Research Article

Molecular and Epidemiological Features of *Peste des Petits Ruminants* Outbreak during Endemic Situation

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Abstract | An outbreak of *Peste des Petits ruminants* (PPR), possibly caused by the introduction of new animals in the herd, was investigated in an unvaccinated mixed herd of sheep and goats. Goats in the herd showed characteristic signs of PPR including nasal and ocular discharges, high temperature, diarrhea and ulcerative lesions in the oral cavity. A total of eighteen goats from a herd of sixty, were affected and two goats succumbed within two weeks. Interestingly, the disease was exclusively observed in goats and all sheep kept in the same herd were serologically positive but did not show any clinical signs of PPR. The active PPR virus (PPRV) infection was confirmed by antigen capture ELISA and RT-PCR in both swab and body tissue samples. The molecular characterization revealed clustering of the PPRV within lineage IV with significant substitutions in the nucleoprotein (NP) gene. Genetic variations within NP gene, and possibly in other proteins which are essentially mediating protective immunity, may explain the extreme infectious nature of the virus and its host-specific pathogenesis. Moreover, understanding the nature of such circulating field viruses is essential to underpin the endemic potential of PPRV and its possible spread to the susceptible wild or domestic small ruminants.

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Introduction

Peste des Petits ruminants (PPR) is a major restraint to small ruminants industry in endemic areas including Pakistan. It is a highly contagious disease of small ruminants and is characterized by high fever in first 3-4 days, followed by ocular and nasal discharges, diarrhea and lesions in the oral cavity, and the morbidity and mortality can reach up to 100% in naïve herds (Munir et al., 2013). The disease is caused by PPR virus (PPRV), which is a member of the genus morbillivirus in the family paramyxoviridae (Gibbs et al., 1979).

In Pakistan, existence of PPR has been recognized since 1991 and many outbreaks have since then been documented based on clinical diagnosis (Athar et al., 1995; Hussain et al., 1998); however, later there was reporting based on laboratory confirmation (Abubakar et al., 2008a, Abubakar et al., 2008b;

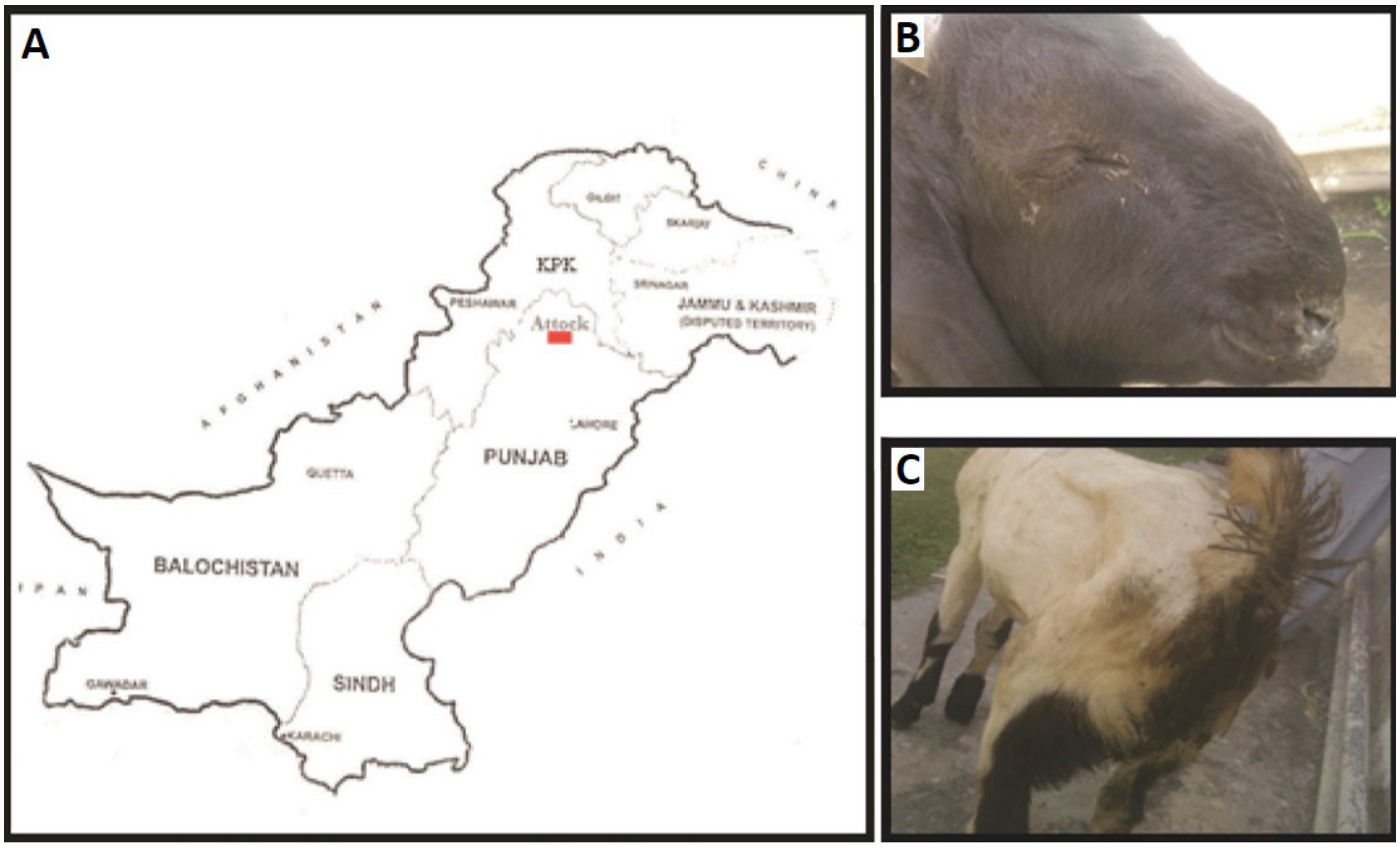

Figure 1: *Area of Outbreak and Main Clinical Signs in Goats*

Abubakar et al., 2011; Abubakar et al., 2012; Abubakar and Munir., 2014). Moreover, PPR is sometimes clinically misdiagnosed as contagious caprine pleura-pneumonia (CCPP), pasteurellosis or contagious ecthyma (Hussain et al., 2002), further necessitating the need of confirmatory laboratory diagnosis, especially in an endemic situation. During last few years, PPR outbreaks have increased in Pakistan to an alarming level involving previously disease free areas with variable damage to livestock (Ali, 2004; Abubakar and Munir, 2014; Abubakar et al., 2015).

In most conventional and semi-modern rearing systems, sheep and goats, which are the most susceptible small ruminant species for PPRV, are raised in close vicinity. This raises the concern on disease transmission between these species. In this regards, a significant number of studies have been conducted to assess the disease potential of PPRV in a species, breed, age, gender and host immune status dependent manners (Munir et al., 2013). Although, genetic background contributes in defining the susceptibility of the host to any infections, our understanding on differential pathogenic potential of PPRV in sheep and goats remain incomplete. Present study was conducted to highlight the species-specific pathogenesis of PPRV in a herd of sheep and goats. The results demonstrate that, beside host factors, genetics of PPRV may play a critical role in selecting the susceptibility of small ruminants, especially in animal abundant areas and disease endemic situations.

Materials and Methods

A suspected PPR outbreak was investigated in a mixed herd of goat and sheep at a semi-organized farm, near district Attock, Punjab province, Pakistan in April 2013 (Figure 1A). The herd originally consisted of 34 sheep and 60 goats. All sheep were healthy and only 18 goats suffered from a disease, which was suspected to be PPR. In the first four weeks, two goats died after severe clinical signs: dullness and depression, ocular and nasal discharges (Figure 1B), lesions on gums (Figure 1B) and severe diarrhea (Figure 1C). Nature of and percentage of animals infected in the suspected outbreak of PPR are detailed in Table 1.

Table 1: *Percentage of Animals showing different clinical signs*

Clinical Signs	Diarrhea	Ocular Discharge	Nasal discharge	Gum lesions
No. of animals affected	13/18	11/18	17/18	6/18
Percent	**72%**	**61%**	**94%**	**33%**

Historical investigations revealed the occurrence of disease outbreak due to introduction of new animals in the flock. Three weeks before the outbreak started, 10 new healthy bucks were purchased from a nearby market and introduced into the flock for fattening purposes. The newly purchased animals were quarantined for 10 days and were routinely vaccinated against CCPP and enterotoxemia (ET); however, these animals remained unvaccinated for PPR.

A total of four swab samples (nasal, ocular, oral and fecal) were collected from four bucks, at the early stages of the disease, and samples were dispatched to the National Veterinary Laboratory, Islamabad for confirmatory diagnosis. None of the four animals were recently purchased. Swabs were prepared in phosphate-buffered saline (PBS) and tested using antigen capture enzyme-linked immune-sorbent assay (ELISA) (BDSL, UK). Further confirmation was performed by conventional polymerase chain reactions (PCR) (Forsyth and Barrett, 1995). The PCR positive samples were sequenced on the Beckman Coulter DNA Sequencing machine (GEXP, USA). The sequences were aligned and edited in BioEdit and phylogenetic analysis was performed in MEMGA5 using neighbour-joining method. Retrospectively, blood samples were collected from four diseased goats and four healthy sheep from the same herd to demonstrate the antibodies against PPRV. Goat samples were found negative for PPRV antibodies whereas sheep showed high antibody titer against PPRV. To follow up the outbreak and health status of sheep and goats, blood samples were again collected from eight convalescent goats 15 days post-infection as well as from two healthy sheep. Sera were separated and tested using competitive ELISA for the detection of antibodies against PPRV (BDSL, UK).

Results and Discussion

Understanding preference of the PPRV for sheep or goats would provide basis in understanding the resistance or susceptible genetic background of small ruminants. As sheep and goats are being raised in close vicinity (most of the time in the same flock), selective clinical outcome of PPRV can be exploited in understanding the transmission dynamics and disease pathobiology. Having an established network of disease reporting and monitoring system, a disease scenario can be followed to address some of these parameters. Such disease scenario was observed in a flock of sheep and goats in which only goats were clinically affected whereas sheep, despite of being seropositive, remained healthy for the duration of the study. Highly infectious nature of PPRV can be realized from the fact that the disease appeared in the flock one week post-introduction of animals. The newly introduced animals were clinically healthy and were kept under quarantine for 10 days. Although the incubation period of the virus ranges from two to ten days, it is hard to conclude that these animals were not originally infected. Since these introduced animals were not vaccinated for PPRV, but only for CCPP and ET, it may be likely that these newly introduced animals were in the stage of late-incubation period. Despite of acute disease and severe clinical outcome, the case fatality rate was low (Table 2 and 3). The possible reasons for the variable disease outcome and case fatality rates are difficult to accurately predict especially when sheep and goats from different age groups are raised in the same flock. In this disease outbreak situation, a higher antibody titter was observed in sheep and adult animals aging between 1-2 years. Early, quicker and prompt induction of antibody production in sheep could possibly explain the potential of sheep being able to control the virus replication in its early stages before uncontrolled virus load establishes. This hypothesis of differing humoral immune response to PPRV between sheep and goats would be interesting to further explore.

Table 2: *Number of animals affected in different age groups*

Age Group (Goats)	Group Size	Animal affected	Vaccination History
Kids	20	2	No vaccination history
Young	25	19	
Adult	15	0	
Total	**60**	**21**	

Table 3: *Case fatality rate of affected flock*

Flock Size	Herd size	Morbidity %	Mortality %	Case Fatality
Goat	60	19 (31.6)	2 (3.3)	10.5%
Sheep	34	0	0	0
Total	**94**	**19 (20.2)**	**2(2.1)**	**10.5%**

Phylogenetically, PPRV can be classified into four lineages based on the NP gene. Each lineage depict some geographical presentation, however, these are not predictive of viral potential of virulence.

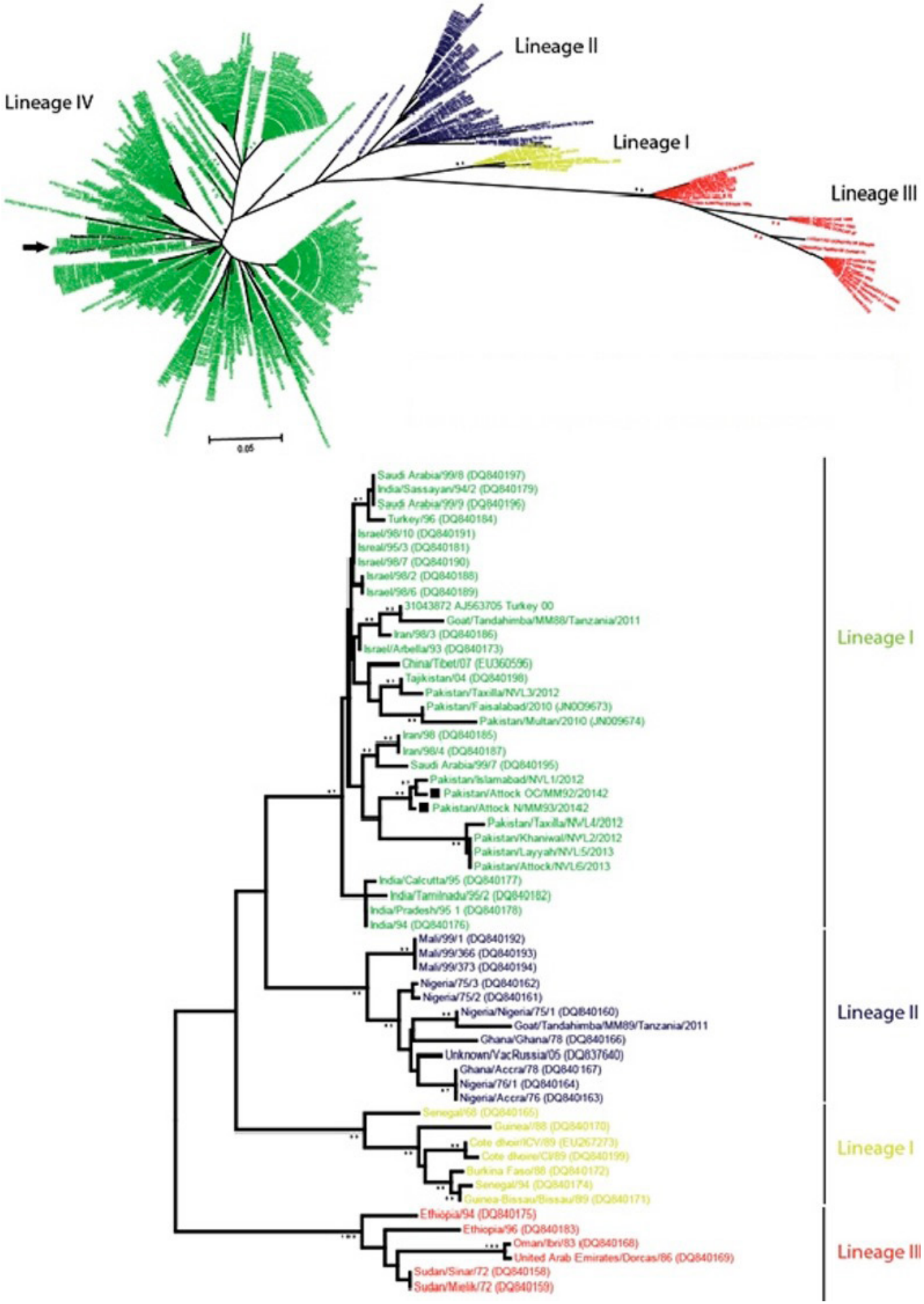

Figure 2: *Phylogenetic analysis of PPRV strain of recent outbreak at Attock district, Pakistan*

To assess the unbiased phylogenetic clustering of PPRV, identified from the clinical material, we collected all available NP genes from public domains. Neighbour-joining based phylogenetic tree construction indicated that the PPRV isolate belonged to lineage IV (Figure 2 upper panel). This lineage is the most prominent lineage in South East Asia and is the emerging group of PPRV that is being reported in previously PPRV-free countries. To have clear clustering patterns, a tree with fewer and representative sequences from each of the four lineages were used. This higher resolution phylogenetic analysis indicated that the PPRV isolate in the current study grouped together with the PPRV isolates reported earlier from Pakistan (Figure 2 lower panel). Infection with any of the four lineages of PPRV can be asymptomatic or produce a wide spectrum of clinical manifestations, ranging from mild and short lived to lethal, depending on many factors, including the strain of virus, sensitivity of the host species, and individual animal susceptibility (Sen et al., 2010). Taken together, genetics of the virus clearly demonstrate that the causative agent of the outbreak is of indigenous origin. The PPRV isolates from the outbreak in the current study clustered closely to PPRV earlier reported from different regions of Pakistan. Hence, it is likely that the newly introduced animals from the animal market were brought from the regions of Punjab.

Table 4: *Sample wise comparison of results of ELISA and PCR*

Test Performed	Type of sample	No. of Samples tested	No. of Samples Positive
ELISA	Nasal Swab	3	3
	Ocular Swab	3	2
	Oral Swab	3	2
	Fecal Swab	3	1
PCR	Nasal Swab	3	3
	Ocular Swab	3	3
	Oral Swab	3	2
	Fecal Swab	3	2

Based on clinical observations and serological monitoring, it is possible to clearly demonstrate a certain level of differential preferences of the virus for sheep and goats (personal communication with field veterinarians; Zahur et al., 2009; Abubakar et al., 2009; Singh et al., 2004, Khan et al., 2008; Balamurugan et al., 2012; Truong et al., 2014; Taylor and Ali, 2005). Based on these observations, and owing to the reason that sheep and goats may harbor viruses of same genetics, it can convincingly be believed that host factors contribute more than the virus-based differences in disease severity in sheep and goats.

The genetic analysis of PPRV was only made based on the NP gene which provides clear clustering patterns of PPRV strains. However, analysis of putative protein sequence of NP is not indicative of virus potential to cause disease in selective animals. This warrants the complete genome sequencing of three isolates that selectively cause disease in sheep or goats or in both. It is also important to keep in mind the roles of hosts in PPR pathobiology; the phylogenetic and polymorphism analyses may provide markers of virus selectivity and would require biological experimentation to underpin this selective nature of PPRV using reverse genetic system, which has recently become available. Moreover, the genetic variations within PPRV proteins, essentially mediating protective immunity, may give the possible explanation of the extreme infectious nature of the virus in the current outbreak and its host-specific pathogenesis.

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Conflict of Interest

There is no conflict of interest among authors.

Authors' Contribution

MA, MM and JJW planned the work and collection of data. MA and SM executed the work and lab testing. MA, ET and MM worked on the analysis of data. JJW and QA helped in results interpretation and write up. All authors read and agreed with the manuscript.

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