Detection of Peste des Petits Ruminants (PPR) virus antibodies in sheep and goat populations of the North West Frontier Province (NWFP) of Pakistan by competitive ELISA (cELISA)

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Abstract

The present study was planned to carry out a seroprevalence study of PPR antibodies in North West Frontier Province (NWFP) including Federally Administered Tribal Areas (FATA), using competitive-ELISA (c-ELISA) kit. A total of 4548 serum samples collected from 26 locations of the NWFP including. Eight hundred twenty eight samples were found positive for PPR antibodies yielding an overall seroprevalence of 18.20 percent in the small ruminant population included in the study. 337 (24.90%) serum samples from sheep and 491 (15.36%) from goats were found positive in PPR c-ELISA yielding a marginal difference in seroprevalence of PPR. The current situation of PPR in Pakistan could be regarded as of low magnitude but constant threat. Incidence may increase to the alarming level due to uncontrolled/ free movement within and from across boarders to the stocks of non-immunized susceptible population of sheep and goats in the country but production systems and economic realities constraint the effective animal movement control within and across the boarders. Based on the limited reports of outbreaks and uneven distribution of occurrence over the years of past decade, the best available options are high level of prevention and objective surveillance. Keeping in view the high risk of PPR, control strategy should be adopted and further study needs to be undertaken to ascertain the extent of PPR virus circulating in the population of goats and sheep in the country. Under existing production and marketing system, sanitation and preventive vaccination are two practicable options for control of PPR in Pakistan. Homologous PPR vaccine is now available which is a freeze dried live vaccine derived from tissue culture from attenuated PPR virus. The vaccine is intended for sheep and goats and is required to be injected to all animals above the age of 3 month. The vaccine may be repeated after one year of the primary vaccination in high risk areas.

Keywords: *Peste des petits ruminants*, Competitive-ELISA, Virus, Seroprevalence, Sheep, Goat.

Introduction

Peste des petits ruminants (PPR) is an acute, highly contagious viral disease of sheep and goats and is characterized by fever, anorexia, ulcerative necrotic stomatitis, diarrhea due to purulent ocular and nasal discharges and respiratory distress (Lefevre and Diallo, 1990) which may associate coughing, pneumonia and death. In non-endemic areas mortality and morbidity may vary depending upon susceptible population and in severe cases can reach up to 90 and 100%, respectively (Hussain *et al.,* 2003). Concurrent bacterial, viral or parasitic infections may aggravate condition and mortality up to 100% (Kitching, 1988), however in endemic mortalities as low as 20% have been described (Roeder and Ubi, 1999). In Asia, mortality between 35-60% has been reported (Dhar *et al.,* 2002).

The causative agent of this economically important disease of small ruminants is a Morbiilivirus, the Peste des petits ruminants virus (PPRV), under the family Paramyxoviridae of order Mononegavirales (Murphy et al., 1999). The virus is closely related to rinderpest virus (RPV), another member of Morbillvirus genus, which causes similar disease in large ruminants (Anderson et al., 1990; Couacy-Hyman et al., 1995).

The virus is also serologically related to Measles

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(MV) and Canine distemper virus (CDV) (Gibbs et al., 1979). A varying degree of cross protection in vivo and serological relationship is known to exist between PPR and RP viruses (Hamdy et al., 1976; Taylor and Abegunde, 1979). Further, the PPR infection in sheep and goats are known to sero-convert and protect in-contact bovines from natural infection, and also may interfere in tissue culture Rinderpest virus (TCRPV) vaccination response (Sudharshan et al., 1995).

The disease has been reported from many countries of the world including most parts of Africa, Middle East (Lefevre *et al.,* 1991), the Arabian Peninsula (Abu-Elzein *et al.,* 1990) and southern Asia (Shaila *et al.,* 1996) and Europe. In Africa, the virus has been found circulating in Benin, Cameron, Central African Republic, Chad, Congo, Eritrea, Ethiopia, Gabon, Ghana Guinea, Ivory Coast, Mali, Mauritania, Niger, Nigeria, Senegal and Togo in addition to Kenya and Sudan (Dhar *et al.,* 2002). In Middle East and Arabian Peninsula; Iraq, Saudi Arabia, United Arab Emirates, Kuwait, Israel, Yemen and Oman are known to harbour infection. In Asia, the disease has been reported in India, Nepal, Bangladesh, Pakistan, Afghanistan and Iran, and in Europe. In Pakistan PPR had its beginning in 1988; which confirmed from 1998 onward. The disease now appears endemic in the region throughout south Asia and beyond including Iran and Afghanistan.

The present study was carried out to detect the PPRV antibodies in the serum samples of sheep and goats by using the monoclonal antibody based c-ELISA.

Materials and Methods

A total of 4548 serum samples from 1353 sheep and 3195 goats collected from 26 locations of North West Frontier Province (NWFP) including Federally Administered Tribal Areas (FATA). The samples were stored at –20^{O}C in the serum bank of National Veterinary Laboratories (NVL), Islamabad, Pakistan.

Competitive ELISA: PPR c-ELISA kit for PPRV antibody detection was obtained from Institute for Animal Health (Pirbright Laboratory, Surrey, UK). The contents of kit included user manual with fact sheet, distilled water (30ml), PBS powder (Sigma, IL), tween -20 (100ml), ELISA plate (Nunc, Maxisorp), anti mouse HRPO conjugate (2ml), substrate H2O2, OPD tablet (30mg), antigen (1ml), negative serum (1ml), weak positive serum (1ml), strong positive serum (1ml) and monoclonal antibody. c-ELISA was performed strictly as per the protocol outlined in the user manual supplied with the kit.

c-ELISA was performed strictly as per the protocol outlined in the user manual supplied with the kit where the method was developed Libeau et al. (1995). PPR antigen was diluted in coating buffer (PBS-0.01 M, pH 7.4), each well of micrtiter plate was charged with 50ul diluted antigen followed by 1 h incubation at 37^{o}Con an orbital shaker. After 3 washing with washing buffer and blot dry, 45ul of blocking buffer (PBS + 0.05% tween 20 + 0.5 negative lamb serum) was added to all wells. According to company directions these chemicals were added: 5ul of blocking buffer to monoclonal control wells, 55ul of blocking buffer to the conjugate control wells and 5ul of test, strong positive, weak positive and negative control sera were added to the corresponding wells. 50ul of mAB (diluted 1/100 in blocking buffer) was added to all the wells except the conjugate control ones followed by incubation of the plate for 1 hour at 37^{o}C on orbital shaker. After 3 washings and blot dryness, 50ul of anti-mouse conjugate were added to all wells. After 1 hour incubation and 3 washings, 50ul of the chromogen/ substrate mixture (OPD/H_2O_2) were added to all wells. After 10 minutes incubation at room temperature, Colour development was stopped by adding 50ul of stop solution (H2SO4, 1M) to all wells. Optival density (OD) values were read at 492nm with ELISA plate reader (Immunoskan BDSL, Thermo Lab. Systems, Finland). The absorbance was converted to percentage inhibition (PI) using the formula with help of ELISA Data Interchange (EDI) software manufactured by FAO/ IAEA.

$$PI = \frac{\text{Absorbance of the test wells}}{\text{Absorbance of the mAb control wells.}} \times 100$$

The test serum samples showing PI value of 50 or above were taken as positive for PPR antibodies.

Results and Discussion

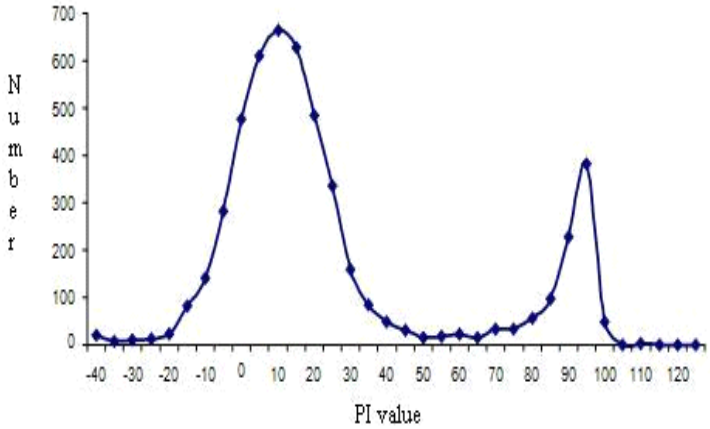
The ELISA plates with control in the expected range were analyzed for the interpretation of c-ELISA results for PPR antibodies. The test serum samples showing more than 50 percent inhibition of mean OD values of monoclonal control wells were considered as positive for PPR antibodies whereas samples having OD values less than 50 were considered negative using the EDI software manufactured by FAO/IAEA (Figure 1). The frequency distribution of of the PI values of the animals tested for PPR antibodies using c-ELISA are given as in Figure 1.

An overall seroprevalence of 18.20 percent was observed in the small ruminant population of the NWFP and FATA. Among the areas sampled, highest seroprevalence of PPR 81.81, 59 and 44 percent was observed in Tank, Kohat and South Waziristan respectively. The lowest seroprevalence was recorded in Abottabad, Bajaur Agency, Malakand and Swat (Table-1). Similarly, significantly higher seroprevalence of PPR was observed by Lefevre *et al.* (1991) in Jordan

Table-1. Locationwise seroprevalence of PPR

Sr.	Location	Sheep		Goat		Overall	
		No. of sample	Positive (%)	No. of samples	Positive (%)	Total	Positive (%)
1.	Abbottabad	5	00 (00)	195	05 (2.56)	200	05 (2.50)
2.	Bajur Agency	40	00 (00)	160	03 (1.87)	200	03 (1.50)
3.	Buner	100	04 (4.00)	100	06 (6.00)	200	10 (5.00)
4.	Chitral	92	08 (8.69)	108	21 (19.44)	200	29 (14.50)
5.	D.I. Khan	85	18 (21.17)	115	19 (16.52)	200	37 (18.50)
6.	F.R. Banu	03	02 (66.66)	197	24 (12.18)	200	26 (13.00)
7.	Hangu	18	10 (55.55)	82	30 (36.58)	100	40 (40.00)
8.	Haripur	107	39 (36.44)	93	23 (24.73)	200	62 (31.00)
9.	Karak	22	01 (4.54)	178	14 (7.86)	200	15(7.50)
10.	Khyber Agency	09	01 (11.11)	91	4 (4.39)	100	5(5.00)
11.	Kohat	26	12 (46.15)	74	47 (63.51)	100	59(59.00)
12.	Kurrum Agency	94	06 (6.38)	106	03 (2.83)	200	09(4.50)
13.	Lower Dir	11	04 (36.36)	89	01 (1.12)	100	05(5.00)
14.	Malakand	11	00	178	20 (11.23)	189	20(10.58)
15.	Mansehra	12	00	188	07 (3.72)	200	07 (3.50)
16.	Mardan	22	08 (36.36)	58	06 (10.34)	80	14 (17.50)
17.	Mohmand Agency	46	01 (2.17)	154	21 (13.63)	200	22 (11.00)
18.	North Waziristan	30	17 (56.66)	167	40 (23.95)	197	57 (28.93)
19.	Noshehra	75	34 (45.33)	125	49 (39.20)	200	83 (41.50)
20.	Orakzai Agency	105	19 (18.09)	95	23 (24.21)	200	42 (21.00)
21.	Peshawar	31	04 (12.90)	167	06 (3.59)	198	10 (5.00)
22.	South Waziristan	89	38 (42.69)	11	06 (54.54)	100	44 (44.00)
23.	Swabi	75	19 (25.33)	112	11 (9.82)	187	30()16.04
24.	Swat	74	00	126	00	200	00 (0.00)
25.	Tank	100	79 (79.00)	98	83 (84.69)	198	162 (81.81)
26.	Upper Dir	71	13 (18.30)	128	19 (14.84)	199	32 (16.08)
	Total	**1353**	**337 (24.90)**	**3195**	**491 (15.36)**	**4548**	**828 (18.20)**

Figure- 1. Frequency distribution of percent inhibition (PI) values



(50.00%). On the contrary, Krishna *et al.* (2001) using the serum neutralization test, observed significantly lower (2.98%) seroprevalence of PPR in Andhra Pradesh of Southern India.

In the present study, 337 (24.90%) serum samples from sheep and 491 (15.36%) from goats were found positive in PPR c-ELISA yielding a marginal difference in seroprevalence of PPR (Table-2). Although, the exact comparison with regards to species wise prevalence could not be made on account of significantly different sizes of the two populations of sheep and goats, the higher prevalence in sheep points at the possible increasing prevalence and the sheep population needs to be given the equal weightage in deriving the estimates and subsequently the strategies to deal with PPR problem in any state.

Detection of PPRV antibodies can confirm the diagnosis of PPR, however, in areas where specific vaccination against PPR is practiced, detection of PPRV antibodies may yield false picture of the prevalence of infection. Presence of maternal antibodies may further contribute to this problem. Thus, in such cases, detection of PPR virus in clinical samples becomes essential. Virus isolation is considered as gold standard test for confirmation of PPR virus but is laborious and requires high technical expertise as well as sterile condition of the samples. Monoclonal antibodies have often been used to develop ic-ELISA, which is rapid and highly sensitive (Libeau *et al.,* 1995; Saliki *et al.,* 1994).

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