Rev. sci. tech. Off. int. Epiz., 2004, **23** (3), 807-819

Prevalence and distribution of peste des petits ruminants virus infection in small ruminants in India

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Submitted for publication: 16 March 2004
Accepted for publication: 21 July 2004

Summary

Peste des petits ruminants (PPR) is an acute febrile viral disease of goats and sheep characterised by mucopurulent nasal and ocular discharges, necrotising and erosive stomatitis, enteritis and pneumonia. The disease is endemic in India and causes large economic losses each year due to the high rates of mortality and morbidity in infected sheep and goats. The present study reports observations from 58 laboratory confirmed outbreaks of PPR and provides details of the prevalence of antibodies to PPR virus (PPRV) in 4,407 serum samples of small ruminants. Most of the clinical specimens used for the study originated from the northern and central parts of India. Serum samples used for the detection of antibodies to PPRV were derived from a greater number of regions within the country, however, these samples may not be a true representation of the target population (unvaccinated sheep and goats over 3 months old). Indigenously developed monoclonal antibody-based diagnostic kits were used for the detection of PPRV antigen (sandwich enzyme-linked immunosorbent assay [ELISA]) and antibody (competitive ELISA). Findings suggested that the disease outbreaks were more severe in goats than sheep and that the frequency of disease outbreaks was greater between the months of March and June (51.7%) as compared to other periods of the year. Based on the screening of the 4,407 sera samples, the antibody prevalence of PPRV in small ruminants in India was 33% (95% confidence interval: 32.3% to 33.7%). The prevalence of antibodies to PPRV was noted to differ between species (i.e. sheep versus goats), age groups and geographical regions. A greater proportion of the sheep (36.3%) versus the goat (32.4%) population was infected with PPRV. The distribution and prevalence of antibodies to PPRV among various age groups of animals indicated that goats were exposed at an earlier age than the sheep, suggesting that goats may be more susceptible to infection with PPRV. A greater number of positive cases were observed in the southern and southwestern part of the country (30%-60%) as compared to northern India (10%-30%). These findings may be correlated with variations in the sheep and goat husbandry practices within different geographic regions, the topography of different states and the socio-economic status of individual Indian farmers.

Keywords

Antibody detection – Antigen detection – Competitive enzyme-linked immunosorbent assay – Incidence – Peste des petits ruminants – Prevalence – Sandwich enzyme-linked immunosorbent assay – Serological surveillance.

Introduction

Peste des petits ruminants (PPR) is an acute febrile viral disease of goats and sheep. Clinical symptoms include mucopurulent nasal and ocular discharge, stomatitis, enteritis and pneumonia (12). The disease is caused by PPR virus (PPRV), a ribonucleic acid virus belonging to the genus *Morbillivirus* and family *Paramyxoviridae* (8). The PPRV is an enveloped virus and, like most enveloped viruses, is sensitive to environmental changes. Rapid inactivation of the virus will occur when exposed to conditions outside of the host environment. Consequently, close contact between infected and susceptible animals is necessary for disease transmission to take place (17).

Peste des petits ruminants has been reported in various parts of Asia and Africa (7, 26). In India, although PPR was believed to have been present in southern India prior to the late 1980s (39), the disease was not officially recorded in this region until 1987 (29). The disease has also been described in parts of northern India (23). Presently, PPR outbreaks are reported regularly (15, 16, 21, 23, 24) and the disease is considered to be endemic throughout India (33).

India is a vast country with a population of 126 million goats and 58.2 million sheep. The ratio of goats to sheep and the population intensity vary greatly under different agro-climatic conditions. In India, sheep and goats play an important role in sustainable agriculture and employment generation (5, 10). Sheep and goat husbandry is primarily the work of small to marginal farmers and landless labourers (28). Due to the endemic status of PPR in India, with a mortality rate of 50% or more in susceptible sheep and goat populations (1, 15, 31), PPR is considered to be one of the main limitations to production in the small ruminant industry. Economic losses due to PPRV have been estimated to be 1,800 million Indian Rupees (US$ 39 million) annually (4).

Information on the prevalence of antibodies to PPRV in small ruminants is available from a number of countries in which the disease is reported, including the Sultanate of Oman (39), Jordan (18), Turkey (25) and various African countries (3, 22). However, the pattern of PPRV infection and its seroprevalence in small ruminants in India has not been systematically studied to date. In the present study, efforts have been made to collect preliminary information on the prevalence of antibodies to PPRV in the sheep and goat population, its distribution in different age groups of animals, the host affinity of PPRV and the socio-economic factors responsible for transmission of the disease in different parts of India. The investigation includes observations from 58 laboratory confirmed PPR outbreaks and data generated from the screening of 4,407 sera samples originating from the sheep and goat population in India. Although the serum samples may not be a true representative of the target population (unvaccinated sheep and goats over 3 months old), this study provides baseline information on the sero-epidemiology of PPRV infection in the small ruminant population in India. This information will be very useful in the development of a PPR control programme using an indigenously developed PPRV vaccine (37).

Materials and methods

Clinical samples for peste des petits ruminants antigen detection

The PPR outbreaks investigated in the present study were recorded from various parts of India between May 2001 and April 2003. The majority of clinical samples were received from the northern and central parts of the country. In most cases, the samples used for laboratory confirmation were transported via special messenger directly from the site of origin of the outbreaks. Occasionally, samples were received from various agencies such as state disease investigation units and research institutes. Tissue samples were prepared in a 10%-20% suspension of phosphate buffered saline (PBS) (0.01M, pH 7.4 ± 0.2) prior to the use of an ELISA to detect the presence of PPRV antigens in the tissues (36). Swab materials collected from animals suspected to be infected with PPRV were extracted in 500 µl of PBS. These specimens were stored at –25°C for further analysis as required. The distribution of the 58 outbreaks from which clinical materials were collected is shown in Figure 1.

Serum samples for the detection of antibody to peste des petits ruminants virus

The serum samples used in the present investigation were either collected for rinderpest sero-surveillance as part of the National Project on Rinderpest Eradication of the Government of India, or obtained as prevaccinate sera as part of field trials for the development of an indigenously developed PPRV vaccine. Some of the serum samples were also collected under a different scheme investigating the prevalence of other diseases that are of concern to the sheep and goat population in India. All of the serum samples tested as part of the present investigation were collected between the years 1998-2003. None of the animals from which the samples were collected had a history of PPR or rinderpest vaccination. The samples used in this study are currently available in the serum repository of the Rinderpest and Allied Diseases Laboratory of the Division of Virology, at the Indian Veterinary Research

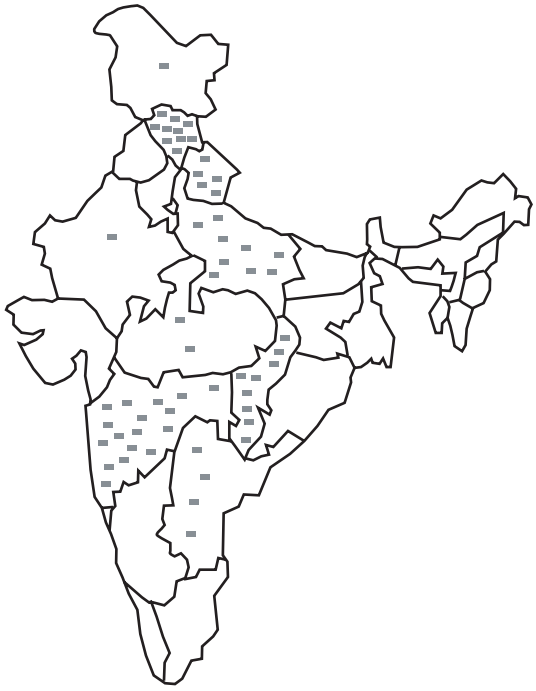

Fig. 1
Geographic distribution of 50 laboratory confirmed peste des petits ruminants virus (PPRV) outbreaks described in the present study of PPR in India (the total number of outbreaks was 58, but in eight of the outbreaks it was not clear which species were involved, i.e. sheep or goats or both)
An outbreak due to PPRV was identified if one of the clinical samples received from a specified area clearly tested positive for antigen to PPRV. The presence of antigen in clinical samples was detected using the sandwich enzyme-linked immunosorbent assay. Each point indicates a single outbreak

Institute (IVRI), Mukteswar campus. The samples originated from both organised (large flocks that are mostly stall fed) and unorganised sectors (scattered animals in rural dwellings). Details of the species and the area of origin from which the samples were collected are presented in Table I. The serum samples used to determine the prevalence of antibodies to PPRV in different age groups of sheep and goats originated solely from organised sectors (Table II). Due to a small sample size and skewed distribution these samples may not be a true representative of the target population.

Tests for detection of peste des petits ruminants antigens and antibodies

Indigenously developed monoclonal antibody (MAb) based ELISAs were used in the present study. These included a competitive ELISA (for the detection of antibodies to PPRV) and a sandwich ELISA (for the detection of antigens to PPRV) (35, 36). The tests used in this study have been proven to have a high efficacy, with diagnostic sensitivities and specificities comparable to internationally accepted tests used for the diagnosis of PPRV (i.e. competitive ELISAs and immunocapture ELISAs for the detection of PPRV antibody and antigen, respectively [3, 19], and virus neutralisation tests [VNT] for the detection of PPRV).

Sandwich enzyme-linked immunosorbent assay

The presence of antigens in clinical samples was detected through the use of a sandwich ELISA employing a detection antibody directed against an epitope of nucleoprotein (N) of PPRV (34). All samples were tested in duplicate as per the protocol previously described by Singh *et al.* (36). Briefly, the ELISA plates were coated with a 1:4000 dilution of capture antibody (100 µl/well) and incubated at 37°C for 1 h with constant agitation. Unbound antibody was washed away using diluted PBS (0.0025 M, pH 7.4 ± 0.2) containing 0.05% Tween 20. Fifty microlitres of blocking buffer were then added to each well followed by the addition of the 50 µl/well of the serum samples. Antigen blank wells (50 µl blocking buffer per well) and positive and negative control wells (50 µl positive or negative control antigen per well) were also included as part of each test plate. The plates were then incubated for 1 h at 37°C with constant agitation. Following incubation, detection antibody (MAb to PPRV N), diluted 1:20 in blocking buffer, was added to each of the wells (100 µl/well) and the plates were incubated at 37°C for 1 h. One hundred microlitres (100 µl) of antimouse-horseradish peroxidase (HRPO) conjugate was then added to each well followed with incubation for 1 h at 37°C. The plates were washed with PBS after each incubation period. In the final step, substrate solution (*O*-phenylenediamine dihydrochloride containing H_2O_2) was added to each well (100 µl/well) allowing 10 min for a colour reaction to develop. The colour reaction was halted with the addition of an equal volume of 1 M H_2SO_4. Optical densities were measured at 492 nm. Samples with greater than twice the optical density of the antigen blank wells were considered to be positive samples. An outbreak was identified as being due to PPRV if one clinical sample received from a specified area clearly tested positive for antigen to PPRV.

Competitive enzyme-linked immunosorbent assay

A MAb-based competitive ELISA was used for the detection of antibodies to PPRV (35). This test was developed using a virus neutralising MAb directed against an epitope of the haemagglutinin protein specific to PPRV. The competitive ELISA used in the present investigation had high diagnostic specificity (99.8%) and sensitivity (90.5%) for the detection of PPRV antibody in convalescent sera when compared with the gold standard VNT (35). All serum samples tested in the present study were processed in duplicate as per the standard protocol

Table I

Prevalence of antibodies to peste des petits ruminants virus (PPRV) in the sheep and goat population of India between 1998 and 2003

A monoclonal antibody-based competitive enzyme-linked immunosorbent assay was used to detect the presence of serum antibodies. Serum samples with antibody levels sufficient to cause a percent colour inhibition of greater than 50% were considered positive for PPRV infection

Indian states	Number of positive samples/total number of samples (percentage of positive samples)					
	Sheep serum		Goat serum		Total	
1. Jammu and Kashmir	11/27	(40.7%)	8/21	(38.1%)	19/48	(39.6%)
2. Himachal Pradesh	15/97	(15.5%)	34/84	(40.5%)	49/181	(27%)
3. Haryana	–		8/57	(14%)	8/57	(14%)
4. Uttaranchal	12/44	(27.3%)	128/630	(20.3%)	140/674	(20.8%)
5. Uttar Pradesh	87/244	(35.7%)	252/1,017	(24.8%)	339/1,261	(26.9%)
6. Rajasthan	60/306	(19.6%)	4/25	(0.16%)	64/331	(19.3%)
7. Madhya Pradesh	0/3		9/30	(30%)	9/33	(27.3%)
8. Chhatish Garh	–		45/102	(44.1%)	45/102	(44.1%)
9. Orrisa	3/4	(0.75%)	11/70	(15.7%)	14/74	(18.9%)
10. Gujarat	8/10	(0.80%)	14/43	(32.6%)	22/53	(41.5%)
11. Maharashtra	216/428	(50.4%)	388/536	(72.4%)	604/964	(62.7%)
12. Karnataka	19/40	(47.5%)	–		19/40	(47.5%)
13. Andhra Pradesh	114/278	(41.%)	20/56	(35.7%)	134/334	(40.1%)
14. Tamil Nadu	0/19		19/37	(51.4%)	29/56	(51.8%)
15. Meghalaya	–		3/145	(2.1%)	3/145	(2.1%)
16. Assam	–		0/54	(0%)	0/54	(0%)
Total	**545/1,500**	**(36.3%)**	**943/2,907**	**(32.4%)**	**1,498/4,407**	**(33%)**
	(CI: 33.9 to 38.7%)		**(CI: 29.8 to 33.1%)**		**(CI: 32.3 to 33.7%)**	

CI: confidence interval

Table II

Peste des petits ruminants virus (PPRV) antibody prevalence in different age groups of sheep and goats

The serum samples used to generate this data originated from organised sectors (large flocks that are mostly stall fed) only and therefore represent a subpopulation of the total 4,407 serum samples screened for the presence of antibodies to PPRV

Age groups	Number of positive samples/total number of samples (percentage of positive samples)					
	Sheep		Goat		Total	
3-6 months	1/64	(1.6%)	0/124	(0%)	1/188	(0.5%)
6-12 months	29/245	(11.8%)	3/16	(18.8%)	32/261	(12.2%)
> 12 months	42/99	(42.4%)	23/106	(21.7%)	65/205	(31.7%)

for the detection of rinderpest antibodies (32). Briefly, the ELISA plates were coated with a 1:100 dilution of PPRV antigen (50 µl/well) derived from Vero cells and the plates were incubated at 37°C for 1 h with constant agitation. Unbound antigen was washed away using diluted PBS (0.0025 M, pH 7.4 ± 0.2) followed with the addition of 40 µl of blocking buffer to each of the wells (PBS containing 0.2% PPRV negative goat serum and 0.1% Tween 20). Twenty microlitres of the test and control serum samples (negative, weak positive and strong positive) were then added (in duplicate) followed with the addition of 40 µl of MAb (except to the conjugate control wells) at a concentration of 1:100 in blocking buffer. The plates were then incubated for 1 h with constant agitation. All of the wells were washed with PBS after each incubation period. Rabbit antimouse-HRPO conjugate, diluted 1:1000 in blocking buffer, was added to each well (50 µl/well) and the plates were incubated for 1 h at 37°C with constant agitation. Substrate solution (*O*-phenylenediamine dihydrochloride containing H_2O_2) was added to each well (50 µl/well) allowing 10 min for a colour reaction to develop. The colour reaction was halted with the addition of an equal volume of 1 M H_2SO_4. Optical densities were measured at 492 nm.

The results of the competitive ELISA were interpreted using software from the Economic Development Institute developed by the Food and Agriculture Organization/ International Atomic Energy Agency for the detection of rinderpest antibody (11, 14). Results were interpreted as previously described by Singh *et al.* (35). Samples with a % colour inhibition (% inhibition of the enzymatic colour reaction) of equal to or greater than 50%, when compared to wells containing the MAb control (no serum), were considered positive. A confidence interval (CI) of 95% level for the prevalence of antibodies to PPRV in the population studied was calculated using standard statistical methods (13).

Results

Disease investigation and clinical findings in diseased animals

Based on information supplied in outbreak records, the most frequent clinical findings in diseased animals were a high body temperature (up to 107°F), severe muco-purulent nasal and ocular discharges, necrotic stomatitis and respiratory distress; diarrhoea was also present in a few incidences. A complete history of disease was not provided for all of the outbreaks. The most common post-mortem lesions reported were necrotic enteritis, pneumonia, splenomegaly and enlargement of the lymph nodes. Interestingly, at least 7 of the 58 outbreaks were associated with either the entry of newly purchased animals from a common market or the intermixing of migratory/nomadic animals with local animals. A complete history of the movement or the nomadic nature of the animals was not available for all of the outbreaks investigated. The introduction of sheep purchased from an area endemic for PPR disease was associated with one of the outbreaks in a goat herd, although the sheep did not develop clinical signs of PPRV infection. Two outbreaks which occurred in sheep flocks from the hilly areas of the sub-Himalayan zone were associated with the movement of the flocks to high altitude pastures during the summer.

Ante-mortem materials (e.g. nasal swabs, buccal swabs and eye swabs) from diseased animals were found to be more suitable than post-mortem tissues for PPRV diagnosis using the sandwich ELISA. Amongst the 58 confirmed outbreaks of PPRV, 32 of the outbreaks involved goats alone, 13 of the outbreaks involved sheep alone, 5 of the outbreaks involved both sheep and goats, and in the remaining 8 outbreaks details of the species involved were not reported. Outbreaks were reported to be more severe in goats than sheep. The greatest frequency of PPR outbreaks reported for the period of May 2001 to April 2003 (i.e. 51.7% of the total number of PPR outbreaks) occurred during the months of March, April, May and June (Fig. 2). The geographical distribution of PPR outbreaks is illustrated in Fig 1.

Prevalence of antibodies to peste des petits ruminants virus

The competitive ELISA 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 competitive ELISAs were plotted as a frequency of the percent colour inhibition (Fig 3). Among the samples considered negative for PPRV (% colour inhibition of less than 50%) the greatest number of samples had a percent 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. A similar distribution was not

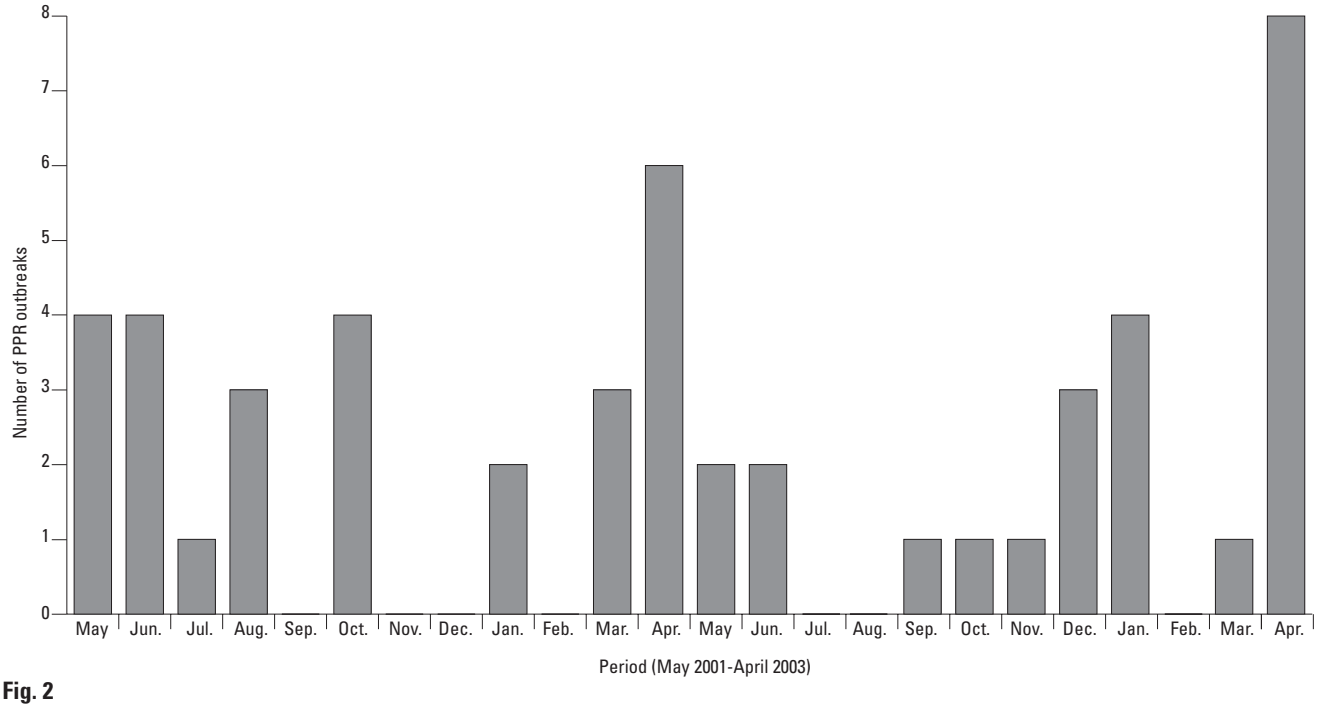

Fig. 2

Frequency distribution of peste des petits ruminants (PPR) virus outbreaks in India (n = 58) between May 2001 and April 2003

A higher frequency of outbreaks is observed between March and June (30/58 = 51.7%) as compared to the other months

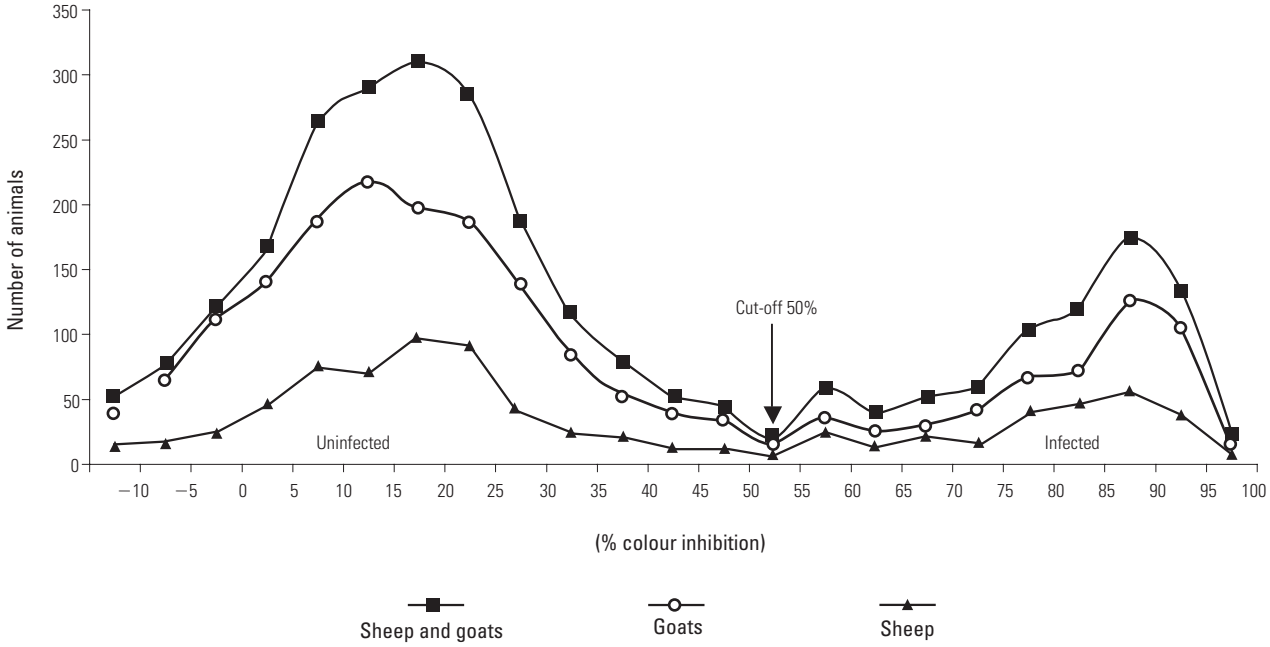

Fig. 3

Identification of uninfected versus infected animals in the sheep and goat population in India

The percent colour inhibition indicated using a competitive enzyme-linked immunosorbent assay provided an indirect measure of antibody levels in the serum samples (n = 2,830 [initially, only 2,830 samples were analysed, later this number increased to 4,407 as samples from a wider geographic area were included in the study]). Samples with antibody levels sufficient to cause a colour inhibition of >50% were considered positive (infected animals)

observed for the sheep population. These 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. This should not be confused with the higher prevalence of antibodies to PPRV (proportion of sera samples with a % colour inhibition of greater than 50%) observed in the sheep population versus the goat population.

The distribution and prevalence of antibodies to PPRV among various age groups of animals was studied using a competitive ELISA. The serum samples used to generate this data originated from organised sectors only and therefore represented a subpopulation of the total 4,407 serum samples screened for the presence of antibodies to PPRV. Findings suggest that the majority of animals were exposed when older than 12 months. The percentage of adult animals (>12 months) that tested positive for PPRV was greater in the sheep (42.4%) versus the goat (21.7%) population. However, a higher proportion (18.7%) of goats between the ages of 6-12 months tested positive for PPRV as compared to sheep from the same age category (11.8%). These results suggest greater susceptibility of the former for infection with PPRV (Fig. 4). The number of PPRV positive samples categorized by age group is shown in Table II.

Based on the screening of 4,407 samples collected from both organised and unorganised sectors, the overall antibody prevalence of PPRV in sheep and goats greater than six months old was 33% (95% CI: 32.3% to 33.7%). This indicates that an average of one out of every three small ruminants was exposed to PPRV. The prevalence of antibodies to PPRV in the sheep and goat population was 36.3% (95% CI: 33.9% to 38.7%) and 31.4% (95% CI: 29.8% to 33.1%), respectively. The percentage of animals that tested positive for PPRV ranged from 20%-60% in the

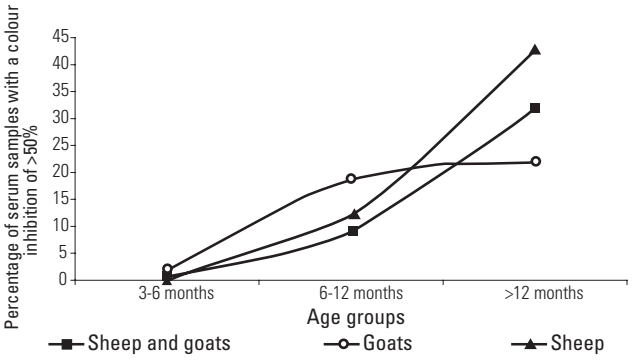

Fig. 4

Prevalence of antibodies to peste des petits ruminants virus (PPRV) in different age groups of small ruminants

The serum samples used to generate this data originated from organised sectors (large flocks that are mostly stall fed) only and therefore represented a subpopulation of the total 4,407 serum samples screened for the presence of antibodies to PPRV

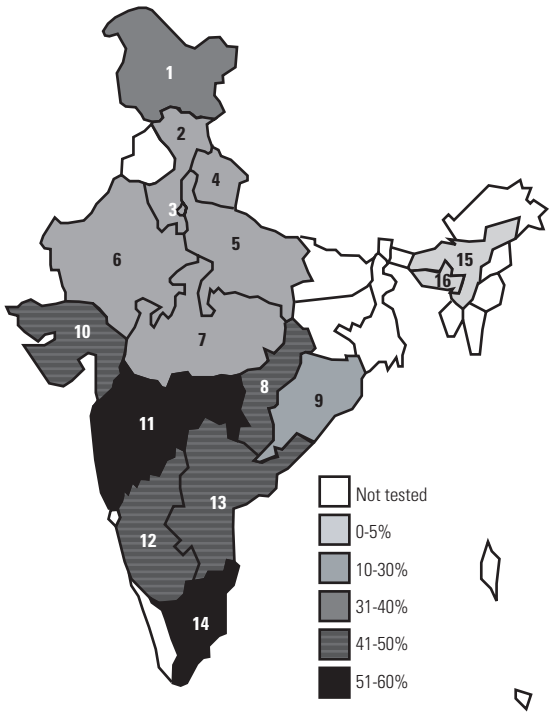

Fig. 5
Antibody prevalence (percentage of positive serum samples) of peste des petits ruminants virus (PPRV) in small ruminants (n = 4,407) in India
Samples with antibody levels sufficient to cause a colour inhibition of >50%, as determined by the competitive enzyme-linked immunosorbent assay were considered positive for PPRV infection. Numbers 1-16 correspond to name of the Indian states as shown in Table I

majority of the geographical regions. A greater number of positive samples were observed in southwestern India as compared to the northern and northeastern regions (Fig. 5). The percentage of positive PPRV samples categorized by state is shown in Table I.

Discussion

Several PPR outbreaks go unrecorded in India due to inadequate animal disease reporting and surveillance systems. The majority of PPR outbreaks in the past have been diagnosed based on clinical signs. An extensive clinical survey of PPRV infection has been difficult up to this point in time because the diagnostic tests that were available for the detection of PPRV were not commonly implemented, possibly because the endemic nature of the disease was not known. The development of a MAb-based sandwich ELISA kit (for PPRV antigen detection) and a competitive ELISA kit (for PPRV antibody detection), both of which were developed in the Rinderpest and Allied Diseases Laboratory at IVRI (34, 35, 36), has greatly facilitated the diagnosis of PPRV. The implementation of these diagnostic tests will aid in tracking PPR outbreaks in different geographical regions, measuring economic losses resulting from PPRV infection, and studying the epidemiology of the disease in susceptible populations. Measurement of the prevalence of antibodies to PPRV in different regions of the country with varying agro-climatic conditions may be helpful in identifying appropriate disease control strategies such as the use of the live attenuated homologous PPR vaccine recently developed at IVRI (37).

Disease transmission: animal movements and climatic factors

The majority (78.2%) of farmers in India have small and marginal land holdings (9). Small ruminants are farmed on free range pastureland, shrubs and forest cover. Due to an ongoing decrease in available pastureland and forest area, these animals will often travel long distances during the dry season in search of fodder and water. Migratory animals have been observed in the sub-Himalayan region (Himachal Pradesh, Uttaranchal and Jammu and Kashmir) as well as in dry land areas such as the states of Rajasthan and Gujarat. The migration of animals from low altitude pasture land in the winter to high altitude pasture land in the summer is common in the sub-Himalayan region (23). PPRV is transmitted through direct contact between infected and susceptible animals and nomadic animals will often come into contact with local sheep and goat populations from whom they may contract the virus (23, 31). Likewise, infected migratory animals may transmit the virus to susceptible local sheep and goats. The movement of animals, therefore, plays an important role in the transmission and maintenance of PPRV in nature, as does the purchase of potentially infected animals and their subsequent introduction into naïve flocks/herds.

The migration of animals in search of food during the hot dry summer when fodder is scarce may be one of the reasons for the higher frequency of PPR outbreaks between the months of March and June. Furthermore, when fodder is limited, animals often become nutritionally deficient, resulting in an increased susceptibility to infection. Consequently, large numbers of animals become infected during this period and these animals then help to maintain the circulation of the virus throughout the year by frequent animal to animal transmission.

Climatic factors favourable for the survival and spread of the virus may also contribute to the seasonal distribution of PPR outbreaks. With the start of the rainy season (between June/July and August/September) the migratory activity of animals is reduced due to the increased availability of local fodder. The nutritional status of the animals also improves, resulting in an increased resistance to infection. These factors may play a key role in limiting the transmission of

PPRV and hence reducing the frequency of PPR outbreaks during this period. Similar observations were also made during a five year study of PPRV in the tropical humid zone of southern Nigeria (40).

Differences in the antibody prevalence among the goat and sheep populations

The higher prevalence of antibodies to PPRV (the proportion of animals with circulating PPRV antibodies at a level sufficient to cause a colour inhibition of >50% using the competitive ELISA) in the sheep versus goat population should not be misinterpreted as an increased susceptibility of sheep to infection with PPRV. Rather, this may be attributed to a higher recovery rate (lower case fatality rate) and/or a greater longevity of sheep versus goats. In another study a case fatality rate of 34.4% versus 46.9% was observed in the sheep versus goat population, respectively (31). Male goats are sold for meat at the local market at approximately one year of age (the age at which the desired body weight is reached), but sheep which are used for both wool and meat production are kept for a longer period of time. Recovered (convalescent) animals will have detectable levels of circulating antibody in their serum. The presence of a large proportion of animals in the sheep population which have recovered from previous infections with PPRV and are maintained in the flock over many years for the purposes of wool production may indirectly account for the high prevalence of antibodies to PPRV detected in sheep (36.3%, 95% CI: 33.9% to 38.7%) as compared to goats (31.4%, 95% CI: 29.8% to 33.1%).

Differences in the prevalence of antibodies in different regions of India

In India the proportion of goats to sheep is approximately 2:1. In the present study, despite the small sample size, the ratio of goats to sheep was also approximately 2:1. (However, as previously stated, the samples may not be representative of the target population, and due to the relatively small number of samples that were tested from some states, differences between states in the PPRV antibody of goats versus sheep may not have much significance.) The overall prevalence of antibodies to PPRV in small ruminants was higher in southern and southwestern India (30%-60%) compared to northern India (10%-30%). With the exception of the state of Kerala, all of the southern states in India (Andhra Pradesh, Tamil Nadu and Karnataka) had a higher proportion of sheep than the national average. A similar situation existed in the western Indian states of Gujarat and Rajasthan. Geographical regions with a higher proportion of sheep than the national average (southern and southwestern states) may appear as having a greater prevalence of antibodies to PPRV.

Between 1987 and 1993 severe outbreaks of PPRV were reported in the southern and southwestern states of India. The remainder of the country was considered to be free of the disease during this period (23). PPR outbreaks were reported in the northern part of India from 1994 onward. The greater length of time that PPRV has been endemic in the southern and southwestern states may account for the higher prevalence of infection observed in this region as compared to other regions in the country. A rate of PPRV antibody prevalence ranging between 0% and 2.1% was reported for the northeastern Indian states of Assam and Maghalaya. The northeastern states of India are geographically isolated and connected to the rest of the country by a very narrow passage of land. These states have a relatively small sheep and goat population, and, although previous outbreaks have been reported in this region following the transport of goats from endemic zones of the country (21), intermixing of these animals with the small ruminant population from the rest of the country is usually limited. The hilly terrain characterizing this region may further restrict the movement of animals and therefore the transmission of the virus between animals. These factors may account for the low PPRV antibody prevalence (<2.1%) reported in this region as compared to the rest of the country. Despite the geographical isolation of northeastern India from the rest of the country, the migration of small ruminants from the surrounding countries of Bangladesh and Burma, both of which have reported PPR outbreaks in the past, should not be ignored (7, 30).

Differences in the severity of disease in the sheep and goat population

The present investigation also revealed differences in the severity of PPRV infection in goats and sheep. In the present study, as well as in previous studies of PPRV isolates originating from north India (23), a higher affinity of the virus for the caprine species versus the ovine species has been observed. These laboratory findings suggest that the same virus capable of inducing high mortality in goats may not produce as severe an infection in sheep, but the reverse may not be true. Further investigation is required to determine reasons for the difference in the pathogenicity of PPRV in caprine and ovine species. Molecular analysis of virus isolates originating from the same geographical region and involving both sheep and goats is necessary to detect differences at the genomic level. The identification of isolates that have a higher affinity for either the caprine or ovine species may account for the observed difference in the severity of disease between these two species. The epidemiological pattern of such viruses (viruses with multiple closely related isolates) may vary as is evident from the closely related strains of rinderpest virus. Rinderpest virus in Asia has a high affinity for cattle, as well as small ruminants (2). However, depending upon the

strain involved, the sheep and goat population in African countries may remain unaffected by rinderpest (6).

The greater severity of the disease in goats may be attributed to a greater susceptibility of the goat population to infection with PPRV. The recovery rate of goats infected with PPRV is considerably less than that of sheep infected with the virus (31). In addition, the fecundity of goats is higher than that of sheep, so newborn kids replace a large proportion of the goat population each year. Newborn animals become susceptible to PPRV infection at three to four months of age (38), corresponding with the natural decline in maternal antibodies (27), and this large number of young susceptible stock may also account for the severity of PPRV infection in the goat population in India. Furthermore, the uniform distribution of the goat population in rural areas, combined with the practice of grazing goat herds over long distances, results in a high rate of contact between animals which increases the potential for transmission of PPRV in the caprine population.

Conclusion

A minimal number of small ruminants (0.2%) in India have been vaccinated using an indigenously developed live attenuated vaccine (37). Current plans for the implementation of an intensive PPRV vaccination programme will probably change the epidemiology of PPRV virus in India. Unlike vaccination programmes for rinderpest virus, which circulates primarily in cattle and secondarily in sheep and goats, vaccination strategies for the control of PPRV would be slightly different. Such a vaccination programme would need to account for the population dynamics of the goat herds in India. The slaughtering of male goats at an early age combined with the high fecundity of the caprine species results in 40% of the goat population being replaced each year. Initially, in order to reduce economic losses due to PPRV, intensive vaccination of the entire population within a specified area would need to be undertaken. Subsequent vaccinations would then be performed on younger animals at approximately 6 months of age.

An average of one out of every three small ruminants in India has been previously infected with PPRV and has subsequently recovered from the disease. Recovered animals are protected from reinfection for the remainder of their lives. Recovery rates from PPRV infection are considerably lower for goats than for sheep, resulting in a low proportion of the goat population being protected from reinfection. A relatively high proportion (70%-80%) of the goat population is therefore at risk of infection, particularly in the northern parts of the country where PPRV antibody prevalence is very low (10%-30%). If these preliminary findings are substantiated by more comprehensive serological surveillance, using an indigenously developed competitive ELISA kit, they may have far reaching implications for PPR control strategies in the country.

Though the sample size in this study was limited and may not be a true representation of the target population, the present investigation provided preliminary information on PPRV infection/outbreak patterns in the small ruminant population of India. More intensive investigations of a similar nature, taking into account disparities in sheep and goat husbandry practices and the agro-climatic conditions affecting the pattern of the natural vegetation, are required. These factors are indirectly influenced by socio-economic factors, the migration patterns of small ruminants in relation to season, flock size and the population intensity of the animals. Such studies are only possible in collaboration with the state animal husbandry departments of respective regions and the co-operation of the local public. Efforts to generate more field data are in progress.

Acknowledgements

The authors are thankful to the staff of the Rinderpest and Allied Diseases Laboratory, Division of Virology, IVRI, Mukteswar, for their help and to the Director of IVRI for the use of the facilities. Critical reading of the manuscript by Dr S.K. Singh, Scientist at IVRI, is gratefully acknowledged. This study was in part funded by the National Agricultural Technology Project of the Indian Council of Agricultural Research and the National Project on Rinderpest Eradication, organised by the Government of India.

Prévalence et distribution de l'infection par le virus de la peste des petits ruminants chez des petits ruminants en Inde

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Résumé

La peste des petits ruminants (PPR) est une maladie virale fébrile aiguë des caprins et des ovins caractérisée par des écoulements nasaux et oculaires mucopurulents, une stomatite érosive et nécrosante, une entérite et une pneumonie. En Inde, cette maladie enzootique entraîne chaque année d'importantes pertes économiques liées aux taux élevés de mortalité et de morbidité chez les caprins et les ovins infectés. Cette étude porte sur les observations de 58 foyers de PPR confirmés par des laboratoires et fournit des informations détaillées sur la prévalence d'anticorps dirigés contre le virus de la PPR (PPRV) dans 4 407 sérums de petits ruminants. La plupart des prélèvements destinés à cette étude avaient été obtenus dans le nord et le centre de l'Inde. Les sérums employés pour la détection des anticorps de PPRV n'étaient pas nécessairement représentatifs de la population cible, même s'ils provenaient d'un nombre plus important de régions. La détection des antigènes (par épreuve immuno-enzymatique [ELISA] en sandwich) et des anticorps (par ELISA de compétition) du PPRV a été réalisée au moyen de trousses de diagnostic fabriquées en Inde avec des anticorps monoclonaux. Les résultats indiquent que les foyers de la maladie étaient plus graves chez les caprins que chez les ovins. La fréquence d'apparition des foyers était plus élevée entre mars et juin (51,7 %) que durant les autres mois de l'année. L'examen des 4 407 sérums a révélé que la prévalence des anticorps du PPRV était de 33 % chez les petits ruminants de l'Inde (32,3 % à 33,7 %, avec un intervalle de confiance de 95 %). La prévalence des anticorps dirigés contre la PPRV variait selon l'espèce (par exemple, les caprins ou les ovins), les groupes d'âge et les zones géographiques. Le taux d'infection par le PPRV était plus élevé dans la population ovine (36,3 %) que chez les caprins (32,4 %). La distribution et la prévalence des anticorps parmi les animaux de groupes d'âge différents ont révélé que les caprins étaient exposés à un âge plus précoce que les ovins, ce qui pourrait traduire une plus forte sensibilité au virus. Les auteurs ont relevé un nombre de cas positifs plus élevé (30 %-60 %) dans le sud et le sud-ouest de l'Inde que dans la partie septentrionale (10 %-30 %). Ces résultats peuvent être corrélés à des pratiques d'élevage différentes des ovins et des caprins selon les zones géographiques, la topographie des États et le statut socioéconomique des éleveurs.

Mots-clés

Détection des anticorps – Détection des antigènes – Épreuve immuno-enzymatique de compétition – Épreuve immuno-enzymatique en sandwich – Incidence – Peste des petits ruminants – Prévalence – Surveillance sérologique.

Prevalencia y distribución de la infección por el virus de la peste de pequeños rumiantes en ovinos y caprinos de la India

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Resumen

La peste de pequeños rumiantes (PPR) es una enfermedad vírica febril, que afecta a caprinos y ovinos y se caracteriza por la presencia de secreciones nasales y oculares mucopurulentas, estomatitis necrosante y erosiva, enteritis y neumonía. Es endémica en la India, y cada año es causa de enormes pérdidas económicas debido a las elevadas tasas de mortalidad y morbilidad en ovinos y caprinos infectados. Los autores estudian los informes sobre 58 brotes de PPR confirmados por pruebas de laboratorio y presentan además en detalle la prevalencia de anticuerpos al virus PPR en 4.407 muestras séricas de pequeños rumiantes. La mayoría de las muestras clínicas utilizadas en el estudio procedían del norte y el centro de la India, mientras que las muestras séricas empleadas para la detección de anticuerpos venían también de otras muchas regiones del país. Es posible, sin embargo, que dichas muestras no sean verdaderamente representativas de la población total de pequeños rumiantes. Se utilizaron kits de diagnóstico basados en anticuerpos monoclonales y fabricados en el país para detectar antígenos del virus PPR (ensayo inmunoenzimático [ELISA] indirecto) y para detectar anticuerpos (ELISA de competición). Los resultados parecían indicar que los brotes infecciosos eran más graves en los caprinos que en los ovinos. Los brotes eran más frecuentes entre los meses de marzo y junio (51,7%) que durante el resto del año. La prevalencia de anticuerpos en pequeños rumiantes en la India, estimada a partir del cribado de 4.407 muestras séricas, se cifraba en 33% (con un intervalo de confianza del 95%: 32,3% a 33,7%). Este parámetro dependía de la especie (ovinos o caprinos), el grupo de edad y la región geográfica. La proporción de ovinos infectados respecto a la población total (36,3%) era mayor que en el caso de los caprinos (32,4%). Por lo que respecta al grupo de edad, la distribución y prevalencia de anticuerpos indicaba que la exposición al virus PPR se producía a una edad más temprana en los caprinos que en los ovinos, lo que lleva a pensar que tal vez los primeros sean más susceptibles a la infección. En la parte sur y suroeste del país se observó un mayor número de casos positivos (30%-60%) que en el norte de la India (10%-30%). Es posible establecer correlaciones entre estos resultados y las diferencias en las técnicas de producción ovina y caprina en distintas regiones geográficas, la topografía de cada estado y la situación socioeconómica de cada explotador indio.

Palabras clave

Detección de anticuerpos – Detección de antígenos – Incidencia – Ensayo inmunoenzimático de competición – Ensayo inmunoenzimático indirecto – Peste de pequeños rumiantes – Prevalencia – Vigilancia serológica.

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