EPIDEMIOLOGICAL STATUS OF *PESTES DES PETITS RUMINANTS* (PPR) VIRUS INFECTION IN SMALL RUMINANTS OF PUNJAB, INDIA

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ABSTRACT

The present study reports the seroprevalence of PPR in sheep and goat populations of the north-western Indian state of Punjab, bordering Pakistan. Serum samples (n=446) from sheep and goats were collected using random sampling software 'survey toolbox' and monoclonal antibody (MAb)-based competitive ELISA was used for the detection of antibodies to PPR virus. Risk factors associated with the prevalence of anti-PPR antibodies were analyzed. The competitive ELISA revealed an overall apparent prevalence of 55.38%. Species seroprevalence was found to be significantly higher (chi square 13.549 $p<0.01$) in goats (63.16%) as compared to sheep (45.73%). Further, prevalence of specific antibodies was found to increase significantly with age (chi square 60.566, $p<0.01$) in both the species and significantly higher (chi square 19.727, $p<0.01$) in females in comparison to males. Animals under grazing had significantly higher antibody prevalence (chi square 74.380, $p<0.01$) in comparison to stall fed ones as indicated by risk ratio (18.676). Observed higher seroprevalence of the disease in the state of Punjab shows the need for implementation of regular vaccination and restriction of interstate movements of small ruminants for effective control of the disease.

Key words: Epidemiology, PPR, goats, sheep

Introduction

Peste des petits ruminants (PPR) is a highly contagious viral disease of domestic and wild small ruminants caused by a virus, peste des petits ruminants virus (PPRV) of genus *Morbillivirus,* family Paramyxoviridae (Gibbs *et al.,* 1979). PPR has been reported from various parts of Africa, Middle East, Indian sub-continent and Central Asia (Diallo *et al.,* 2007). The disease is one of the major transboundary animal diseases posing a severe threat to international trade (Domenech *et al.,* 2006). PPR has been identified as one of the priority animal diseases whose control is considered important for poverty alleviation in Western Africa and Southern Asia (Perry *et al.,* 2002). Though the disease was reported for the first time in India by Shaila *et al.* (1989), analysis of previous data suggested the presence of disease in southern India since 1980s (Taylor *et al.,* 2002). The disease has been reported in neighboring Pakistan more recently (Athar *et al.,* 1995; Hussain *et al.,* 1998). Since then, outbreaks of disease in small ruminants have been reported from both the countries (Joshi *et al.,* 1996; Nayak *et al.,* 1997; Rao *et al.,* 1998; Shankar *et al.,* 1998; Aruni *et al.,* 1999; Katoch *et al.,* 1999; Dhand *et al.,* 2002; Kumar *et al.,* 2002; Sharma *et al.,* 2007, Zahur *et al.,* 2008, Abubaker *et al.,* 2009). Abubaker *et al.* (2009) have reported the sero-prevalence and risk factors associated with the incidence of PPR in small ruminants in Pakistan including the state of Punjab bordering India. However, studies on sero-prevalence of the disease in India are limited (Hinsu *et al.,* 2001; Singh *et al.,* 2004a; Raghavendra *et al.,* 2008) and no reports are available from the state of Punjab. The state of Punjab is situated in the north-western part of India bordering Pakistan in the west. Though the state has no major population of small ruminants, prevalence studies in the state will help in understanding the epidemiology of PPR in surrounding regions like, Rajasthan and Jammu and Kashmir (in India) and Pakistan where the disease is responsible for severe economic loss. Hence, the present study was designed to investigate the seroprevalence of the disease in Punjab (India) using random sampling software 'survey toolbox' (Cameron, 1999). This is the first report on seroprevalence of the disease from the Indian state of Punjab. Further, the study appears to be first of its kind in India using such software in small ruminants and hence the results from the study could reflect those at the state and national level.

Materials and Methods

Study area

Punjab is the north-western state of India bordering Pakistan on the west and situated between the 29.30°N to 32.32°N latitude and 73.55°E to 76.50°E longitude. Climatically, the state has 5 seasons- spring (February- March), summer (April to June, maximum temperature 43.6°C), rainy season (July-August), autumn (September to November) and winter season (December-January, minimum temperature 4.4°C). Punjab state is broadly divided into 3 agro-climatic zones viz. Sub Mountain Region (annual rainfall 800-900 mm), Central Plain Region (annual rainfall 500-800 mm) and Southern-Western Region (<400 mm). Approximately 70% of human population lives in villages and agriculture is the main occupation. India has 200 million cattle, 76 million buffaloes, 110 million goats and 46 million sheep, of which Punjab has 2.64 million cattle, 6.17 million buffaloes, 0.41 million goats and 0.44 million sheep (www.husbandrypunjab.org).

Selection of villages and animals for sero-surveillance

To draw a simple random sample from the population of animals, two-stage sampling procedure was adopted. In the first step, villages were selected followed by selection of animals. A computerized list of the villages of the state was used as the sampling frame and villages were selected (n=48) from the state using simple random sampling, without replacement using 'Random Village' program of Survey toolbox (Cameron, 1999). The animals were selected using systematic random sampling and approximately 10% animals (n= 446) were selected from the selected flocks.

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Sample collection and serology

Blood samples (5 ml) were collected from the jugular vein of healthy animals; the sera were separated and stored at -20°C until they were tested for antibodies to PPR virus using competitive ELISA (cELISA). A standardized questionnaire for animals sampled at the farms was filled at the time of sampling. A monoclonal antibody (MAb)-based cELISA was used for the detection of antibodies to PPRV having sensitivity of 90.5% and specificity of 99.8% (Singh *et al.,* 2004b).

Statistical analysis

True prevalence was calculated at 95% confidence interval (CI) using the 'True prevalence' program of survey toolbox, in which sensitivity, specificity and sample size were taken into consideration. The data was analyzed using SPSS (Statistical Package for Social Sciences) for Window version 11.0.1©SPSS Inc. USA computer software program. The associations were evaluated between binary outcome variable and a variety of risk factors such as species, age, sex, and feeding patterns.

Results

For seroprevalence study a total of 446 animals were selected from 48 villages of Punjab. The serum samples from these animals were analyzed with compitative ELISA, which revealed prevalence of 63.16% and 45.73% in goats and sheep, respectively; the overall apparent prevalence of disease being 55.38%. Given the sensitivity and specificity of test at 90.5 and 99.8%, respectively, true prevalence was calculated to be 61.11% (95% CI 58.68-63.53).

The cELISA used in the present study to detect the presence of antibodies to PPRV was able to clearly differentiate the exposed (infected) population from the unexposed (not infected) population. However, considerable differences were observed between the exposed sheep and goat populations when the results of the cELISAs were plotted as a frequency of the per cent colour inhibition. Among the samples considered negative for PPRV (% colour inhibition of less than 50%) the greatest number of samples had a per cent colour inhibition of between 6 to 16 and 11 to 21 for goats and sheep, respectively. Alternatively, among the samples considered positive for PPRV (% colour inhibition of greater than 50%) a peak frequency distribution of between 81% and 91% colour inhibition was observed for the goat population (Fig. 1).

Various host factors associated with the prevalence of anti-PPR antibodies were studied. Species (Table 1) prevalence was found to be significantly higher (chi square 13.549 $p<0.01$) in goats (63.16%) as compared to sheep (45.73%). Prevalence was significantly higher (chi square 19.727, $p< 0.01$) in females in comparison to males and risk of infection was twice more in females (Table 2). In different age groups (<1 year, 1-2 years, 2-3 years, 3-4 years, 4-5 years and >5 years) prevalence of specific antibodies to PPR virus was found to increase significantly with age (chi square 60.566, $p<0.01$) in both the species (Fig. 2) indicating exposure of animals to infection during the lifetime. Grazing animals had significantly higher antibody prevalence (chi square 74.380, $p<0.01$) in comparison to stall fed animals (Table 3) as indicated by risk ratio.

Discussion

Though Punjab is not a major small ruminant rearing state and this report is a small sample study, the information obtained is of epidemiological significance since the state has some major small ruminant migration routes across its territory. The state has geographical continuity and similar ecological features as bordering Pakistan, where the disease has greater significance to the livestock industry. Sero-prevalence of PPR has been reported recently from southern peninsular India (Raghavendra *et al.,* 2008) using samples collected from most parts of India (Singh *et al.,* 2004a). However, the state of Punjab was not included in their study as the state has a small ruminant population. Though no animal trade occurs across the border between Indian Punjab and Pakistan, trading of farm goods is practiced leading to movement of men and materials related to agriculture at Wagha (International border between India and Pakistan). The endemic nature of PPR in Pakistan and the high prevalence the disease in the plains of Pakistan Punjab has been reported recently (Zahur *et al.,* 2008; Abubaker *et al.,* 2009). Unusual increase in the occurrence of the outbreaks in the months April to June coincides with the movement of sheep flock of nomads from the desert state of Rajasthan. Since the April-June period is hot and dry summer in Rajasthan, it is usual for nomads in this region to migrate towards the Himalayan plane of Jammu through the state of Punjab. By the time the Punjab region has finished harvesting the wheat crop and it is possible that wheat stumps in the harvested fields might cause abrasion of oral mucosa during grazing making them more prone for the entry of virus (Dhand *et al.,* 2002; Sharma *et al.,* 2007). Interestingly, in a similar study a higher incidence of PPR was reported in the months of March and April in Pakistan during 2006-2008 (Abubaker *et al.,* 2009). Molecular characterization of virus isolates from either side of the border is needed for investigating the possible cross border transmission of the virus.

The prevalence (55.38%) in the present study is quite higher compared to earlier studies. In similar studies from southern peninsular India Raghavendra *et al.* (2008) had reported the sero-prevalnce of 41.35% in sheep and 34.91% in goats. However, Singh *et al.* (2004a) reported the lower prevalence in northern India (10-30%) compared to southern and south-western India (30%-60%). Interestingly, similar to the present observation (Abubaker *et al.,* 2009) has reported the sero-prevalence of 54.09% in sheep and 44.15% in goats from Pakistan. The high prevalence of antibodies to PPR virus in the absence of vaccination indicates the widespread infection in the small ruminants of the state.

The cELISA used in the present study to detect the presence of antibodies to PPRV was able to clearly differentiate the exposed (infected) population from the unexposed (not infected) population. However, considerable differences were observed between the exposed sheep and goat populations when the results of the cELISAs were plotted as a frequency of the per cent colour inhibition. The findings suggest that only those goats capable of mounting a strong humoral antibody response to PPRV (high percent colour inhibition) were capable of surviving infection.

Our results show that the risk of disease was twice as high in goats in comparison to sheep. Similar studies conducted earlier have also reported higher prevalence of disease in goats as compared to sheep (Rajeshwari *et al.,* 2000; Singh *et al.,* 2004a), although, Abraham *et al.* (2005) reported higher antibody prevalence in sheep (13%) in comparison to goats (9%). Thus, for any control programme to be successful, both the species will have to be included in the programme. The observed prevalence of specific antibodies which was found to be increasing with age indicates the exposure of animals to infection during the lifetime.

Table 1: Species wise antibody prevalence of PPR

Species	Total	Positive	% Prevalence
Goat	247	156	63.16
Sheep	199	91	45.73
Total	446	247	55.38

Chi square - uncorrected	13.549 (P < 0.01)
Chi square-Mantel-Haenszel	13.518 (P < 0.01)
Chi square-corrected (Yates)	12.852 (P < 0.01)
Odds ratio	2.035 (95% CI=1.392-2.974)
Risk ratio	1.381 (95% CI=1.161-1.648)

Table 2: Sex wise antibody prevalence of PPR

Sex	Total	Positive	% Prevalence
Female	389	231	59.38
Male	57	16	28.07
Total	446	247	55.38

Chi square - uncorrected	19.727 (P < 0.01)
Chi square-Mantel-Haenszel	19.682 (P < 0.01)
Chi square - corrected (Yates)	18.480 (P < 0.01)
Odds ratio	3.746 (95% CI=2.045-6.859)
Risk ratio	2.116 (95% CI=1.443-3.297)

Table 3: Association of feeding pattern with antibody prevalence of PPR

Feeding pattern	Total	Positive	% Prevalence
Grazing	387	245	63.31
Stall-fed	59	2	3.39
Total	446	247	55.38

Chi square - uncorrected	74.380 (P < 0.01)
Chi square - Mantel-Haenszel	74.213 (P < 0.01)
Chi square - corrected (Yates)	71.975 (P < 0.01)
Odds ratio	49.173 (95% CI=13.021-185.206)
Risk ratio	18.676 (95% CI=5.554-67.901)

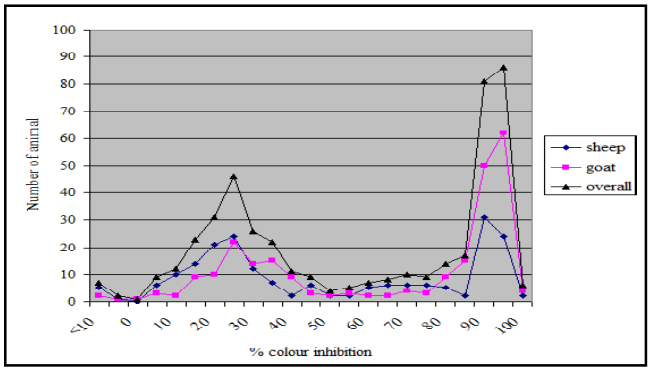

Fig. 1: Identification of uninfected versus infected in sheep and goat populations

Similar results had been reported by various workers (Singh *et al.,* 2004a; Agrawal *et al.,* 2006). Evidence of infection was twice more frequent in females compared to males which were similar to earlier reports (Hinsu *et al.,* 2001; Agrawal *et al.,* 2006). The reason

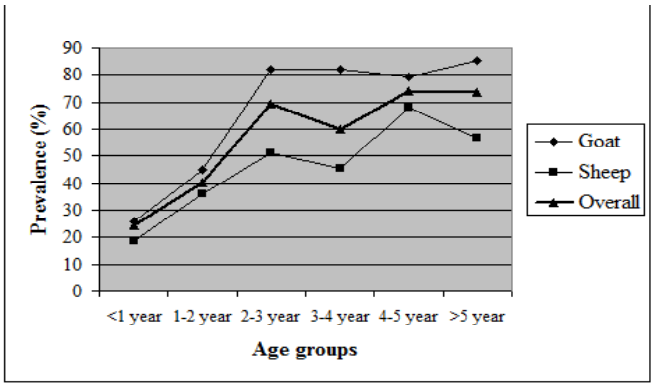

Fig. 2: Age-wise prevalence of antibodies to PPRV

for higher antibody prevalence in females could be that most of the males are sold at much earlier age for meat purpose retaining only a few for breeding, whereas females are mostly kept for reproduction and milk production. Feeding pattern has significant impact on PPR antibody prevalence which was reflected in the observation that grazing animals had significantly higher antibody prevalence in comparison to stall fed animals as indicated by risk ratio. As the grazing animals have higher chance of picking up the infection from direct and indirect contact with source of the virus.

Observations of the study suggest that PPR is endemic in the state of Punjab and outbreaks reported were coinciding with the annual migration of animals from across the states. Since, similar pattern of outbreaks and sero-prevalence of the disease were reported from neighboring Pakistan it seems that similar epidemiological factors operate on either sides of the border. Further molecular studies of the virus isolates from the outbreaks are required to investigate the possible transmission of the virus across the borders. Strict movement restrictions and regular vaccination of both goat and sheep populations is required for effective control of the disease in the region. Further, regional and international approaches are needed for prevention and control of the disease and to reduce its economic impact.

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