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EPIDEMIOLOGICAL INVESTIGATIONS OF A *PESTE DES PETITS RUMINANTS* (PPR) OUTBREAK IN AFGHAN SHEEP IN PAKISTAN

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ABSTRACT

Epidemiological and virological investigations were carried out during an outbreak of *Peste des petits ruminants* (PPR) in Afghan (Bulkhi) sheep in Pakistan. The overall morbidity, mortality and case fatality rates were 41.0, 1.2 and 3.0%, respectively. The epidemic curve was plotted and the values for basic reproductive number (R0) and herd immunity threshold (HIT) for the affected flock were estimated to be 6.85 and 85.4%, respectively. The morbid material analysis by immuno-capture ELISA (Ic-ELISA) and haemagglutination assay (HA) revealed the presence of PPR virus. The PPR virus was isolated and identified through cytopathic effects, Ic-ELISA and transmission electron microscopy (TEM).

Key words: PPR, sheep, basic reproduction number, herd immunity threshold, epidemic curve.

INTRODUCTION

Peste des Petits Ruminants (PPR) is a disease of major economic importance and imposes a significant constraint upon sheep and goat production owing to its high mortality rate (Asim *et al*., 2008; 2009). It is an acute, highly contagious and frequently fatal disease of sheep and goats caused by PPR virus (PPRV), a member of genus morbillivirus of family Paramyxoviridae. Therefore, it poses serious threat to the development of small ruminants production in many countries where it is endemic and is a source of great financial loss to the farmers and livestock owners.

The disease was first reported in Pakistan during 1994 when the confirmatory diagnosis was made by polymerase chain reaction (Amjad *et al*., 1994). This was further validated when the PPR virus (PPRV) antigen was detected during outbreaks at different areas using Ic-ELISA (Hussain *et al*., 2002).

An outbreak of PPR occurred in the Bulkhi sheep of Small Ruminant's Research Programme, Livestock Research Station, National Agricultural Research Centre, Islamabad, Pakistan during June, 2006. The epidemiological data, clinical/pathological features of this outbreak and recovery and identification of PPR virus are reported in this paper.

MATERIALS AND METHODS

Epidemiological observations

Data regarding flock size, age, sex, vaccination history and possible source of virus transmission were recorded. The flock comprised a total of 82 sheep. Of these 26 were lambs (≤3.5 months old), 20 young stock (>3.5 months to 18 months old) and 36 adults (>18 months old). There were 21 males and 61 females. Adults were vaccinated against PPR vaccine (Jovac, Jordan), while the other two groups (lambs and young stock) were not vaccinated (Table 1).

Table 1: Parameters of affected Afghan (Bulkhi) sheep flock

Age group (months)	Group size	Males	Females
Lambs (≤ 3.5)	26 (32)	13	13
Young (>3.5 -18)	20 (24)	5	15
Adults (>18)	36(44)	3	33
Total	82	21(26)	61(74)

Numbers in parenthesis show percentages.

The epidemiological curve was drawn to see the magnitude and progression/ time course of the outbreak. The basic reproduction number (R0) and herd immunity threshold (HIT) values were estimated following Anderson and May (1992):

R0 = 1/ proportion susceptible

HIT = 1-1/R0

Clinical and post-mortem examination

Clinical examination of the affected flock and necropsy of dead animal was carried out and ocular (n = 6) and nasal (n = 6) swabs from all suspected live animals were collected. A piece of lung, spleen, kidney, liver and mesenteric lymph nodes (MLNs) from one dead animal were collected for laboratory confirmation.

Laboratory analysis

Samples were tested by Ic-ELISA for detection of PPRV antigen, following Anonymous (2002) and HA was performed following Wosu (1995). The results of Ic-ELISA were read by ELISA Data Interchange (EDI) software.

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Table 2: Various epidemiological parameters of PPR infection in affected Afghan (Bulkhi) sheep flock

Age group (Months)	Group size	Vaccine history	Morbidity	Mortality	Case fatality
Lambs (≤ 3.5)	26	No	23 (88)	1 (4)	1 (4.4)
Young (>3.5 -18)	20	No	11 (55)	0	0
Adults (>18)	36	Yes	0	0	0
Total	82	--	34 (41)	1 (1.2)	1 (3)

Numbers in parenthesis show percentages.

Samples positive by Ic-ELISA (from lungs, spleen and MLNs) were processed and cultured onto VERO cells (African Green Monkey Kidney cells) to obtain virus isolates following Ozkul *et al.* (2002). The PPRV was identified by cytopathic effects (CPE), Ic-ELISA for PPR virus and transmission electron microscopy (TEM), following standard operating procedures (SOPs).

RESULTS

Epidemiological observations

The flock consisted of 82 sheep of different age groups (Table 2). The vaccinated animals (36) did not experience any morbidity or mortality. Only the non vaccinated animals (46) experienced the disease. Of 26 lambs, one died resulting in a group case fatality of 4.4%. However, lambs experienced the highest morbidity of 88% (23/26), showing overt disease. There were 55% (11/20) cases among young stock. The overall morbidity, mortality and case fatality were 41, 1.2 and 3%, respectively (Table 2).

The epidemiological curve was plotted to see the pattern of outbreak (Fig. 1). It resembles a typical propagated outbreak with peaks of primary, secondary and tertiary generations of cases spaced by incubation period. The most probable period of exposure of the susceptible animals to PPRV was calculated from 3 to 5 days before the index case was identified (Fig. 1). The R0 and HIT values for affected flock were estimated to be 6.85 and 85.4%, respectively.

Clinical and post-mortem findings

The clinical examination of the affected animals revealed high fever (106-107°F), mild conjunctivitis, congestion of the third eye lids and mild ocular and nasal discharges (Fig. 2a). Erosive lesions were present on the inner side of the upper lip (Fig. 2b). All animals exhibited diarrhea. On the external examination, the carcass was dehydrated (sunken eyes) along with the soiling of hind quarters. While on internal examination red raw area was observed on the dental pad beneath the incisors. Both lungs were pneumonic particularly the cardiac lobe (Fig. 2c). Haemorrhages were seen on liver (Fig. 2d), kidneys, mucosal surface of abomasum (Fig. 2e) and on the mucosa of large intestine (Fig. 2g)

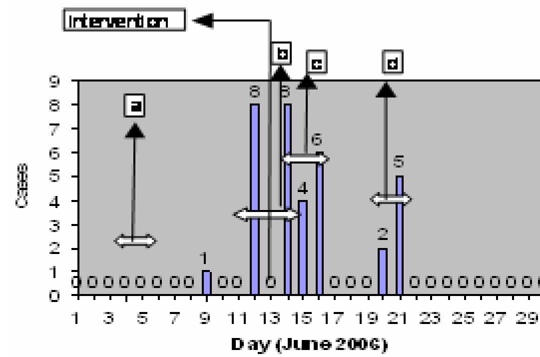


Fig. 1: Epidemic curve of PPR outbreak in Afghan sheep: a) Most probable time of exposure to PPRV (day 3-5); b) Primary cases which acquired infection from index case on day 9. All expressed clinical signs after a 4-6 days incubation period; c) Secondary cases which acquired infection from primary cases. Most probably, these were infected between day 11-13; d) Tertiary cases. Intervention my have resulted in reduction in the number of cases evident by lower frequency of cases here.

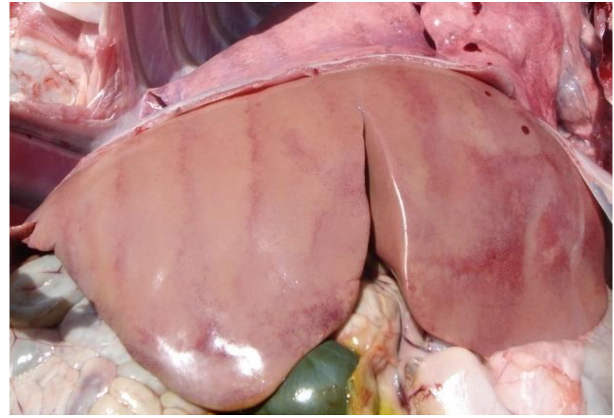
and the intestinal contents were watery. Lymph nodes (LN) particularly the mesenteric lymph nodes (MLN) were found reactive (inflammed).

Laboratory analysis

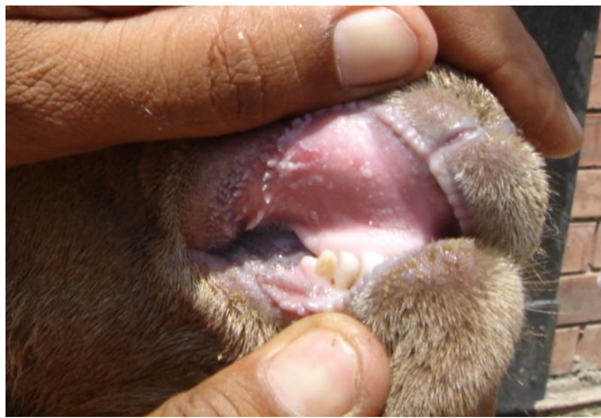
All the ocular and nasal swabs and lungs, spleen and MLNs were tested positive for PPRV antigen by Ic-ELISA. The spleen and MLNs homogenates exhibited agglutination of chicken erythrocytes at the concentration of 0.5% ranging from1:8 to 1:16. However, the ocular and nasal swabs from live animals did not demonstrate HA activity.

The PPR virus was isolated from spleen and MLNs. The cytopathic effects (CPE) were observed on day 5 post-inoculation. Initially, there was rounding of cells and later on clumping and elongation of VERO cells (Fig. 3a, b, c). The PPRV was harvested when 80% of the cells showed CPE. The cell culture supernatant, when tested by Ic-ELISA, was positive for PPRV. The TEM displayed a virion with a lipid envelope (Fig. 3d).

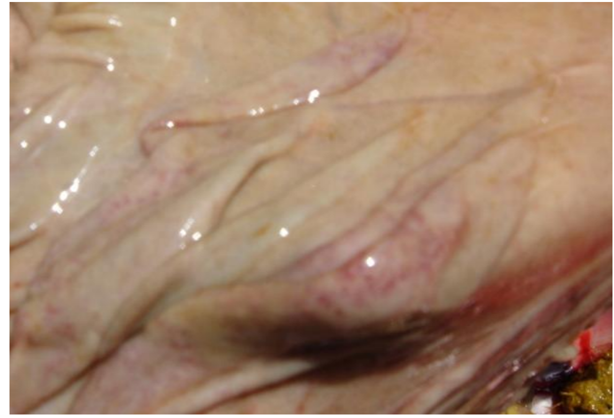
a) Mild conjunctivitis and nasal discharge



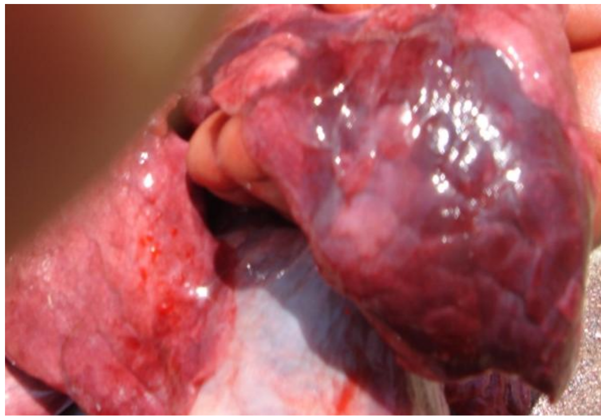
d) Haemorrhages on liver



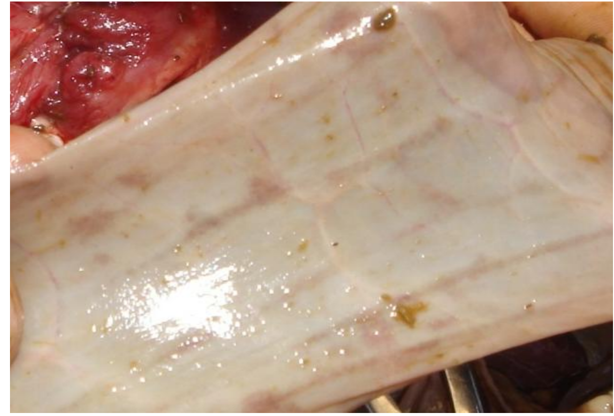
b) Erosions on the inner side of upper lip



e) Haemorrhages in abomasum



c) Congestion of cardiac lobe of lungs

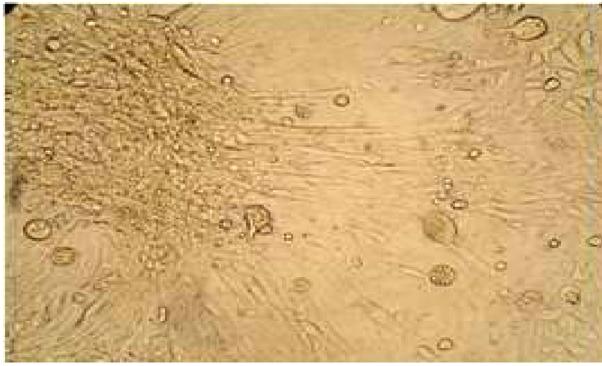


g) Haemorrhages in large intestine

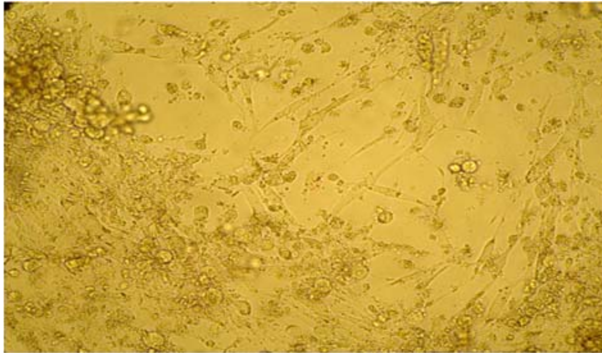
Fig. 2: Clinical signs and necropsy findings of PPR outbreak in Bulkhi sheep

DISCUSSION

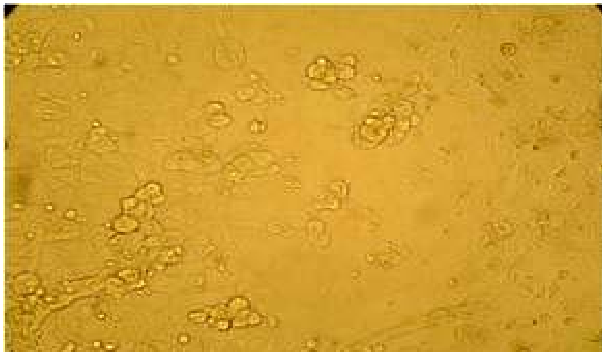
PPR has been reported in sheep in a number of countries in the region including Afghanistan, Iran and India (Shaila *et al*., 1989; Majok, 2001; Abdollahpour *et al.,* 2006). Although the clinical and postmortem findings may be sufficient for the diagnosis of PPR in the endemic areas, yet the laboratory confirmation is essential for definitive diagnosis. In the outbreak under study, the clinical signs, postmortem findings and epidemiological observations clearly indicated the presence of PPR virus. However, conjunctivitis/



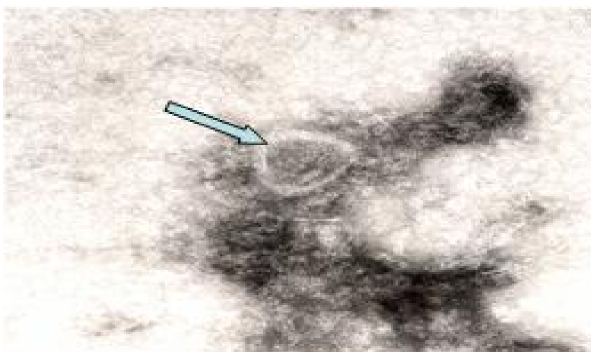
a) CPE –Rounding of VERO cells



b) CPE-Elongated VERO Cells



c) CPE-Clumping of VERO Cells



d) Transmission Electron micrograph (TEM) of PPR virus

Fig. 3: Cytopathic effects (CPE) on VERO cells due to PPRV and TEM of PPR virion isolated from Bulki sheep.

congestion of third eyelid along with ocular/ nasal discharge was very mild. Lung lesions in PPR, i.e., interstitial pneumonia are generally complicated by secondary infections. Such lesions are not seen in small ruminants suffering from rinderpest in the subcontinent (Abdollahpour *et al*., 2006).

The haemagglutination activity of the PPRV noticed in the present study using samples collected at necropsy further confirms the reports made earlier by Wosu (1995). It may prove to be a very convenient and cost effective diagnostic tool in developing countries. The CPE produced by the PPR virus were very similar to those produced by rinderpest virus on VERO cells (Gopilo, 2005). Similar observations were made by Ozkul *et al.* (2002), who reported initial rounding of VERO cells and later on development of syncitia.

The Ic-ELISA proved suitable for both diagnosis from field samples and identification of PPRV in cell culture supernatant. The TEM of PPRV displayed a typical structure for family paramyxoviridae (Losos, 1989).

During the outbreak, the epidemic curve revealed that cases occurred over more than a single incubation periods. The shape and other features of an epidemic curve can suggest hypotheses about the time and source of exposure, the mode of transmission, the causative agent, incubation period and the efficacy of control measures (Dicker and Gathany, 1992). The outbreak had a successive series of peaks reflecting increasing or decreasing numbers of cases in each generation. The epidemic waned after three generations, probably because either the number of susceptible animals fell below some critical level, or the zoo-sanitary measures taken to control the disease became effective. The most probable period of exposure of the susceptible animals to PPRV ranges between 3 and 5 days when the index case must have been infected, perhaps during grazing with the other small ruminants. The R0 value indicates the transmissibility of a pathogen. The estimated R0 value (6.85) for PPRV seems to be very close to those for other morbillivirus. Anderson (1995) documented that the value of R0 varies with the virulence of the pathogen and susceptibility of the host population. It was further reported that rinderpest virus can establish infection in relatively small populations of susceptible species but large populations are necessary for the infection to sustain. In the present outbreak, the susceptible population was very small and the interventions were instituted on the 4th day of the outbreak. This however, is not applicable for the field conditions where the chances of contact transmission are great (extensive production system) and farmers usually do not recognize the disease in its early stages.

Morbidity among lambs (88%) was on the higher side and mortality (1.2%) was almost negligible. Diallo (2006) reported that morbidity and mortality rates due to PPR may vary from 0 to 90% depending on the

animal husbandry, breed, age and other factors. In Pakistan, goats react severely to the exposure of PPRV like other parts of the world where the disease is endemic. It has been reported that during an outbreak of PPR in Pakistan, no clinical signs were observed in sheep kept with the sick goats in the same premises under one roof but they got sero-converted only (Hussain *et al*., 2002). Diallo (1997) reported that the reasons for these outbreaks in different epidemiological condition, like those where flocks consist of only sheep or goats or both, are unknown and was further reported that partial N and H gene sequences of the virus isolate involved in sheep or goat outbreaks have not revealed a clue to explain this situation. It highlights the need for concerted efforts to study the role of Bulkhi sheep in the epidemiology of the disease.

In the present study, lambs and young stock were affected among the susceptible population, while those which were vaccinated 18 months earlier remained un-affected. These observations are in agreement with Awa *et al.* (2002), who reported that maternal immunity decayed after 12 weeks in lambs from experimentally vaccinated ewes. It was also elucidated that attenuated PPR vaccine is capable of providing protection for at least 1.5 years and possibly for the whole economic life of small ruminants (about two years). Further studies may be needed to investigate this issue. The HIT is the proportion of susceptible population needed to be immune for a disease to become stable. The HIT for the flock indicated that we need to achieve more than 85.4% vaccination coverage for control of PPR infection in sheep population. For rinderpest, HIT has been estimated as 75-80% (Rossiter and James, 1989).

It is therefore recommended that before opting for vaccination as a national policy for controlling PPR, R0 value and HIT for both sheep and goat population may be estimated in endemic areas.

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