Short Communication

Serological Detection of Peste des Petits Ruminants Virus (PPRV) in Sheep and Goats of Muzaffargarh District in South Punjab, Pakistan

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Abstract | Peste des petits ruminants virus (PPRV) causes an economically important disease of small ruminants that causes extensive livestock losses across areas where it is endemic. A serological survey on the prevalence of antibodies to PPRV was carried out in sheep and goats of Jatoi and surrounding areas in Muzaffargarh districts South Punjab of Pakistan. A total of 965 serum samples were collected from sheep and goat's farms from January to December 2015 with no history of vaccination. Haemagglutination inhibition (HI) test was performed using PPRV antigen to determine antibody titres level. The overall prevalence of antibodies to PPRV in goats and sheep were 66.22% (521 samples) and 65.76% (444 samples), respectively. Sero-prevalence of anti-PPRV antibodies was found higher in adults (69.14% with GMT 17.91) than in young (62.47% with GMT 12.85). Furthermore, seasonal seropositivity seasons was recorded and sero-prevalence during summer was found to be higher (72.84% with GMT 20.18) than other seasons with an overall PPRV seroprevalence of 66.01%. Moreover, related to sex discrimination, 69.80:63.83% (GMT 14.83:15.92) antibody titre has been screened in female and male, respectively. So, based on the sero-prevalence, the evaluation of intrinsic (age groups and sex) and extrinsic factors (season fluctuation) clearly indicated the occurrence of previous outbreaks of PPR virus due to persistency of virus in endemic regions.

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Peste des petits ruminants (PPR) also known as goat plaque (KATA), is a highly contagious viral disease that attacks many species of domestic and wild animals (Dhar et al., 2002; Jubb, 2007; Asim et al., 2009). The French acronym PPR is commonly used worldwide. The causal agent is the PPRV, which is a negative-sense non-segmented ssRNA virus belonging to the genus Morbillivirus in family *Paramyxoviridae* (Gibbs et al., 1979). Based on molecular analysis of the fusion protein (F-gene), PPRV has been divided into four different lineages I, II, III, IV (Shaila et al., 1996; Muniraju et al., 2014). The PPRV epidemics has been observed worldwide including developing countries (FAO, 2013) after first noticed in Ivory coast in 1942 during 2nd world war (Gargadennec and Lalanne, 1942). The first outbreak of PPR has been observed in Pakistan since 1991 (Ather et al., 1995), while further PPRV epidemics were also documented (Pervez et al.,1993; Tahir et al., 1998; Hussain et al., 2003; Zahur et al., 2008).

In Pakistan, PPR infection is a major constraint against

the development of both national and rural socio-economic production because of loss annually (Abubakar and Munir, 2014). In regards to clinic-pathology, the said virus showed per-acute and acute form of the disease with oro-nasal discharge, hyperemia, diarrhoea, abortion (Abubakar et al., 2011). The morbidity rate is 100 % and in severe outbreaks, mortality reaches up to 100 % (Radostits et al., 2000). The transmission of PPRV occurs through newly introduced unscreened animal, selling or giving away sick animal, exposure to fecal and other excretions from infected to healthy animal and contact with contaminated feed, water, equipment and clothing (Abu Elzein et al., 2004). Due to the high amount of virus shed through secretion and excretion by morbid animal (Braide, 1981), PPRV gets disseminated to other healthy animals. Geographically, some factors also have a significant role in the transmission of the virus (Abubakar et al., 2009; Ul-Rahman et al., 2016). A survey on PPR reported that the virus has become an endemic infection of sheep and goats fand widely distributed all across the country (Zahur et al., 2011). Previously, the control of PPR involved the utilization of heterologous rinderpest (RP) vaccine (Plowright and Ferris, 1962) and later replaced by the homologous vaccine (Dialo et al., 2004). Vaccination has been reported as the only safeguard against endemic PPRV (Bourdin, 1983). Following the successful global eradication of rinderpest virus, peste des petits ruminants virus has also been targeted for global eradication by OIE (2008).

After rinderpest eradication from Pakistan, the use of rinderpest vaccine for the control of PPR in small ruminants has been restricted in order to avoid complications in disease sero-surveillance. However, a homologous vaccine (Nigeria 75/I) is being introduced to immunize the susceptible population (sheep and goats) against this highly contagious disease (Asim et al., 2009). The infection still occurs in Pakistan every year in the form of epidemic in domestic population, creating one of the major constraint in goat farming in Pakistan (Abubakar and Munir, 2014). In order to formulate an appropriate vaccination schedule and control measures, the serological status of PPRV among domestic small ruminants need to be elucidated.

The disease is endemic in Pakistan and causes considerable economic losses due to its high morbidity and mortality. The study was undertaken to investigate current immune status of PPR virus circulating in unvaccinated animals for future vaccination programme.

Materials and Methods

Study Area

Punjab province (31°N 72°E) is located along the North-western edge of the geologic Indian plate in South Asia. Punjab province is located 139 m above sea level, and it is the most populous province (approx. 56% of Pakistani lives in this area). Geographically, the province has been divided into smaller units that comprise several parts. Administratively, South Punjab include 13 Districts, Muzaffargarh district was selected (Jatoi, Alipur, Kalarwali, Ruhilawali and Khan garh cities) based no previous reports on sero-prevalence of PPRV antibodies to be studied.

Sample Collection

This study was conducted jointly by Veterinary Research Institute and Quality Operation Laboratory, University of Veterinary and Animal Sciences, Lahore, Pakistan from January to December 2015. A total of nine hundred and sixty-five samples (goats 521 and sheep 444) were collected randomly from different farms (25 samples from each farm). Approx. 2-3 ml of blood was collected aseptically from jugular vein of each animal and allowed to clot. The sera were separated and stored at -20°C until the haemagglutination inhibition test was carried out.

Haemagglutination Inhibition (HI) Test

To check the antibody titre against PPR virus, this test was performed using a reference strain of PPR (PAK-13/VRI) as control antigen obtained from Veterinary Research Institute, Ghazi Road Lahore. HI test was done according to the protocol described by (Alexander and Chettle, 1977) by 2-fold serial dilution of serum samples using 4 haemagglutinating (HA) unit of PPR virus. The antibody titre of serum samples was measured by observing the button formation due to settling of RBC's. To estimate the HI titre of serum samples, haemagglutination inhibition was considered as an end point at which sera were at maximum dilution. Using OIE recommended criteria in our work, Samples were categorized as seronegative if sera had titre of <1:16 and sera with >1:8 titres were considered to be positive (Musa et al., 2009). For the evaluation of risk factors for the spread of disease, the collected data were distributed into various categories of intrinsic (Breed, Sex, Age) and extrinsic factors (Seasons).

Statistical Analysis

For descriptive statistics, collected data were entered

into computer program MS Excel (Microsoft Co.). Geometric mean titre (GMT) of HI was calculated as designated by (Brugh, 1978). Univariable analysis was carried out by 95% confidence interval (CI) and Chi-square analysis.

Results and Discussion

Hemagglutinin inhibition test was performed in the present study as per instructions of OIE (2009), to detect the PPRV antibodies titre which fall between $4\log^2$-$9\log^2$ range. A total of 965 serum samples (goats= 521, sheep= 444) were collected from different farms and were subjected to HI test. Out of them, Goats=66.22% and Sheep=65.76% were found positive for presence of PPRV antibodies (Table 1). High sero-prevalence of antibodies to PPRV observed in goats as compared to sheep (Rashid et al., 2008). 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 in contrast with the findings of Khan et al. (2008b), who observed the high sero-positive in sheep (51.3%) than the goats (39%) using monoclonal c-ELISA.

Regards to PPRV-HI titre in females, results showed variation from 2^4 to 2^9 as respective percentage 16.40, 17.59, 13.63, 110.87, 7.91 and 1.58% with a geometric mean titre (GMT) of 14.83 and 69.80 % sero-positivity but in male HI antibody titres varied from 2^4 to 2^8 (their respective percentages were 11.98, 8.71, 14.81, 14.38, 7.19 and 6.75%) with a GMT of 15.932 and 63.83% sero-positivity (Table 2). Sex based determination of PPRV antibodies, female showed high prevalence of antibodies as compared to males with high GMT in males. In concordance to the previous studies, Khan et al. (2008b) and Ul-Rahman et al. (2016) reported the female animals were at most risk with high existence of antibodies to the males. This may be related to the physiological differences between female and male, where females reveal some degree of infection resulting from stress due to milk production and pregnancies. Due to significance of productivity potential, females maintained for a longer period of time as compared to males, thus increasing the likelihood for female animals to be exposed to PPRV over time.

There is variation in PPRV infection among animals based on age group, the current study revealed the high existence of PPRV antibodies in adult animals of >1 year (69.14%) as compared to young animals of <1 year (62.47%). With higher proportions of sero-positive, percentage of HI titre in >1 year adult animals were 14.65,11.33, 16.99, 13.67, 9.18 and 3.32% associated to 17.91 GMT. Concerning young animals <1 year, the percentage of HI titre were 13.91, 15.67, 11.04, 11.26, 5.74 and 4.86% with 12.85 GMT (Table 3). These findings are in agreement with previous studies (Abubakar et al., 2011) from Pakistan, in which PPRV antibodies based prevalence found higher titres in adult animals of 1-2 years and > 2 years rather than < 1 years of age. Similarly, previous report by Ishag et al. (2014) reported a high antibody prevalence of 69.9% in 1-2 y old animals when compared to other age group.

Table 1: *Variation of GMT related to same age but in different species*

Species	Positive samples	Antibody titre obtained by HI test									GMT	Mean±SE	C.I 95%	X^2
		2^1	2^2	2^3	*2^4	2^5	2^6	2^7	2^8	2^9				
Goat	521(66.22%)	37	74	65	79	72	71	69	31	23	15.07	57.50± 9.79	32.32;82.68	0.486 NS
Sheep	444(65.76%)	8	70	74	59	57	66	52	42	16	15.63	48.67±7.30	29.90;67.44	
Total	965(66.01%)	45	144	139	138	129	137	121	73	39	15.49	107±13.99	32.78;69.32	

*: *Protective threshold;* **HI:** *Hemagglutinin inhibition;* **GMT:** *Geometric mean titre;* **SE:** *Standard error;* **CI:** *Confidence Interval;* **NS:** *Non-significance*

Table 2: *Antibody titre of tested sera based on sex distribution*

Sex	Positive samples	Antibody titre obtained by HI test									GMT	Mean±SE	C.I 95%	X^2
		2^1	2^2	2^3	*2^4	2^5	2^6	2^7	2^8	2^9				
F	506(69.98%)	35	71	56	83	89	69	55	40	08	14.83	57.55±12.29	25.72;88.95	0.577NS
M	459(63.83%)	10	73	83	55	40	68	66	33	31	15.92	48.83±6.70	31.61;66.06	

For abbreviations see Table 1

Table 3: *Age wise distribution of PPRV antibodies*

Age group	Positive samples	Antibody titre obtained by HI test									GMT	Mean±SE	C.I 95%	X^2
		2^1	2^2	2^3	$*2^4$	2^5	2^6	2^7	2^8	2^9				
Age>1 year	512(69.14%)	8	73	77	75	58	87	70	47	17	17.91	59.00±10.12	33.00;85.00	0.399 NS
Age<1 year	453(62.47%)	37	71	62	63	71	50	51	26	22	12.85	48.40±9.76	19.30;72.50	

For abbreviations see Table 1

Table 4: *Season fluctuation, relationship between GMT and sero-prevalence*

Season	Positive samples	Antibody titre obtained by HI test									GMT	Mean±SE	C.I 95%	X^2
		2^1	2^2	2^3	$*2^4$	2^5	2^6	2^7	2^8	2^9				
Mar-May (Summer)	232(72.84%)	-	34	29	36	38	35	29	20	11	20.18	28.17± 4.34	16.99;39.34	0.854 NS
Jun-Aug (Rainy)	274(61.68%)	26	43	36	23	40	43	30	20	13	13.77	28.17±4.78	15.87;40.47	
Sep-Nov (Autumn)	245(64.89%)	19	28	39	48	19	33	32	18	09	14.03	26.50±5.68	11.89;41.11	
Dec-Feb (Winter)	212(65.09%)	-	39	35	31	32	26	28	15	06	14.13	23.00±4.21	12.18;33.82	

For abbreviations see Table 1

Most outbreaks have been observed in humid condition perhaps due to virus survival in low temperature environment. In regards to seasonal fluctuations, higher (72.84%) PPRV antibodies were observed in summer followed by 65.09% in winter, 64.89% in autumn and 61.68% in rainy season. As concern to HI test titre in summer season (Mar-May), the percentage were 15.52, 16.38, 15.09, 12.50, 8.62 and 4.74% with a high GMT of 20.18 has been observed against rainy season (Jun-Aug) GMT of 13.77 to 8.39, 14.58, 17.41, 10.95, 7.30, and 4.74% with respective titre level (Table 4). In the autumn season (Sep-Nov), the HI test showed 14.03 GMT with the percentage of respective titre were 19.59, 7.75, 13.47, 13.06, 7.35 and 3.67% as compared to high GMT (14.13) in the winter season (Dec-Feb) related to the titre percent in respective well as 14.62, 26.45, 21.49, 13.21, 12.39 and 2.83%. The issue of seasonal prevalence is controversial regards incidence of PPRV antibodies because of managemental practices, environmental, nutritional and socioeconomic conditions, under which animal is kept. In a previous study (Khan et al., 2008a), the high existence of PPRV has been observed in winter season with high antibodies titre potentially in contrast to our findings. Climatic factors favourable for the survival and spread of the virus likely contribute to the seasonal distribution of PPR outbreaks. The presence of PPRV infection in Punjab has been reported already by Tahir et al. (1998) and Hussain et al. (1998) with significance observation in unvaccinated animals, but information from South Punjab is still scarce. There is need of time to investigate the immune status of animals of such areas where PPR is endemic and South Punjab is one of them.

Conclusion

In endemic areas, the extrinsic factors such as season fluctuation has impact on PPRV epidemics, which is actually a significant rural socio-economic loss. The present study reveals that Muzaffargarh district is at most risk as an endemic region of PPR disease. But documentation on prevalence and sero-prevalence are not available before the present study. In addition, based on HI titre results, it is estimated that previously outbreaks infected small ruminants population in the same region. So, there is need of candidate vaccine programme to eradicate PPRV from these areas. In fact, several parameters related to vaccine efficacy and field circulating virus should be evaluated for the implementation of a successful PPRV vaccination program before and after vaccination. Moreover, the livestock and dairy development (L&DD) department of Pakistan should raise significant steps to improve productivity of small ruminants by controlling the PPRV outbreaks in endemic regions, particularly in Muzaffargarh district.

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Conflict of Interests

The authors declare that they have no competing interests.

Authors' Contributions

AR and MA apprehended the idea and drafted the skeleton of manuscript. AR and FY collected samples and NA tested in laboratory. MA did editing and final checking of errors and all authors read and approved the final manuscript.

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