



Serological Detection and Confirmation of PPR Among Sheep and Goat Kept under Different Production Systems

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ABSTRACT

The current study was executed to explore the prevalence of PPR in different production systems of sheep and goat in district Nowshera, KPK. A total of 246 serum samples were randomly collected from three age groups (<1 year, 1-2 year and >2 years) of sheep and goats. Competitive ELISA was performed to evaluate sero-prevalence of PPR in goats and sheep. The swab and tissue samples were collected from the surrounding areas from suspected sheep and goats to confirm the PPRV. Out of total 246 sera, 144 (58.54%) samples were found seropositive for PPRV. PPR was more prevalent in sheep (61.82%) as compared to goats (55.88%). In sheep population, the highest prevalence was recorded in the transhumant production system with 73.3% seropositive for PPRV. Younger animals seemed to be more susceptible to PPRV infection, 58.82% goats of age less than 1 year were seropositive for PPRV as compared to 51.43% of goats above 2 years. Results of antigen detections showed that the outbreaks were going in study population and confirmed positive for PPRV by I_cELISA and RT-PCR (N-gene). The study revealed a comparison of PPRV occurrence in the different production system of small ruminants and on-going disease outbreaks in the target area.

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Authors' Contribution

AK and AHM conceived the idea. AI, JKZ and MA conducted the experiment and all authors participated in write up and approved the manuscript.

Key words

Peste des petits ruminants, Production system, Sheep, Goat, Competitive ELISA

INTRODUCTION

Goat and sheep maintain particularly, a valuable economic and ecological place in Asian agriculture. The population of goats and sheep in Pakistan is about 68.4 and 29.4 million heads, which produce 44.6,000 tons of wool, 25.8,000 tons of hair and 671,000 tons of mutton annually (PES, 2014-2015). In Pakistan, sheep and goat are raised in various ways depending on the territorial conditions, availability of water and food stuff and change of weather. The production system of goats and sheep is classified into three types i.e. nomadic, transhumant and sedentary.

Peste des petits ruminants (PPR) is an acute, contagious disease caused by peste des petits ruminants virus (PPRV), a morbillivirus (Asim *et al.*, 2009). Two primary hosts of PPRV are sheep and goats (Khan *et al.*, 2008).

Mortality rate ranges from 40-80% in acute cases, but morbidity and mortality rates are generally lower in endemic areas (Balamurugan *et al.*, 2014). The disease is transmitted by direct contact involving secretions or excretions from infected animals to healthy animals in close proximity. PPR was reported in Pakistan in 1994 and the confirmatory diagnosis was made by polymerase chain reaction and further validated when the PPRV antigen was detected during outbreaks in different areas using I_cELISA (Hussain *et al.*, 2002).

A serological study showed 54.09% sheep and 44.14% goats of Pakistan are sero-positive for antibodies against PPRV (Abubakar *et al.*, 2008). PPR is a disease of major economic importance and imposes a significant constraint upon sheep and goat production owing to its high mortality rate (Zahur *et al.*, 2009). Loss of rupees 20.5 billion (US\$, 0.24 billion) has been reported from Pakistan due to the PPR (Abubakar *et al.*, 2015).

A tentative diagnosis of PPR is made based on clinical signs whereas laboratory confirmation of PPR is carried out by immuno-capture ELISA (I_cELISA) and competitive

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ELISA (cELISA). Reverse transcription polymerase chain (RT-PCR) has been shown to be useful for the rapid detection of morbillivirus RNA in sample submitted for laboratory diagnosis (Shaila *et al.*, 1996). The present study was designed to evaluate PPR prevalence among sheep and goat reared under different production systems in nowshera, khyber pakhtunkhwa, Pakistan.

MATERIALS AND METHODS

Study designs

The study was carried out in the district Nowshera, Khyber Pakhtunkhwa (KPK) and its surroundings during 2016 where sheep and goats were kept under different production systems. A total of 246 (110 from sheep and 136 from goat) serum samples were randomly collected from three different age groups of sheep and goats, <1 year, 1-2 years and >2 years. The collected samples were labeled accordingly to allow for identification of each animal and flock.

Serum samples testing

Competitive ELISA was performed to check antibody titer against PPRV in sheep and goats as per protocol outlined in the user manual supplied with commercial PPR cELISA kit (IDvet, Grabels-France) in National Veterinary Laboratory (NVL), Islamabad, Pakistan.

Antigen detection of PPRV by RT-PCR and IcELISA

During the course of above serum samples collection, there were reports of PPR suspected outbreaks in the surrounding areas. Therefore, additionally, 19 swab samples from nasal, eye discharges, saliva and oral ulcers of suspected animals were collected. Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) was performed of these swab samples by one-step RT-PCR Kit (Qiagen, USA) using designed primers of fusion protein (N) gene. Anti-N monoclonal antibodies based ELISA was also performed with the help of Kit produced by BDSL Company, UK with the collaboration of Flow Laboratories and CIRAD, EMVT, France.

Data analysis

Data collected, tabulated and analyzed by using excel sheet of Microsoft version 2010 of all the PI values obtained by ELISA plate reader (Multiskan Plus, LabSystem, Finland).

RESULTS

Sero-prevalence of peste des petits ruminants (PPR)

A total of 246 serum samples were examined by cELISA to estimate the prevalence rate of PPR in sheep and goats. Table I shows the overall prevalence of PPR among sheep and goats in the study area. An overall prevalence rate of 246 (58.54%) was recorded in both species i.e. sheep and goats. The prevalence of PPR was recorded higher 61.82% in sheep than goats 55.88%.

Table I. Prevalence of PPR in sheep and goats of district Nowshera, KPK (n=246).

S/No.	Animals	No. of animals examined	No. of animals infected	Prevalence %
1.	Sheep	110	68	61.82
2.	Goats	136	76	55.88
Total		246	144	58.54

Prevalence of PPR in different production systems of sheep and goats

The prevalence of PPR in different production systems of sheep and goats of district Nowshera is depicted in Table II. Our findings revealed a prevalence of 62.86, 62.00 and 56.00 percent in Nomadic, Transhumant and Sedentary production systems of sheep as compared with prevalence of 54.55, 73.33 and 53.03 percent in goats, respectively.

Age wise prevalence of PPR

As depicted in Table III, the prevalence rate of PPR was observed in three age groups, i.e. <1 year, 1-2 years and >2 years of sheep. Our data showed that the prevalence was higher in age group <1 year (65.45%), whereas the lowest in age group >2 years (41.38%) followed by age group 1-2 years (61.54%). Age wise prevalence of PPR in different age groups of goats revealed 58.82%, 57.58 and 51.48% in <1 year, 1-2 year and >2 year age groups respectively (Table III).

Antigen detection of PPRV by RT-PCR and IcELISA

A total of 19 samples from nasal, ocular discharges, saliva and oral ulcers of clinically sick sheep and goats were found positive for PPR virus. According to the results the prevalence percentage of positive samples were 78.95% by RT-PCR technique and 68.42% through Ic-ELISA.

Table II. Prevalence of PPR in different production systems of sheep and goats.

S. No.	Animals	Nomadic			Transhumant			Sedentary		
		No. of animals examined	No. of animals infected	%	No. of animals examined	No. of animals infected	%	No. of animals examined	No. of animals infected	%
1.	Sheep	35	22	62.86	50	31	62.00	25	14	56.00
2.	Goats	55	30	54.55	15	11	73.33	66	35	53.03
Total		90	52	57.78	65	42	64.62	91	49	53.85

Table III. Age wise prevalence of PPR in different production systems of sheep and goat.

S. no.	Production systems	<1 year			1-2 years			>2 years		
		No. of animals examined	No. of animals infected	%	No. of animals examined	No. of animals infected	%	No. of animals examined	No. of animals infected	%
Sheep										
1	Nomadic	20	12	60.00	12	7	58.33	3	1	33.33
2	Transhumant	25	16	64.00	10	7	70.00	15	7	46.67
3	Sedentary	10	8	80.00	4	2	50.00	11	4	36.36
Total		55	36	65.45	26	16	61.54	29	12	41.38
Goat										
1	Nomadic	38	20	52.63	7	4	57.14	10	5	50.00
2	Transhumant	5	4	80.00	5	3	60.00	5	4	80.00
3	Sedentary	25	16	64.00	21	12	57.14	20	9	45.00
Total		68	40	58.82	33	19	57.58	35	18	51.43

DISCUSSION

The aim of this study was to determine the seroprevalence of PPR among sheep and goats kept under different production systems of district Nowshera, KPK. PPR is considered as one of the major constraints in augmenting the productivity of small ruminants in developing countries and severely affects poor farmer's economy (Balamurugan *et al.*, 2012). According to the Food and Agriculture Organization (FAO) PPR is a target animal disease for eradication from the world and about 62.5% of the global domestic small ruminant population is at risk of being infected by PPRV (FAO, 2009). Due to its nature and consequent capacity of rapid spread, PPR was regarded as List 'A' disease by the Office of International Des Epizooties (OIE, 2000).

In the present study, the highest prevalence of PPR (61.82%) was observed in sheep as compare to goats (55.88%). The findings of this study are in accordance with the results of Nizamani *et al.* (2015), Jalees *et al.* (2013), Abubakar *et al.* (2009) and Khan *et al.* (2008) who determined the overall prevalence rate of 37.2% and

34.78%, 51.5% and 46.5%, 56.80% and 48.24%, 54.9 % and 44.15 % in sheep and goats respectively. The possible reason for the increased prevalence rate in sheep could be that the sheep are more common in the study area which may have more exposure to PPRV during the animal movement.

During the current study, prevalence rate of PPR in sheep and goats were documented as 64.62% in transhumant production system followed by nomadic (57.78%) and sedentary (5.085%). Zahur *et al.* (2011) described the production systems of Pakistan as nomadic (44.00%), transhumant (38.00%) and sedentary (18.00%), respectively.

The results of the current study indicated that sheep and goats under one year of age are more susceptible to PPRV infection. Comparable results have been reported by Sreenivasa and Gopal (1996), Saliki *et al.* (1993), Goswami *et al.* (1998), Anjaneyalu and James (1999) and Kumar *et al.* (2002). They described that animal of younger age <1 year age group appeared to be more susceptible to PPRV infection than older animals. The results are in contrast to those reported by Abubakar *et al.* (2014), Nizamani *et al.*

al. (2015) and Zahur *et al.* (2011) who reported that adult animals aged >2 years might be more exposed to PPR infections as compared to younger animals. During this study both RT-PCR and IcELISA techniques were used to confirm the PPRV presence in clinically ill sheep and goats. The results revealed higher (78.95%) prevalence rate in RT-PCR technique. Whereas, the prevalence percentage of positive samples tested through IcELISA were 68.42 percent. A similar type of study has been conducted by Chauhan *et al.* (2014) in Gujarat, India. They detected 48.00% swab samples positive by RT-PCR and 23.80% by IcELISA. Bahadar *et al.* (2009) described the overall prevalence of PPR antigen as 50.00% (60/120) in various samples. RT-PCR coupled with ELISA have also been used to increase the analytical sensitivity of visualization of RT-PCR products and to overcome the drawbacks of electrophoresis based detection such as use of ethidium bromide, exposure to UV light (Kumar *et al.*, 2007).

Prevalence of PPR was high in sheep as compared to goats. The highest prevalence was recorded in the transhumant production system. Younger animal below one year of age seems to be more susceptible. The RT-PCR technique was more sensitive than IcELISA for detection of PPRV.

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Statement of conflict of interests

Authors confirm that there is no conflict of interest.

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