

Screening of peste des petits ruminants virus in a population of district Khairpur, Pakistan

A.K. Maitlo¹, J.A. Ujan¹, S.A. Ujjan¹, M. Ruk¹, B.A. Memon²,
A.A. Mahar⁴ and A.A. Ujjan³

¹Department of Zoology, Shah Abdul Latif University, Khairpur Mirs, Sindh, Pakistan

²Department of Microbiology, Shah Abdul Latif University, Khairpur, Sindh, Pakistan

³Pest and Disease Research Laboratory, Department of Agriculture and Agribusiness, University of Karachi, Karachi, Pakistan

⁴Department of Microbiology, Northwest A&F University, Shaanxi, China

Corresponding author: J.A. Ujjan
E-mail: javedujjan@yahoo.com

Genet. Mol. Res. 16 (3): gmr16039679

Received March 20, 2017

Accepted August 15, 2017

Published September 28, 2017

DOI <http://dx.doi.org/10.4238/gmr16039679>

Copyright © 2017 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution ShareAlike (CC BY-SA) 4.0 License.

ABSTRACT. Goats are the Pakistan's fastest growing ruminants, and Pakistan is the third largest goat producer in the world after India and China. Goat meat preference is the main reason for its increased demand. In the country, there are 25 goat breeds and two wild relatives such as Mark and Goats. At present, Pakistan has 53.8 million goats, according to the 2006 GOP report, and their population growth rate was more than 3% per year (37, 23, 22, and 18% of the goat population in Punjab, Sindh, Balochistan, and NWFP, respectively). Peste des petits ruminants virus (PPRV) belongs to the family Paramyxoviridae and is considered to be one of the major constraints on increasing the productivity of goats and sheep in the areas where they exist and become local. It is closely related to cattle and buffalo rinderpest virus,

dogs and other wild predator distemper virus, human measles virus, and marine mammalian measles virus. The present study aimed to determine the screening of the PPRV, *Capra Hircus* Lin. population, in the Khairpur Mirs District, Sindh, Pakistan. We selected 290 goats for serum sample collection and analysis using competitive ELISA kits according to the manufacturer's instructions. Our results showed that 59 (64%) of the 92 clinical cases were positive and 33 (36%) were seronegative. The study concluded that PPR might be more prevalent in the Khairpur District. Furthermore, it is highly recommended to use homologous PPR-attenuated vaccines to prevent lethal virus attacks that control PPR in the country.

Key words: *Capra hircus* Lin.; Khairpur population; Peste des petits ruminants virus; PPRV screening

INTRODUCTION

The peste des petits ruminants virus (PPRV) is a member of the Paramyxoviridae family, and it is the causative agent for the peste des small ruminant viral disease. It is a powerful and urgent viral disease in goats and sheep (Luka et al., 2012). The clinical PPRV observation and determination were based on sudden fever (41°C), anorexia, respiratory distress, necrotizing stomatitis catarrhal inflammation, ulcer mucosa, cough, pneumonia, gastrointestinal inflammation, diarrhea, and death (Adombi et al., 2011).

The PPRV was first described in Côte d'Ivoire (Gargadennec and Lalanne, 1942), and later the same disease was found and recognized in many sub-Saharan Atlantic states and the Red Sea (Diallo et al., 1995; Chauhan et al., 2009). The same disease was officially recognized in Nigeria and the Tibetan People's Republic of Tibet in July 2007 (Wang et al., 2009). The disease is mainly through close contact, including excretion from infected animals to close healthy people and secretion (Muse et al., 2012).

There are four major phylogenetic groups, three of which are in Africa, while the fourth report is in the Indian subcontinent, but the third group is also reported in the Middle East (Chauhan et al., 2009). The PPRV was reported and confirmed for the first time in 1987 in the Village of Arasur, Villupuram District in Tamil Nadu, southern India. Shaila et al. (1989) prevented the PPRV from entering southern India in 1993, and the PPRV epidemic began in northern India. Afterward, the disease is recognized from part to part and is believed to be a pandemic (local) disease that causes the great jolt of small ruminants in the country. Some PPRV workers accredited and reported around Gujarat (Chauhan et al., 2009). In 2008, northern Tanzania reported the same disease as PPRV (Muse et al., 2012). In the summer of 2008, the same disease in Morocco has been met and recognized. The identification of the character (PPRV) strain was established in Sudan from 2000 to 2009 (Kwiatek et al., 2011). PPRV is mainly caused by single-stranded RNA viruses. PPRV can be divided into four genetic lines based on the N-gene of the nucleocapsid. PPRV is a natural disease, especially in West Africa, which is considered to be a mandatory constraint on livestock (Munir et al., 2012).

The PPRV and rinderpest virus show a shared correlation of antigenic components, which are neutralized by virus testing (Hamdy and Dardiri, 1976). PPRV was also found in Afghanistan in 2001-2006 and Iran (Zahur et al., 2009). The disease was described in Pakistan

in the early 1990s as the basis for clinical and epidemiological interpretation (Pervez et al., 1993; Athar et al., 1995) by using polymerase chain reaction (Amjad et al., 1996). PPRV was serologically established in many parts of Pakistan, and many readings have described a total incidence of close to 50% in small ruminant populations (Khan et al., 2007; Abubakar et al., 2009; Aslam et al., 2009). Further identification and detection by competitive enzyme-linked immunosorbent assay (cELISA) were performed when the PPRV virus and its antigen were detected (Zahur et al., 2009). PPRV was found stable even at pH 4.0 to 10.0, it is destroyed by most disinfectants (substances that kill bacteria and viruses) but remains active in freezing and (chili) freezing and tissue. Healthier goats that are free from PPRV in world has very high impact on economy of world and can lead the animal husbandry up to 40% of agricultural gross domestic product (Steinfeild et al., 2006).

Pakistan's total population is 80.28 million small ruminants (sheep: 26.48 million, goats: 53.78 million; Anonymous, 2005-2006). The contribution of the oil sector plays a vital role in the economy, increasing the agricultural sector by 50 and nearly 11% in Pakistan, and between 33 and 35 million people in the country for livestock such as sheep and goats, nearly 25.5 and 61.9 million equivalent to a total of 31 million tons of milk, 782.1 tons of wool, 215,000 tons of hair, and 51.2 million skin/year) (Anonymous, 2005-2006). Due to high mortality and morbidity, annual PPRV losses exceed US\$342 million, reducing genetic populations (Hussain et al., 2008). In some countries, the production of small ruminants resulted in significant losses, which was the cause of local capital losses (Zahur et al., 2009). Molecular and serological tests involved the identification of viruses by different techniques such as agar gel immunodiffusion, cELISA, polymerase chain reaction, Penside kit method, and hemagglutination inhibition. However, the use of cELISA to detect PPRV has allowed rapid, accurate, and cost-effective methods approved by WHO, and therefore, this study involved the use of the cELISA method. The highest prevalence of PPR was also reported in Tank and South Waziristan cohorts. The lowest prevalence was recorded in Abbottabad, Bajaur Agency, Malakand, and Swat (Mehmood et al., 2009).

To observe the prevalence of PPRV in Sindh Province, Pakistan, the highest prevalence of PPR was reported in different regions of Sindh Province including the Dadu River (69.3%), Tallaharayar (60.3%), Hyderabad and Haierpur in the eastern area of Tapalkar, near the Nara and Kolstam (Dadu) hills. While in the southern coastal areas of Baden (15.7%) and Anderson (30.9%) it was found the lowest incidence. These occurrences are coincident with the investigation of Abubakar et al. (2011).

As far as we know, there is no study on the PPRV epidemics in the Khairpur region of Sindh Province, Pakistan. Therefore, this study was conducted to screen the population of PPRV (*Capra hircus* Lin.) in goats in Pakistan.

MATERIAL AND METHODS

Sample collection

A total of 92 serum samples were collected at random from the two villages of Taluka Kingri and Khairpur, including Pir Mangio, Garhi Mori, Waris Gambhir, Muhammad Yousuf Pittafi, Baberoli and Jumo Gadhi in the Khairpur region (see Google map in Figure 1). Figure 1. Geographic map of sample collection sites of the Khairpur Mirs District. At first, a blood sample was collected from suspected goats by puncturing the jugular vein with a 10-mL

syringe using an adjunct to the veterinary reserve test. The samples were named according to the proper identification of each animal sample and placed in an inclined position overnight to allow separation of serum from the clotted blood sample; then, it was kept in a clean and sterile vacuum tube and stored at 20°C. The samples were stored frozen in a freezer at the Gameel Ur Rehman Genome Research Center where serological analysis of PPRV infection and photographs of the collected samples were performed at the Department of Animal Sciences, Shah Abdul Latif University Khairpur (Figure 2).

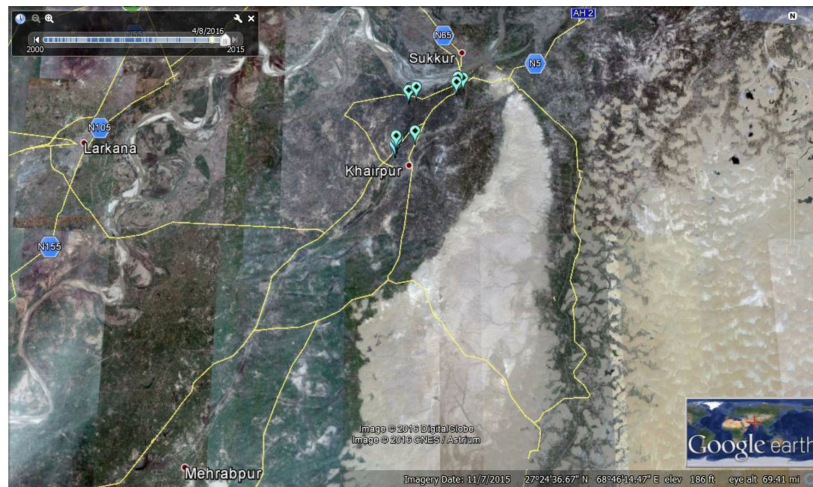


Figure 1. Geographic map of sample collection sites of district Khairpur mirs.



Figure 2. Photos of PPRV infection and sampling.

Sample diagnosis with ELISA

The stored PPRV blood samples were then transferred from the Genetics Laboratory at the Department of Zoology, Khairpur Shah Abdul Latif University to the University of Karachi. ELISA was performed using a kit from the Thermo Scientific Company (Figure 3 shows various photographs taken during the experiment).



Figure 3. Photos taken during the experiment.

Data analysis

The ELISA microplate was read using an immuno-scanning reader (Flow Laboratories, UK) with an inductive filter of 492 nm, as shown in the photographs, and the reader was attached to a microplate loaded with the ELISA Data Information software (FAO/IAEA, Vienna, Austria) for automatic reading and calculation of percent inhibition (PI) values. The optical density (OD) value was converted to PI using the following formula:

$$PI = 100 - (OD \text{ control} / \text{test serum}) \times 100 (OD \text{ monoclonal control})$$

Serum samples showing a PI value of 50 or higher were considered positive for PPRV antibodies, and a value below 50 was measured as negative. Besides, we use computer excel program percentages and pie charts.

RESULTS

We observed 290 goats from all 9 villages under study and 92 goats were initially diagnosed with the PPRV disease based on signs and symptoms. Of the 92 infected samples tested for cELISA, 59 samples (64%) were positive, and 33 samples (36%) were seronegative. Data showed 59 cases of positive samples, 13 cases of males (22%), 46 cases of females (78%).

In the first category, the highest number of PPRV positive (+ ve) cases was 35 (59.32%) with 10 to 12 months. In the second group, 18 animals (30.5%) were with 4 to 8 months. The third group included 6 cases (10.16%) with 2 to 4 months of age, and this was due to the lower passive immunity of the older age group (detailed results are given in Tables 1-4 and Figures 1 and 2).

Table 1. Total number of goats examined.

Serial number	Village name	Total number of goats examined
1.	Pir Mangio	45
2.	Garhi Mori	26
3.	Garhi Ranghal Shah	22
4.	Babarloi Bypass	8
5.	Waris Ghambhir	113
6.	M. Ramzan Theri	13
7.	M. Mithal Phulpoto	18
8.	Jumu Gadhi	31
9.	M. Yusuf Pitafi	14

Table 2. Total number of samples tested with cELISA.

Serial number	Village name	Total number (N = 92) of samples in each village	PPRV Positive (+ ve)
1.	Pir Mangio	16	7
2.	Garhi Mori	18	13
3.	Garhi Ranghal Shah	12	8
4.	Babarloi Bypass	8	1
5.	Waris Ghambhir	21	17
6.	M. Ramzan (Theri)	2	2
7.	M. Mithal Phulpoto	4	3
8.	Jumu Gadhi	8	6
9.	M. Yusuf Pitafi	3	2
Total		92	59

Table 3. Gender wise number of positive and negative PPRV cases.

Serial number	Village name	Total No. of positive PPRV males	Total No. of positive PPRV females
1.	Pir Mangio	1	6
2.	Garhi Mori	6	7
3.	Garhi Ranghal Shah	1	7
4.	Babarloi Bypass	0	1
5.	Waris Ghambhir	2	15
6.	M. Ramzan (Theri)	0	2
7.	M. Mithal Phulpoto	0	3
8.	Jumu Gadhi	2	4
9.	M. Yusuf Pitafi	1	1
Total		13	46

Table 4. Species, gender, and age wise PPRV cases.

Species/gender/age groups	No. of PPRV-positive samples	Percentage of PPRV-positive cases
Goat		
Male	13	13
Female	46	46
Age group (months)		
2 to 4 months	6	(10.16%)
4 to 8 months	18	(30.5%)
10 to 12 months	35	(59.32%)

DISCUSSION

Our study described PPRV disease outbreaks in the Khairpur region of the Khairpur Mirs region in two taluka, Pakistan and described more prevalent rates of PPRV in the Khairpur region (64%) compared to Khan et al. (2008) and Misbah et al. (2009) in Pakistan by the same method (cELISA). Only 50 to 51% of the positive and negative results were found.

In addition, our study shows that the PPR seroprevalence of small ruminants was highest in Waris Ghambir (80.95%), consistent with the findings of Abdalla et al (2012). This observation may be attributed to the PPR as a transboundary disease, as well as in the provinces and other parts of the country, because animals (small ruminant) are in frequent movement.

Our study also predicted that the overall positive seroprevalence of females was 78%, while that of males was 22%. There was a difference in seropositivity between the females and males of the tested small ruminants. This has no biological possibility and agrees with the findings of Kivaria et al. (2013), that animal sex has no effect on the development of PPRV antibodies. The fact that small ruminant producers keep more females for breeding purposes can explain this observation. Thus, females are more likely than males to be exposed to PPRV throughout their lifespan. The results showed that PPR had been circulating in most provinces before the implementation of the large vaccination campaign. Since goat farming is an important source of economic activity in our province, we should pay full attention to the widespread transmission of this viral disease, which could otherwise be out of control.

CONCLUSION

This study concluded that PPR might be more prevalent in Khairpur Mir, especially in the 10- to 12-month age group than in older age groups (2 to 8 years), because of the passive immunization with colostrum. Besides, our study also showed that females are more affected because of their number.

ACKNOWLEDGMENTS

I have great honor for Dr. Javed Ahmed Ujan and Dr. Kamran Azim for their help and support.

REFERENCES

- Abdalla AS, Majok AA, Malik KH and Ali AS (2012). Sero-prevalence of peste des petits ruminants virus (PPRV) in small ruminants in Blue Nile, Gadaref and North Kordofan States of Sudan. *J. Public Health Epidemiol.* 4: 59-64. <https://doi.org/10.5897/JPHE11.213>

- Abubakar M, Jamal SM, Arshed MJ, Hussain M, et al. (2009). Peste des petits ruminants virus (PPRV) infection; its association with species, seasonal variations and geography. *Trop. Anim. Health Prod.* 41: 1197-1202. <https://doi.org/10.1007/s11250-008-9300-9>
- Abubakar M, Arshed MJ, Hussain M and Ali Q (2011). Evidence of peste des petits ruminants in serology of sheep and goats from Sindh, Pakistan. *Transbound. Emerg. Dis.* 58: 152-156. <https://doi.org/10.1111/j.1865-1682.2010.01193.x>
- Adombi CM, Lelenta M, Lamien CE, Shamaki D, et al. (2011). Monkey CV1 cell line expressing the sheep-goat SLAM protein: a highly sensitive cell line for the isolation of peste des petits ruminants virus from pathological specimens. *J. Virol. Methods* 173: 306-313. <https://doi.org/10.1016/j.jviromet.2011.02.024>
- Amjad H, Qamar-ul-Islam, Forsyth M, Barrett T, et al. (1996). Peste des petits ruminants in goats in Pakistan. *Vet. Rec.* 139: 118-119. <https://doi.org/10.1136/vr.139.5.118>
- Anonymous (2005-2006). Pakistan Economic Survey. Financial department, economic adviser, government. Pakistan, Islamabad.
- Aslam M, Abubakar M, Anjum R, Saleha S, et al. (2009). Prevalence of PPRV in Mardan, hangu, and Kohat district of Pakistan; Comparative analysis of PPRV suspected serum samples using competitive ELISA (cELISA) and Agar gel Immunodiffusion (AGID). *Vet. World* 2: 89-92.
- Athar H, Muhammad G, Azim F, Shakoor A, et al. (1995). An outbreak of peste des petits ruminants-like disease among goat in Punjab (Pakistan). *Pak. Vet. J.* 15: 140-143.
- Chauhan HC, Chandel BS, Kher HN, Dadawala AL, et al. (2009). Peste des petits ruminant's virus infection in animals. *Vet. World* 2: 150-155.
- Diallo A, Libeau G, Couacy-Hymann E and Barbrun M (1995). Recent developments in the diagnosis of rinderpest and peste des petits ruminants. *Vet. Microbiol.* 44: 307-317. [https://doi.org/10.1016/0378-1135\(95\)00025-6](https://doi.org/10.1016/0378-1135(95)00025-6)
- Gargadennec L and Lalanne A (1942). La peste des petits ruminants. *Bull. Serv. Zoo Tech. Epizzot. AOF* 5: 16-21.
- Hamdy FM and Dardiri AH (1976). Response of white-tailed deer to infection with peste des petits ruminants virus. *J. Wildl. Dis.* 12: 516-522. <https://doi.org/10.7589/0090-3558-12.4.516>
- Hussain M, Irshad H and Khan MQ (2008). Laboratory diagnosis of transboundary animal diseases in Pakistan. *Transbound. Emerg. Dis.* 55: 190-195. <https://doi.org/10.1111/j.1865-1682.2008.01026.x>
- Khan HA, Siddique M, Arshad MJ, Khan QM, et al. (2007). Serum Prevalence of Plague Ruminant (PPR) Virus in Sheep and Goats in Punjab, Pakistan. *Pak. Vet. J.* 3: 109-112.
- Khan HA, Siddique M, Abubakar M and Ashraf M (2008). The detection of antibody against peste des petits ruminants virus in sheep, goats, cattle and buffaloes. *Trop. Anim. Health Prod.* 40: 521-527.
- Kwiatek O, Ali Y, Saeed H, Khalafalla I, et al. (2011). Asia follows the plague ruminant virus. *Africa Emerg. Infect Dis* 17: 1223-1231. <https://doi.org/10.3201/eid1707.101216>
- Kivaria FM, Kwiatek O, Kapaga AM, Swai ES, et al. (2013). The incursion, persistence and spread of peste des petits ruminants in Tanzania: Epidemiological patterns and predictions. *Onderstepoort J. Vet. Res.* 80: 01-10.
- Luka PD, Erume J, Mwiine FN and Ayebazibwe C (2012). Molecular characterization of peste des petits ruminants virus from the Karamoja region of Uganda (2007-2008). *Arch. Virol.* 157: 29-35. <https://doi.org/10.1007/s00705-011-1135-4>
- Mehmood A, Ali Q, Gadahi JA, Malik SA, et al. (2009). Pestdes Petits ruminant (PPR) virus antibodies in sheep and goat populations Northwest Frontier (NWSUAF) of Pakistan by competitive ELISA (cELISA). *Vet. World* 2: 333-336.
- Misbah A, Muhammad A, Rehana A, Shamim S, et al., (2009). Prevalence of Peste des Petits Ruminants Virus (PPRV) in Mardan, Hangu and Kohat District of Pakistan; Comparative analysis of PPRV suspected serum samples using competitive ELISA (cELISA) and agar gel immunodiffusion (AGID). *Vet World* 2: 89-92.
- Munir M, Zohari S, Suluku R, Leblanc N, et al. (2012). Genetic characterization of peste des petits ruminants virus, Sierra Leone. *Emerg. Infect. Dis.* 18: 193-195. <https://doi.org/10.3201/eid1801.111304>
- Muse EA, Karimuribo ED, Gitao GC, Misinzo G, et al. (2012). Epidemiological investigation was introduced and the factors of Peste Des petits spread were ruminants, southern Tanzania. *Onderstepoort J. Vet. Res.* 79: 49-54. <https://doi.org/10.4102/ojvr.v79i2.457>
- Pervez K, Ashraf M, Khan MS, Khan MA, et al. (1993). Rhabdoid disease in goats in Punjab, Pakistan. *J. Livestock Res* 1: 1-4.
- Shaila MS, Purushothaman V, Bhavasar D, Venugopal K, et al. (1989). Peste des petits ruminants of sheep in India. *Vet. Rec.* 125: 602.
- Steinfeld H, Gerber P, Wassenaar T, Castel V, et al. (2006). Livestocks long shadow, FAO, Rome, Government. Pakistan (2006). Livestoc Census, A.C., ed. 2006, Lahore.
- Wang Z, Bao J, Wu X, Liu Y, et al. (2009). Peste des petits ruminants virus in Tibet, China. *Emerg. Infect. Dis.* 15: 299-301. <https://doi.org/10.3201/eid1502.080817>
- Zahur AB, Ullah A, Irshad H, Farooq MS, et al. (2009). An epidemiological survey of the outbreak of Peste Des Petits Ruminants (PPR) in Pakistan's Afghan sheep. *Pak. Vet. J.* 29: 174-178.