importance of timely and effective responses to epidemiological findings, including massive vaccination campaigns, restricted movement specially in district of high incidence, and public awareness campaign, to stop the spread of PPR virus. Out of 755 animals investigated during the study 120 were diseased cases suspected for PPR whereas 635 were healthy. Seroprevalence of antibodies in healthy cases (28.98%) suggests prior exposure of animals to this infection. Seroprevalence of (51.67%) in diseased cases indicated the presence of PPR infection in population (Table II). This prevalence exceeds the results reported by Allahvirdizadeh *et al*. (2020) who found seroprevalence of 37% among diseased sheep in a study conducted in Iran.

Overall seroprevalence

The pre-vaccinated seroprevalence of PPR in small ruminants of AJK using c-ELISA is summarized in Table III.

Table I. Suspected PPR out-breaks recorded during study period in AJK.

Divisions/ Districts	2022	2023	Total no. of outbreaks
Muzaffarabad			
Muzaffarabad	4	5	9
Jhelum Valley	3	2	5
Neelum	4	-	4
Total	11	7	18
Poonch			
Haveli	0	0	0
Bagh	0	1	1
Poonch	1	0	1
Sudhnoti	0	0	0
Total	1	1	2
Mirpur			
Mirpur	12	10	22
Bhimber	10	0	10
Kotli	7	0	7
Total	29	10	39
Overall	41	18	59

Out of 755 ruminant samples analyzed, 32.58% were found to be seropositive for PPRV antibodies, with 246 samples testing positive. Specifically, the seropositivity rate for goats was 33.44%, while sheep showed a rate of 28.78%. Our results were significantly higher than 9.93% reported in earlier study by Abubakar *et al*. (2017) in small ruminants of AJK. The significant increase in the spread of the PPR virus over approximately five years suggests a rapid expansion of PPRV in this area. This indicated insufficient vaccination and preventive measures to manage the PPRV in AJK. The interventions carried out in various years, which were marked by insufficient targeted disease control measures, are blamed for the dramatic increase in PPR virus transmission among small sheep and goat populations in AJK that has been recorded. There is a crucial need for continuous surveillance, research and immediate massive vaccination and others control measures in whole state to curb the spread of PPRV not only in sheep and goat but also in other small ruminants to safeguard livestock health and agricultural livelihoods.

Previously other studies conducted in Pakistan demonstrated different seroprevalence rate as 54% (Abubakar *et al*., 2018) and 48.5% (Zahur *et al*., 2011). In Sindh province of Pakistan, overall PPR seroprevalence in goats was observed 56.3% and sheep was 49.5% (Abubakar *et al*., 2011). The differences in prevalence rates observed across various studies could be linked to the volume of sera and the specific collection techniques employed. Furthermore, the PPRV may spread among unvaccinated goat and sheep populations due to their unrestricted movement and inadequate quarantine protocols during sample collection during suspected outbreaks (Habib *et al*., 2019).

District-wise seroprevalence

In our investigation, all districts in AJK exhibited positive PPRV antibodies (as indicated in Table III). Notably, Mirpur demonstrated significantly higher seropositivity (43.10%) compared to Haveli (18.18%; P= 0.038; χ^2= 0.845) and Poonch (25.80%; P= 0.045; χ^2= 0.830). Additionally, in the case of goats, significantly different seroprevalence was observed among districts (P= 0.014). Chi-square analysis indicated significantly different prevalence of PPR between Mirpur vs. Neelum ($P < 0.05$); Jhelum Valley vs. Neelum ($P < 0.05$); and Muzaffarabad vs. Neelum ($P < 0.01$) districts. However, no significant difference ($P > 0.05$) was observed across different districts regarding PPR virus seroprevalence in sheep.

In present results Mirpur and Jhelum valley demonstrated significantly higher seropositivity but in an earlier study, Abubakar *et al*. (2018) reported the highest seropositivity in district Kotli (80%) followed by Muzaffarabad and Mirpur from Azad Jammu and Kashmir. Our research supports the findings of Baloch *et al*. (2021), who similarly noted greater incidence of PPR in the Tharparkar (35.38%) and Umerkot (28.64%) districts from Pakistan's semi-arid and arid regions than the Hyderabad (18.27%) and Thatta (25.71%) districts. Tajpara *et al*. (2022) also reported highly significant ($P < 0.00001$) seroprevalence rates in India. Similarly, Rahman *et al*. (2023) reported significantly different seroprevalence from different agro-ecological zones of Bangladesh. Previous research has shown that a variety of environmental

Table II. Seroprevalence of PPR in goats and sheep with respect to health status.

Health status	Total samples screened	Negative	Positive	Sero positive (%)	P value	χ^2	DF	CI
Diseased animals	120	58	62	51.67	< 0.00001 ***	23.66	1	95%
Healthy animals	635	451	184	28.98				
Total	755	509	246	32.58	-	-	-	-

Table III. District wise, species wise, sex wise, and age wise pre-vaccinated seroprevalence of PPR in small ruminants of AJK using c-ELISA.

Districts	Total samples screened	Goats		Sheep		Total samples positive	Total sero-positive (%)
		Samples screened	Seropositive (%)	Samples screened	Seropositive (%)		
District wise							
Mirpur	58^{a}	58	25 (43.10)	--	--	25	43.10
Jhelum Valley	44^{b}	34	15 (44.12)	10	3 (30.00)	18	40.90
Muzaffarabad	172^{c}	133	56 (42.11)	39	9 (23.08)	65	37.79
Sudhnoti	79^{d}	79	28 (35.44)	--	--	28	35.44
Kotli	80^{e}	80	26 (32.50)	--	--	26	32.50
Neelum	135^{f}	58	13 (22.41^{a*,b*,c**})	77	24 (31.17)	37	27.40
Bhimber	61	61	16 (26.23)	--	--	16	26.23
Bagh	42	42	11 (26.19)	--	--	11	26.19
Poonch	62	62	16 (25.81)	--	--	16	25.80^{a*}
Haveli	22	9	0	13	04 (30.77)	4	18.18^{a*}
Overall	755	616	206 (33.44)	139	40 (28.78)	246	32.58
		P= 0.014*; χ^2=1.000		P= 0.865; χ^2=0.83		P= 0.135; χ^2=13.65	
Species wise							
Goats	616		206 (33.44)			P-value=0.289; χ^2=0.590	
Sheep	139		40 (28.78)				
Sex wise							
Female	614	501	171 (34.13)	113	33 (29.20)	204	33.22
Male	141	115	35 (30.43)	26	7 (26.92)	42	29.79
		P= 0.448; χ^2=0.574		P= 0.816; χ^2=0.053		P= 0.432; χ^2=0.616	
Age wise							
Kids (<6 month)	64	55	9 (16.36)	9	3 (33.33)	12	18.75
Young (6 month-5 year)	656	545	185 (33.94^{a**})	111	34 (30.63)	219	33.38
Adult (>5 year)	35	16	12 (75.00^{a***})	19	3 (15.79)	15	42.86^{a*}
		P= <0.0001***; χ^2=1.00		P= 0.398; χ^2=0.891		P= 0.024*; χ2=0.988	

*= P < 0.05; **= P < 0.01; ***= P < 0.0001. a = kids vs other age groups.

conditions, including soil type, temperature, humidity, and rainfall, can induce variations in the seroprevalence of PPR virus across different zones (Hota *et al*., 2018).

Species-wise seroprevalence

With respect to species specificity, seroprevalence was found to be non-significantly higher (P=0.289) in goats (33.44%) compared to sheep (28.78%) in AJK (Table III). These results align with the findings of Kihu *et al*. (2015), Kgotlele *et al*. (2016), Rahman *et al*. (2017), Tajpara *et al*. (2022), and Ejigu *et al*. (2023) who, in their respective studies conducted in Kenya, Tanzania, Pakistan, India, and Ethiopia. Conversely, divergent findings were noted in studies conducted by Nizamani *et al*. (2015), Woma *et al*. (2016), Saeed *et al*. (2018), and Gelana *et al*. (2020), where higher seroprevalence of PPRV was reported in sheep as opposed to goats. These investigations were carried out in Pakistan, Nigeria, Sudan, and Ethiopia, respectively. The contrasting outcomes emphasize the impact of geographical and environmental variables on the patterns of PPRV prevalence in small ruminants, highlighting the need for region-specific strategies in the management and control of this infectious disease. In others studies it was found that goats are less resistant to PPRV compared to sheep which tend to manifest a milder form of infection owing to their innate resistance (Farougou *et al*., 2013; Yalew *et al*., 2019). Recovery rate is also higher in sheep compared to goats (Mahmoud *et al*., 2016). A trend of higher percentage of seropositivity in goats than sheep observed in the current research possibly attributed to the inherent resistance of sheep to the PPR virus. The farmers may be advised to focus more on sheep rearing because of comparatively a higher resistance of this animal to PPR virus compared to goats.

Sex-wise seroprevalence

In the context of small ruminants, a slightly different seroprevalence with no statistical significance (P=0.432) between females (33.22%) and males (29.79%) was observed. Similarly, when comparing sex in goats and sheep, we found females in both species were non significantly more seropositive than males (as shown in Table III). Our results align with previous studies conducted by Rasheed *et al.* (2020) in Gilgit Baltistan, Pakistan, where they reported a similar pattern of sex-wise seroprevalence (69.96% in females versus 63.92% in males). Similar trends were also observed by Bello *et al.* (2016); Patel *et al.* (2017); Gebre *et al.* (2018) and Nkamwesinga *et al.* (2023). Conversely, our results are at contrast with those of findings by Tajpara *et al.* (2022) and Nizamani *et al.* (2015), who revealed higher levels of seropositivity in males compared to females. The female reproductive stress could raise the risk of PPR virus infection (Munir *et al*., 2013).

Age-wise seroprevalence

The disparity in peste des petits ruminants seroprevalence across various age groups exhibited statistical significance (P= 0.024; χ^2= 0.98) as shown in Table III. The highest seroprevalence, standing at 42.86%, was discerned in adult animals (> 5 years old), closely followed by young (6 months to 5 years old) at 33.38%. Conversely, the age group of < 6 months (kids) displayed the lowest seroprevalence at 18.75%. Similarly, adult goats (75%) showed significantly higher (P<0.0001) susceptibility compared to kids (16.36%) and young animals (33.94%). Conversely, a contrasting pattern was observed in sheep, with lambs (33.33%) showing higher susceptibility than adults (15.79%).

The present findings align with a prior study carried out in Pakistan by Rasheed *et al.* (2020) who also suggested higher seroprevalence in adult animals (69.02%) compared to young animals (62.94%) from Gilgit Baltistan province. Similar trends were also observed by De *et al.* (2016); Dubie *et al.* (2022); and Nkamwesinga *et al.* (2023) from Nigeria, India, Ethiopia and Uganda respectively. Conversely, Tajpara *et al.* (2022) documented that young age category showed highest seroprevalence. The higher susceptibility of adult animals to PPR virus may be due to long lifetime allowing more likelihood of being exposed to PPR virus (Salih *et al*., 2014). Higher susceptibility in young sheep is also supported by the evidence of Bello *et al.* (2016) and Mahmoud *et al.* (2017) who also reported greater seroprevalence in young animals compared to adults. The lack of a statistically significant association between age and positivity in sheep suggests that antibodies are present across all age categories, and the virus circulates consistently among sheep of all ages. This observation is supported by the notion that animals in the most susceptible age group (adults) typically succumb to the virus upon exposure, while only those with some degree of resistance survive. Consequently, the detection of antibodies in serum samples from age groups other than adults is reasonable (Shuaib *et al*., 2014).

Analysis of risk factors

Table IV shows the risk factors associated with PPR seroprevalence i.e. herd size, body condition and season.

Table IV. Chi-square analysis of associated risk factors of PPRV seroprevalence.

Risk factors	Total samples screened	Goats		Sheep		Total samples positive	Total seropositive (%)
		Samples screened	Seropositive (%)	Samples screened	Seropositive (%)		
Herd size							
Large (n= >100)	181	100	49 (49.00)	81	31 (38.27)	80	44.19
Medium (n= >50-100)	137	104	42 (40.38)	33	2 (6.06*)	44	32.12
Small (n= <50)	437	412	115 (27.91)	25	7 (28.00)	122	27.91
		P= <0.0001***; χ^2=18.79		P= <0.0026*; χ^2=11.88		P= 0.0004***; χ^2=0.99	
Body condition							
Good	628	508	152 (29.92)	120	32 (26.67)	184	29.29
Poor	127	108	54 (50.00)	19	8 (42.11)	62	48.81
		P= <0.0001***; χ^2=16.13		P= 0.167; χ^2=1.908		P= <0.0001***; χ^2=0.992	
Season							
Summer	231	212	94 (44.34)	19	7 (36.84)	101	43.72
Winter	524	404	112 (27.72)	120	33 (27.50)	145	27.67
		P= <0.0001***; χ^2=17.25		P= 0.403; 0.698		P= <0.0001***; χ^2=0.996	

Herd size

The results of this study revealed the highest seroprevalence of PPR infection in large herds (44.19%; n= >100), while ruminants in smaller herds (n= <50) showed the lowest seroprevalence (27.91%). All findings were statistically significant, with a p-value of <0.0001 (Table V). A similar trend was observed in the case of goats. On the contrary sheep in small herds (28%) were significantly more affected than medium herds (6.06%; n= >50-100) at P= (0.022). The present results agree with the findings of Selvaraju (2014), Gebre *et al*. (2018) and Dubie *et al*. (2022). This direct association proves the highly contagious nature of disease and rapid transmission in overcrowded herds. The observed significant correlation between herd size and PPRV cELISA positivity may stem from the common practice among owners and herders, irrespective of herd size, of engaging in communal grazing and/or watering activities. Consequently, during these communal interactions, all animals are equally susceptible to PPRV infection by virtue of being in proximity to potentially infected animals (Shuaib *et al*., 2014).

Table V. Prevalence of PPRV antigen using sandwich ELISA.

Sample type	Total samples	Positive samples	% Positive
Nasal swabs	18	16	88.88
Tissues	8	3	37.50
Fecal samples	8	1	12.5
Total	34	20	58.82

Body conditions

In the present study all goats and sheep were classified into two categories based on their body conditions: poor and good. Chi-square analysis revealed a noteworthy association between body condition and the prevalence of peste des petits ruminants. The overall prevalence of PPR was found to be higher in animals with poor body conditions (48.81%) compared to those with good body conditions (29.29%). Similar trend was also observed in the case of goats (P<0.0001) and sheep (P=0.167). Our findings are in alignment with the research outcomes reported by Rath *et al*. (2020) who observed greater seroprevalence in sheep and goats with moderate body condition (97.55%) compared to good ones (4.55%). The poor body condition could be associated with incidence of PPR infection (Dubie *et al*., 2022). The heightened vulnerability to disease observed in small ruminants with inadequate body conditions is linked to nutritional imbalances and pre-existing parasitic infestations, as previously documented (Selvaraju, 2014).

Season

Rapid fluctuations in weather conditions, including sudden temperature shifts, prolonged droughts, and persistent rainfall, negatively impact the disease dynamics (Rahman *et al*., 2023). In summer season 43.72% of seroprevalence was observed which is highly significant (P<0.0001) compared to winter season (27.67%) as presented in Table V. Similar trends were observed in overall small ruminants as well as in goats at P<0.0001. Sheep were also more affected in summer, but the difference was non-significant (P=0.403). Rasheed *et al*. (2020) and Rath *et al*. (2020) also reported higher seroprevalence in summer season compared to winter. Rahman *et al*. (2023) reported higher seroprevalence in rainy and summer season and observed variation could stem from distinct geographic locations and study durations. Establishing a scientifically robust disease prevention policy could leverage insights into the seasonal fluctuations of PPR. For instance, scheduling vaccination campaigns prior to the onset of winter and rainy seasons could be considered as a preventive measure.

Prevalence of PPRV antigen

Out of 34 swab samples tested for prevalence of PPRV antigens confirmed the presence of infection in population. It was observed that 20 samples were positive for PPRV antigen with prevalence of 58.82% (Table III). The research conducted in Khyber Pakhtunkhwa, Pakistan, Kabir *et al*. (2020) reported higher prevalence of PPRV antigen (78.95%) than our findings (58.82%). In a latest study Abubakar *et al*. (2018) reported 54.17% prevalence in AJK. This indicated a gradual increase in the disease status.

CONCLUSIONS

The study reveals 32.58% seroprevalence of PPR among goat and sheep population in Azad Jammu and Kashmir. The higher prevalence shows previous exposure of sheep and goats to PPR virus that emphasizes a greater vulnerability of small ruminants and establishes a variable impact of PPR across different districts, species, sex, and age groups. Additionally, the data demonstrated that goats are more vulnerable to the PPRV than sheep. These findings highlight the need for a comprehensive vaccination campaign, biosecurity measures, restricted animal movement. This study emphasizes the crucial role of continuous surveillance and modified strategies to address the disease effectively, aiming to protect livestock health and support not only the livelihoods of local herders in AJK but also the adjoining regions. Additional investigations are required to enhance our comprehension of the epidemiology of PPR and to perform phylogenetic characterization of the circulating viruses.

DECLARATIONS

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IRB approval

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

Ethical approval

Approval for ethical issues was obtained from Advanced-Studies and Research, Board at the University of Azad Jammu and Kashmir, Muzaffarabad.

Statement of conflict of interest

The authors have declared no conflict of interest.

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