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Research Article

Sero-prevalence of Peste des Petits Ruminant (PPR) Virus in Sheep and Goat Population of Gilgit Baltistan Province of Pakistan

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MR conducted the research. TA supervised this study. NM, MFS MI and SY helped in sample collection and reviewing the article.

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Goat, HI, PPR, Seroprevalence, Sheep

Abstract | Peste des petits ruminants (PPR) caused by PPR virus (PPRV), is a contagious disease of domestic and wild small ruminants. The disease is endemic in developing countries of African and Asian worlds including Pakistan, where several clinical cases in small ruminants (sheep and goat) have been frequently reported. Despite PPRV is endemic in Pakistan, information on disease serosurveillance of prevailing strains in Gilgit-Balitistan (GB) territory is scarce. Therefore, the current study was designed to assess the seroprevalence of PPRV and to evaluate potential risk factors involved in the transmission of PPR disease in four distinct locations of GB province. We reported occurrence and risk factor analysis of PPR in small ruminants (n=1000) originating from different places in district Gilgit using Hem agglutination Inhibition (HI) test followed by risk analysis through Open-Epi software. Serum samples including goats n=500 and sheep n=500 were collected from different herds situated at Naltar lake, Tattovat, Fairy meadows, Bangle, and Naltar. Overall a comparable prevalence was identified for both goat and sheep (46% vs 44%, P > 0.05). Future studies are necessary to further ascertain the study outcomes and elucidate the molecular epidemiology of prevalent strains in the said geographical locations for better disease control and management interventions.

Novelty Statement | It is the first report from Gilgit-Balitistan which ascertain necessary intervention such as vaccination on mass scale, animal movement control etc for disease management in future.

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Introduction

Peste des petits ruminant's (PPR), also called goat plague or Kata prevalent in domestic and wild animals (Banyard *et al.*, 2015); however, it also has been reported in large animals such as buffalos, cattle and camel. The virus belongs to genus *Morbillivirus* within family *Paramyxoviridae* (Couacy-Hymann *et al.*, 2007). The disease is highly infectious and is endemic throughout the Middle East and many Asian regions (Banyard *et al.*, 2016) with significant economic losses (Albina *et al.*, 2013). The disease is considered notifiable owing to its economic importance and food security risk. Since it can cross boundaries such as evidenced in countries bordering Pakistan, Afghanistan, Iran, India and China (Wang *et al.*, 2009; Munir *et al.*, 2015; Mohebbi *et al.*, 2018), It is transboundary in nature. The PPR has been reported by FAO and OIE as class one disease to be eradicated (OIE and FAO, 2015). PPR virus affects sheep, goats and related

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species, also effect camel from large animals and becoming a highly contagious viral disease. The most important finding of pathological process of PPR can be observed in the respiratory and digestive system. Morbidity and mortality rates also give an idea about the pathogenicity measure of virus. Vaccination is a way to control PPR, required on mass scale; losses can be limited by prevention but for this, a deep knowledge of pathogenesis of disease is very important. Pakistan has an agriculture-based economy. According to Economic survey of Pakistan (GOP, 2017), among the meat-producing animals, goats and sheep are regarded as "poor-man cow", and the need for HALAL meat and its export has increased the importance of these animals. However, the biggest obstacle is the irresistible disease, the lack of a legitimate and the appropriate vaccine. A high cost of rearing is associated with the occurrence of PPR in a herd in China (Banyard *et al.*, 2014) and same could be considered true for similar setting in Pakistan e.g., Gilgit-Baltistan, where a large number of farmers are dependent for their routine livelihood on small ruminant. By seeing highly contagious nature of disease, its burden in affected population and subsequent economic losses, studies elucidating the nature of infection and its prevalent status to devise appropriate interventions is much required.

Materials and Methods

This research was performed to evaluate the rate of occurrence of PPR virus, in small ruminants from different areas in Gilgit Baltistan and included Tattovat, Fairy-Meadow, Naltar Lake and Bangle Naltar. Also, the study analyzed a potential association among a number of risk factors that could predispose occurrence of disease in animals and its subsequent spread in the surrounding susceptible population. The risk factors included animal species, age, gender and season. The study samples were collected during a period of one year from 2016 -2017 and were processed and analyzed at Faisalabad Institute of Research Science and Technology (FIRST), Faisalabad.

Collection of blood samples

The study involved aseptic collection of 1000 blood samples (500 for goat, 500 for sheep), through jugular vein. These samples were collected from animals that were reared under open-house grazing method, exhibited flu like symptoms such as cough, nasal discharge, high temperature and pustules were clinically suspected for PPR. Briefly, the test animal was properly restrained at the spot and the left side of the throat of animal was pressed to find the jugular vein. The neck was shaved to locate the jugular vein position as assess sero-positivity in small ruminants, 1000 samples (500 for goat, 500 for sheep) of blood, collected aseptically through jugular vein of sheep and goats reared under open-house grazing method (Forsyth and Barrett, 1995). The parameter like age of animal, breed, gender and geographical location along with locality was also noted during sampling. Blood samples were transferred to EDTA vacutainers for plasma separation. All samples were properly labeled by placing date and sample ID on the vacutainers. The ID was traced from the data file containing information about the age, breed, gender, locality and geographical location of collected sample. The samples were transferred to research lab of Microbiology, FIRST in ice packs.

Figure 1: A map showing geographical location of Gilgit-Baltistan and sampling sites indicated by red-circles.

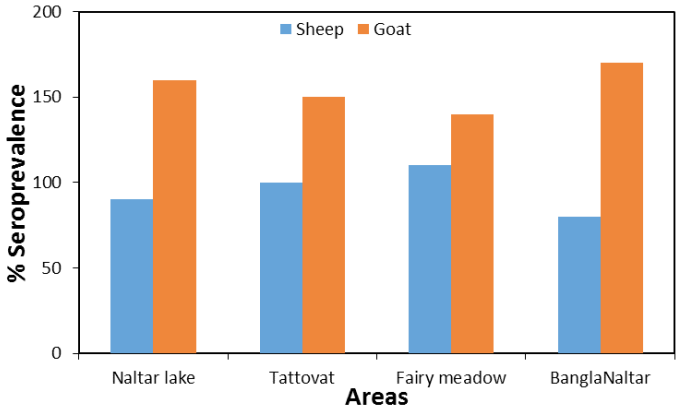

Figure 2: A graphical description of prevalence of disease in different areas of Gilgit Baltistan, Pakistan.

Processing for plasma separation

Once reached in the laboratory, the blood samples were processed for plasma separation. The vacutainer was centrifuged at 4000 rpm for 10 minutes at 4 °C (Dhar *et al.*, 2002). The plasma (supernatant) was separated and aliquots were made in pre-labeled sterile plastic microfuge tubes having capacity of up to 2 ml after that all samples were stored at -20 °C till its further use for serology. A brief history of herd animal health was taken and according to that animals were healthy apparently.

Table 1: Specie wise prevalence of PPR virus in sheep and goat population of Gilgit District, Gilgit-Baltistan, Pakistan.

Results for specie wise prevalence in Gilgit Baltistan area							
Specie	Samples	Positive	Negative	df	OR	95% CI	P value
Sheep	500	220	280	1	0.9224	0.7189-1.1834	0.5240
Goat	500	230	270	1			
Total	1000	450	550		-	-	-

Table 2: Gender wise prevalence of PPR virus in sheep and goat population of Gilgit district, Gilgit-Baltistan, Pakistan.

Gender	Samples	Positive	Negative	Df	OR	95%CI	P value
Female	526	268	258	1	1.6666	1.2954-2.1441	0.08
Male	474	182	292	1			
Total	1000	450	550	-	-	-	-

Table 3: Age wise prevalence of PPR virus in sheep and goat population of Gilgit District, Gilgit-Baltistan, Pakistan.

Age	Samples	Positive	Negative	Df	OR	95% CI	P value
>1 year	552	381	171	1	1.311	1.008-1.7066	0.0431
<1 year	448	282	166	1			
Total	1000	663	337	-	-	-	-

Table 4: Season wise prevalence of PPR virus in sheep and goat population of Gilgit district, Gilgit-Baltistan, Pakistan.

Season	Samples	Positive	Negative	Df	OR	95% CI	P value
Summer	242	25	217	1	0.0903	0.0582-0.1399	0.1842
Winter	758	425	333	1			
Total	1000	450	550	-	-	-	-

Hemagglutination inhibition assay

The hemagglutination inhibition test was performed to check the sero-positivity of blood samples for corresponding antibodies against PPR as per method taken from (Anderson and McKay, 1994), in Microbiology laboratory of FIRST, Pakistan.

Statistical analysis

All the data collected was entered into MS Excel (Mic Co.) computer program and Geometric mean titre (GMT) was calculated as suggested by (Brugh, 1978). An overall analysis was carried out by Chi-square analysis and 95% confidence interval (CI).

Results and Discussion

Since the study employed antibody-based detection of PPR, the findings of the study could be implicated to express current as well as previous exposure of animals to PPR. For this particular purpose, the study used HI for detection of PPRV antibodies and the outcome which falls within the range of $4\log_2$-$9\log_2$. Out of total samples processed, though the prevalence rate was almost comparable and a non-significant difference was observed for both animal species, it was higher in goat than sheep (46% vs 44%, P> 0.05). A brief summary of prevalence rate according to location in district Gilgit, is presented in (Table 1). While considering sex-based prevalence of antibodies corresponding to PPR, the occurrence was found to be significant (P < 0.05) again, however, it was higher in female (69.96%) as compared to male (63.92%). Nevertheless, evidenced by the calculated GMT, the amount of antibody titer was higher in male (15.92) than female (14.83) indicating their capacity to generate a strong immune response upon exposure to infection. Adult animals with age > 1 year (69.02) as compared to young animals with age <1 year (62.94%). Further, while the influence of age was analyzed, a high antibody titer was observed for animals greater than one year of age (17.91) as compare to GMT from the animals of less than one year of age (12.85). As influence of season in further dissemination of disease is concerned, there was a non-significant difference (P>0.05) between the prevalence rate according to season. The study shows higher seroprevalence of PPR virus in summer season 72.72% followed by 65.09% in winter, 64.91% in autumn and 61.73% in rainy season. The GMT was found to be higher (20.18) in summer season (Dec-Feb), 14.03 in autumn season (Sep-Nov) and lowest GMT (13.77) during rainy season (Jun-Aug).

The main goals of the present study were to determine the seroprevalance of PPRV in domestic animals in an area near such as Gilgit Baltistan bordering the neighboring countries. As documentation on prevalence and seroprevalance on PPRV are not available from these areas before the present study. The current study has revealed a high seroprevalance of antibodies to PPRV were observed in goats as compared to sheep (Rehman *et al*., 2011). The findings of the present study are in agreement with previous reports on epidemiological studies of PPRV in domestic small ruminants, in which antibodies to PPRV showed high incidence in goats rather than sheep using various techniques (Dhar *et al*., 2002; Ozkul *et al*., 2002). But our study results are contrasting with the findings through another study (Khan *et al*., 2011), who observed a high seropositivity in sheep (51.3%) than the goats (39%) using monoclonal c-ELISA.

The present study also showed that, high prevalence of antibodies was detected from the animals of older than one year of age as compare to younger animals. These findings are in agreement with previous studies (Abubakar *et al*., 2017) from Pakistan, in which PPRV antibodies based

prevalence found higher titers in adult animals of 1-2 years and > 2 years rather than < 1 years of age. Similarly, previous report by (Aziz ul Rehman *et al*., 2016) reported a high antibody revalence of 69.9% in 1-2 y old animals when compared to other age group. Keeping in view of the passive immunity from the vaccinated mother also contributed in the protection of younger animals aged up to 4 months from the disease outbreak from PPRV. Most outbreaks have been observed in humid condition perhaps due to virus survival in low temperature environment, seasonal prevalence is controversial regards incidence of PPRV antibodies because of manage mental practices, environmental, nutritional and socioeconomic conditions, under which animal is kept.

In conclusion, the study provides a preliminary insight towards the presence of PPR infection in small ruminant in Gilgit-Baltistan area. Further studies with a large dataset covering a wide geographical region is much necessary to better elucidate disease control and management strategies.

Conflict of interest

The authors have declared no conflict of interest.

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