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SEROPREVALENCE OF *PESTE DES PETITS RUMINANTS* IN GOATS OF KERALA

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Thesis submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I hereby declare that the thesis entitled "**SEROPREVALENCE OF *PESTE DES PETITS RUMINANTS* IN GOATS OF KERALA** is a record of research work done by me during the course of research and this thesis has not previously formed the basis for the award of any degree, diploma, fellowship or associateship or other similar title, of any other University or Society.

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CERTIFICATE

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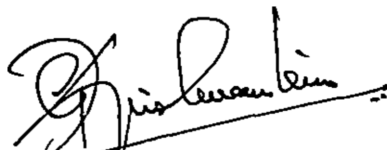

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EXTERNAL EXAMINER

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Introduction

1. INTRODUCTION

India is basically an agricultural country and livestock production activities provide a robust support to the economy of the enterprise of the farmers. Besides cattle, small ruminants contribute substantially to the rural economy of India. Goat is a very important livestock species in India, because of it's significant contribution to the national economy. The incessant increase in goat population in India shows the potentiality of this species for the economic upliftment of the rural poor. The present goat population in India is 126 millions and India occupies the first place in goat population and Kerala has a goat population of 18.6 lakhs (Report, Animal Husbandry Department, Kerala).

Peste des petits ruminants (PPR) plays an important role among the economically important diseases of sheep and goats. It is a severe, fast spreading viral disease of mainly domestic small ruminants. *Peste des petits ruminants* virus has been classified in the family paramyxoviridae and genus morbilli virus, which includes measles, rinderpest (RP), canine distemper, porcine distemper and the morbilli viruses found in whales, porpoises and dolphins. The disease is characterized by the sudden onset of depression, fever, discharges from the eyes and nose, sores in the mouth, distended breathing and cough, foul smelling diarrhoea and death. The disease is endemic in the sub Saharan region of Africa extending to the Arabian Peninsula. The disease was first reported in India in 1987 (Shaila *et al.*, 1989), subsequently several outbreaks have been confirmed in different parts of India.

Laboratory diagnosis of PPR can be done by virus isolation or by the detection of antigen or antibody. Serologic tests like neutralization tests, agar gel immunodiffusion test (AGID) and enzyme linked immuno sorbent assay (ELISA) were employed for detection of PPR antibodies. Sensitivity and specificity of ELISA

in detecting antibodies is more than that of the other serological tests. Seroprevalence studies showed that PPR has gained establishment in the small ruminants of the country (Sudharshana *et al.*, 1995; Hinsu *et al.*, 2001; Singh *et al.*, 2004; Saha *et al.*, 2005; Sunilkumar *et al.*, 2005; Agrawal *et al.*, 2006; Dorairajan *et al.*, 2006).

The seroprevalence of the disease in Kerala was reported by Sunilkumar *et al* (2005). After that no systematic study has been done to detect the seroprevalence of PPR in Kerala. The present study was undertaken with the following objectives .

1. Assessing the seroprevalence of PPR in the goat population of Kerala using competitive ELISA.
2. An epidemiological analysis of the disease in Kerala.

Review of Literature

2. REVIEW OF LITERATURE

2. 1 HISTORY

In India the disease was first detected in sheep in an outbreak in Arasur village of Tamil Nadu in 1987 (Shaila *et al* ., 1989).

In the early 1940s Gargadennec and Lalanne, working in the Ivory cost studied a fatal disease of goats and labelled it as '*peste des petits ruminants*' because of the high mortality rate. *Peste des petits ruminants* has many synonyms- Erosive stomatitis and enteritis of goats, Goat catarrhal fever, Kata and Stomatitis pneumoenteritis complex (Scott, 1990).

Elhag Ali and Taylor (1984) isolated PPR virus from Sudan and thus extended the geographical range of this virus.

The virus was initially isolated in Senegal in 1962 by Gilbert and Monnier (Wamwayi *et al.*, 1995).

The disease has been reported in various parts of Africa and Asia since then (Roeder and Obi, 1999; Dhar *et al* ., 2002).

2. 2 ETIOLOGY

Etiology of Stomatitis pneumoenteritis complex in Nigerian Dwarf goats was identified as *Peste des petits ruminanats* virus (Hamdy *et al.*, 1976).

The virus is antigenically related to rinderpest virus, human measles virus and canine distemper virus (Gibbs *et al.*,1979).

Chandran *et al.* (1995) showed adaptation of PPR virus in vero cells with visible cytopathogenic effect 36 hours post infection. Acidophilic intracytoplasmic and intranuclear inclusion bodies were observed.

Mondal *et al.* (2001) demonstrated the ability of PPR virus to induce apoptosis in goat peripheral mononuclear cell culture.

Dhar *et al.* (2002) reported that, of the four known lineages of PPRV, lineage 1 and 2 viruses have been found exclusively in West Africa. Viruses of lineage 3 found in East Africa, Arabia and Southern India. Fourth lineage virus spread across the middle east and Asia.

All members of the genus Morbilli virus *inhibit the proliferation of human B lymphoblast cell line (Heany, 2002).*

PPR virus resists 60^0 C for 60 minutes , is stable between the pH 4 and 10, is susceptible to alcohol, ether and detergents, survives for long periods in chilled and frozen tissue and is susceptible to most disinfectants (OIE, 2002).

The virus has been classified in the family paramyxoviridae and genus morbilli virus (OIE, 2002).

John *et al.* (2006) reported that the Vero cell adapted PPR virus can be adapted to BHK 21 cell line by 15 serial passages.

2.3 GENOME ORGANIZATION

There is a marked difference in the apparent molecular weight of the nucleo capsid protein between PPR and RP and the N protein of PPR is almost identical to the N protein of measles virus and canine distemper (Diallo *et al.*, 1987).

Morbilli viruses are pleomorphic particles with sizes ranging from 350-600 nm. They have one known nonstructural protein (C) and six structural proteins; large protein(L), hemagglutinin (H), phosphoprotein, (P), nucleocapsid protein (N), fusion protein (F) and matrix protein (M). They are negative stranded nonsegmented RNA viruses (Diallo, 1990).

Mc Cullogh *et al.* (1991) reported that anti N protein Monoclonal antibody of rinderpest virus (RPV) reacted with RPV isolates but not with PPR isolates.

Cell attachment of the virus is mediated through the hemagglutinin neuraminidase protein and the fusion protein mediates biological fusion (Murphy *et al*., 1999).

Seth and Shaila (2001) reported that fusion protein of PPR virus mediated biological fusion in the absence of hemagglutinin neuraminidase protein.

Rahaman *et al.* (2003) reported that F proteins of paramyxoviruses were important components of the fusion process.

Choi *et al.* (2005) determined the epitopic profile of the N protein of PPRV.

Bailey *et al.* (2005) published the full genome sequence of PPRV at the nucleotide level, which was most similar to that of Rinderpest. At the protein level, five of the six structural proteins and the V protein showed a great similarity to the dolphin morbilli virus.

2. 4 INCIDENCE

2. 4.1Global

Taylor (1979b) screened 100 sheep and goat sera in Nigeria and it was found that 38 samples neutralized PPRV and thus confirmed the serological evidence of PPR in Nigeria.

Taylor (1984) suggested that PPR occurred in a belt across Africa, immediately south of the Sahara and extended on to the Arabian peninsula.

Furley *et al.* (1987) reported an outbreak of PPR in a zoological collection in the Arabian gulf.

Abu Elzein *et al.* (1990) confirmed the first PPR virus isolation from diseased goats in Saudi Arabia and thus extended the geographical distribution of the disease east of the African continent and across the red sea to Saudi Arabia.

Taylor *et al.* (1990) reported serological evidence to show that PPR was widely distributed in Omani sheep and goats.

Lefevre *et al.* (1991) detected antibodies to PPR in Jordan in 30 sheep and goat sera samples of the 8520 sera samples tested.

Wamwayi *et al.* (1995) recorded the occurrence of PPR specific antibodies in the sera of goats in East Africa.

Roger *et al.* (2001) showed a global seroprevalence of 7.8 per cent for PPR antibodies in 90 Etheopean camels.

Ozkul *et al.* (2002) reported a seroprevalence of 29.2 per cent in sheep and 20 per cent in goats of the total 1607 animals examined in Turkey.

In a seroprevalence study in African grey duikers the prevalence rate was 10. 5 per cent (Ogunsanmi *et al* ., 2003).

Afaleq *et al.* (2004) conducted the first serological survey for PPR and RP antibodies in sheep and goats in Soudi Arabia. The prevalence of PPR virus antibodies was 0 .6 per cent and 3.1 per cent respectively in goats and sheep.

Couàcy-Hymann *et al.* (2005) reported a seroprevalence of PPR less than 1 per cent in 247 sera samples from a wild life population at Cote de Ivoire.

In Iran, Morshedi *et al.* (2006) conducted a seroprevalence study of 130 sera samples and 74 samples were found positive for PPR antibodies.

Traore and Wilson (2006) revealed the existence of PPR in West Africa.

2. 4. 2 India

In India Sudarshana *et al.* (1995) reported 5.3 and 11.11 per cent seropositivities of PPR among sheep and goats respectively.

Krishna *et al.* (2001) reported the over all prevalence of PPRV in small ruminants of Andhra Pradesh as 2.98 per cent.

Hinsu *et al.* (2001) reported the seroprevalence of PPR among sheep, goats, buffaloes and cattle in Gujarat as 55.29, 100, 4.76 and 0 per cent respectively.

Dhand *et al.* (2002) recorded PPR for the first time in Punjab, where presumptive diagnosis was made by c ELISA.

The over all prevalence of PPR antibodies in small ruminants of India was detected as 33 per cent among 4, 407 sera samples tested (Singh *et al* ., 2004a).

Saha *et al.* (2005) reported a seroprevalence of 30.7 per cent among 866 goats in and around Kolkata.

Agrawal *et al.* (2006) reported an overall seroprevalence of 9. 2 per cent in an epidemiological investigation of PPR in goats in Uttaranchal state.

In Tamil Nadu Dorairajan *et al.* (2006) conducted a seroprevalence study in goats. Out of 800 sera samples tested , 98 samples were positive.

A seroprevalence study in Gujarat by Kanani *et al.* (2006) revealed a prevalence rate of 69. 06 per cent in sheep and 28. 73 per cent in goats.

2. 4. 3 Kerala

Abraham *et al.* (2005) reported an outbreak of PPR in a goat farm in Kerala

In Kerala, Sunilkumar *et al.* (2005) observed less than 1 per cent seroprevalence of PPR among 536 goat samples tested.

2. 5 EPIDEMIOLOGY

2. 5. 1 Host range

2. 5. 1. 1 Goats and sheep

Several workers have reported that the virus can infect goats and sheep resulting in mucopurulant nasal and ocular discharges, necrotizing and erosive stomatitis, enteritis and pneumonia (Kulkarni *et al.*, 1996; Shaila *et al* ., 1996; Singh *et al.*, 1996; Rao *et al.*, 1997; Rao *et al.*, 1998b; Abraham *et al.*, 2005).

Seroprevalence studies suggested that the disease is widespread all over the world in sheep and goats (Taylor *et al.*, 1979b; Lefevre *et al.*, 1991; Sunilkumar *et al.*, 2005; Morsheidi *et al.*, 2006).

2. 5. 1. 2 Cattle and buffaloes

PPRV was not pathogenic for cattle and protected them from challenge with virulent rinderpest virus. Neither replication nor excretion of PPRV in cattle could be demonstrated, although serum antibody was produced (Gibbs *et al.*, 1979).

Govindarajan *et al* (1997) isolated PPR virus from a case in an Indian buffalo.

Cattle and buffaloes can become infected but there is little evidence of disease associated with infection (Roeder and Obi., 1999).

Hinsu *et al.* (2001) conducted a seroprevalence study where prevalence rate in cattle was found to be 0 per cent.

Brindha *et al* . (2007) carried out a seromonitoring study of PPR in cattle in Tamil Nadu, where an over all positivity of 28. 8 per cent was obtained .

2. 5. 1. 3 Pigs

Pigs could be subclinically infected with PPRV by inoculation or contact with infected goats. There was no evidence that the virus could be spread to uninfected pigs or goats and pigs are not considered important in the epidemiology of PPR (Nawathe and Taylor, 1979).

2. 5. 1. 4 Camels

Roger *et al.* (2001) detected a seroprevalence of 7. 8 per cent for PPR antibodies among Etheopean camels.

2. 5. 1. 5 Wild animals

Furley *et al.* (1987) reported an outbreak of PPR in a variety of zoo animals such as gazelles, ibex, sheep and gemsbok in the Arabian gulf.

Ogunsanmi *et al.* (2003) reported a seroprevalence of 10. 5 per cent in African grey duikers.

A seroprevalence of less than 1 per cent was estimated in the wild life population of Cote de Ivoire (Couacy- Hymann *et al.*, 2005).

2. 5. 2 Transmission and spread

A close link between the appearance of the disease and the introduction of new stock was reported (Taylor, 1984; Barua *et al.*, 2004).

PPR virus in an aerosol invades the body through the tissues lining the upper respiratory tract, gets disseminated before the onset of clinical signs and is shed in the nasal secretions, tears, saliva and urine (Scott, 1990).

The movement of animals play an important role in the transmission and maintenance of PPRV in nature (Shaila *et al.*, 1989; Anjaneyalu and James, 1999; Singh *et al.*, 2004a).

Taylor *et al.* (1990) suggested that close contact between the goats and sheep of neighbouring owners would be sufficient to promote virus transmission.

The discharges from eyes, nose, and mouth as well as loose faeces contain large amounts of the virus. Fine infective droplets are released into the air from these secretions and excretions, other animals inhale the droplets and are likely to become infected (Roeder and Obi, 1999).

Appearance of PPR in apparently healthy animals could be associated with herding of animals from different locations, varying degrees of stresses and sudden change in the environment and feeding habits (Kumar *et al.*, 2001).

2. 5. 3 Season and managemental practice

In the humid tropics, the onset of rain causes managemental changes ; owners sell their surplus kids and tether the remaining animals indoors to protect the growing crops. The goats dislike rain and hurdle close together under the shelter. This behaviour favours rapid spread of the virus by the aerosol route. In arid and semi arid areas surplus animals are marketed in the dry season because of the scarcity of feed. Infection is readily acquired in the market and spreads to settled flocks and herds (Scott, 1990).

An epidemiological study of PPR outbreak in Oman revealed that sick animals belonged to small holder farmers who could not prevent contact between their own animals or those of their neighbours (Taylor *et al.*, 1990).

Kumar *et al.* (1997) reported that incidence was high during rainy season and remained high up to early winter.

The disease was found to be highly prevalent in winter season (51.7 per cent) followed by summer and rainy seasons (40 per cent) (Saha *et al.*, 2005).

2. 5. 4 Age

The mortality rate was highest in 3-6 month of age group of animals (60 per cent) followed by 0-3 (42.5 per cent) and 6-12 months (17.1 per cent) of age group (Rana *et al.*, 1998).

In an outbreak of PPR in Andhra Pradesh, Mohankumar *et al* (2002) observed higher rate of infection in young (59.4 per cent) compared to adults (46.12 per cent) in both sheep and goat.

The disease was more severe in young ones as compared to adults. The morbidity, mortality and case fatality rates in young ones were significantly higher than adults (Bhikane *et al.*, 1997; Dhand *et al.*, 2002).

A higher proportion of goats between the ages of 6 to 12 months were positive for PPRV as compared to sheep from the same category (Singh *et al.*, 2004a).

The prevalence of PPR antibodies in tested animals appeared to increase with age (Afaleq *et al.*, 2004).

The highest prevalence of the disease was observed in the age group of 5 to 12 months (65 per cent) followed by 12 months and above (23 per cent) and 0 to 4 months (12 per cent) (Saha *et al.*, 2005).

2. 5. 5 Sex

Mondal *et al.* (1995) reported an outbreak of PPR in a goat farm in West Bengal where no sex variation irrespective of the breed was observed in the affected goats.

The attack rate and case fatality were higher in males than in females in goats and sheep affected with PPR (Shankar *et al.*, 1998).

No sex variation was seen in the prevalence of PPR in Kolkata (Saha *et al.*, 2005).

2. 5. 6 Breed

Mondal *et al.* (1995) recorded a higher prevalence of PPR (67.24 per cent) in Black Bengal goats than Jamunapari breed.

The prevalence of the disease was found to be highest in the Jamunapari breed (52 per cent) as compared to Black Bengal (20 per cent) and non descript breeds (28 per cent) (Saha *et al.*, 2005).

2. 6 MORTALITY AND MORBIDITY

In the first reported field outbreak in Arasur Village of Tamil Nadu, the morbidity, mortality and case fatality rates were 10, 2.5 and 25 per cent respectively (Shaila *et al.*, 1989).

Mondal *et al.* (1995) reported a mortality rate varying from 23.8 per cent to 38.2 per cent in an outbreak in West Bengal.

Shaila *et al.* (1996) confirmed an outbreak of PPR in goats of Kalakkanmai village of Tamil Nadu, where, out of 500 animals in a village 200 animals were affected and 40 of them died.

In an outbreak in Andhra Pradesh, the mortality rate was higher in goats (30 per cent) compared to sheep(15 to 20 per cent) (Rao *et al.*, 1997).

The overall morbidity, mortality and case fatality rates of PPR were 30.56, 13.2 and 43.2 per cent respectively in an organized sheep farm in Andhra Pradesh (Sreeramulu, 2000).

Saha *et al.* (2005) reported a mortality rate of 24 % and morbidity rate of 30.7 per cent in an outbreak at Kolkata.

2. 7 ECONOMIC SIGNIFICANCE

Morbilli virus infections cause significant mortality in human beings and animals. Measles virus is responsible for up to two million child hood deaths annually while rinderpest and *peste des petits ruminants* cause severe epizootics in domestic and wild ruminants in areas of the world where they remain endemic (Barrett , 1999).

2. 8 PATHOGENESIS

Hamdy *et al.* (1976) reported that experimental inoculation of PPR virus produced cytopathogenic effect which was characterized by cell rounding, clumping into grape like clusters, syncitia formation and the appearance of stellate or spindle cells with long fine, often anastomosing processes.

The virus in aerosol invaded the body though the tissues lining the upper respiratory tract, was disseminated before the onset of clinical signs and was shed in nasal secretions, tears, saliva and urine. When diarrhoea supervenes fecal excretion occurred . Animals that recovered did not become carriers (Scott, 1990).

Shaila *et al.* (1990) experimentally produced frank clinical reactions characterized by moderate to high pyrexia, diarrhoea and nasal discharges in three goats but none of the three cross bred calves.

Brown *et al.* (1991) observed viral inclusions in tracheal, bronchial and bronchiolar epithelium, type 2 pneumocytes, syncitial cells and occasionally alveolar macrophages and reported that inclusions both in cytoplasm and nucleus.

According to Kulkarni *et al.* (1998) the onset of disease accompanied marked leukopaenia in four kids dying after two weeks after the experimental inoculation and the degree of leukopaenia was initially positively correlated to the death of the animal.

Wadhwa *et al.* (2002) reported hypoglycaemia, hypoproteinemia, markedly elevated blood urea nitrogen and aspartate amino transferase, in an outbreak in migratory sheep and goats in Himachal Pradesh.

Kumar *et al.* (2004) reported lymphocytolysis and occasional syncitia formation in the lymphoid tissues, in an experimental *peste des petits ruminants* virus infection.

Pawaiya *et al.* (2004) reported degeneration and disorganization of the epithelial cells of the stratum spinosum and stratum granulosum evincing pyknotic nuclei, karyorrhexis and focal changes in stratum spinosum were quite intense involving even cells of the basal layer; the degenerating cells conspicuously contained intracytoplasmic and intranuclear eosinophilic inclusion bodies.

Purushothaman *et al.* (2006) reported haemoglobinaemia, leucopaenia and lymphocytopaenia with normal protein and albumin levels, in a PPR outbreak in Tamil Nadu.

2. 9 CLINICAL SIGNS

The onset of illness was manifested by the initial appearance of watery occular and nasal discharge followed by mucopurulant discharge as the disease signs progressed. Fever of more than 40 ^{0} Celcius persisted for 5 to 7 days. Nasal discharge was pronounced after the onset of fever and persisted for 2 to 7 days (Hamdy *et al.*, 1976).

Symptoms on progression of infection include severe diarrhoea, often profuse but not haemorrhagic, dehydration, emaciation, bronchopneumonia, dyspnoea and abortion followed by hypothermia and death (Taylor, 1984; Bundza *et al.*, 1988 and Scott, 1990).

Clinically the affected goats showed symptoms such as erosive stomatitis, pyrexia (up to 105 ^{0} F), occulonasal discharges, conjunctivitis, coughing, watery diarrhoea. As the diarrhoea increased in severity, it was accompanied by abdominal pain, tachypnoea followed by dehydration, prostration and death (Bundza *et al.*, 1988; Brown *et al.*, 1991).

Sheep and less commonly goats develop sub acute reactions after an incubation period of about 6 days, the illness being manifested by a low grade fever, nasal catarrh, recurring mucosal erosions and intermittent diarrhoea. After a course of 10 to 14 days, the animals usually recover. Acute reactions begin after an incubation period of 3 to 4 days and per acute reactions follow incubation periods that are often as short as 2 days (Scott, 1990).

Joshi *et al.* (1996) reported outbreaks of PPR among migratory Gaddi sheep and goats in Himachal Pradesh and the affected animals invariably exhibited high rise of temperature (107 – 108 ^{0} F), general debility, stomatitis, broncho pneumonia and severe diarrhoea.

Kulkarni *et al.* (1996) described the symptoms of PPR observed during an outbreak in goats in Maharastra such as hyperthermia (40- 43^{0}C), anorexia, dullness, leg weakness, watery nasal discharge, yellowish thick mucopurulent and at later stages catarrhal and bloody lachrymation, ocular discharges and stomatitis with necrotic lesions in the buccal cavity involving cheeks, gums, dental pads and tongue. Diarrhoea persisted for 4 to 7 days until death and exhibited a pasty and non pelletd type. Classic symptoms were not seen in kids and most died after diarrhoea, oronasal discharges and fever.

Shaila *et al.* (1996) reported the symptoms in goats in Tamil Nadu affected with PPR. The affected goats showed initial rise of body temperature (40- 41^0 C) followed by lachrymal and nasal discharges. Lachrymal discharge became mucopurulent causing eyelids to stick together. Erosions were seen on gums and white pulpy coating on tongue followed by streaming diarrhoea and the course of disease was 7 to 10 days.

Singh *et al.* (1996) observed symptoms such as increase in body temperature (106-107 ^{0} F), ulcerative stomatitis, blood tinched nasal catarrh, laboured breathing and blackish diarrhoea during an outbreak of PPR in sheep in Rajastan.

Anorexia, fever, diarrhoea, nasal discharges and pneumonia were the constant features of PPR in affected goats at Central institute for research on goats, but buccal lesions, conjunctivitis and corneal opacity were observed only in few cases. (Kumar *et al.*, 2001)

2. 10 DIAGNOSIS

2. 10. 1 Detection of viral antigen and nucleic acid

Many techniques have been used to detect the virus, which include Agar gel immunodiffusion, counterimmunoelectrophoresis, indirect or passive haemagglutination, immunofluorescence or immunoperoxidase staining, single radial haemolysis, complement fixation and virus isolation in cell culture. (Diallo *et al.*, 1995).

2. 10. 1. 1 Agar gel immunodiffusion Test (AGID)

Agar gel immunodiffusion test is extremely useful as an initial test for detection of PPR antigen but it does not discriminate between PPR and RP viruses. Further AGID is not sensitive enough to detect low quantities of excreted PPR virus in pathological samples as in the case with mild form of the disease (Diallo *et al.*, 1995).

Katoch *et al.* (1999) detected morbilli virus antigens in tissue samples by positive AGID test against hyperimmune rinderpest serum.

Detection of viral antigen by AGID test is a relatively simple, fast and cheap process (Roeder and Obi.,1999; OIE., 2004).

2. 10. 1. 2 Counter immunoelectrophoresis (CIE)

Tissue samples from spleen and lymphnodes were found positive for PPR antigen by Agar gel precipitation and CIE (Singh *et al* ., 1999).

Counterimmunoelectrophoresis is the most rapid test for PPR viral antigen detection (OIE., 2004).

Purushothaman *et al.* (2006) confirmed the presence of PPRV antigen by CIE using PPRV specific antiserum.

Rahaman *et al.* (2004) detected PPR antigen in mesenteric lymphnodes of goats by modified CIE.

2. 10. 1. 3 Virus neutralization test (VNT)

Virus neutralization test is sensitive and specific . It is the prescribed test for international trade. The standard neutralization test is carried out in roller tube cultures of primary lamb kidney cells or verocells, but it is time consuming (OIE., 2004).

2.10.1. 4 Fluorescent Antibody Test (FAT)

Sumption *et al.* (1998) detected PPR viral antigen in conjunctival epithelial cells obtained from goats by use of a specific monoclonal antibody to PPR in FAT. The viral inclusion had a bright apple green fluorescence, which were found in the cytoplasm and nucleus of the epithelia.

Brindha *et al.* (2001) confirmed the presence of PPR virus in vero cell line by immunofluoroscence test using monoclonal antibody against PPR virus nucleoprotein and observed clear cytoplasmic fluorescence in positive cases.

2. 10. 1.5 Enzyme linked Immunosorbent Assay (ELISA)

A PPR virus specific neutralizing monoclonal antibody and double antibody sandwich ELISA was developed for specific detection of PPR virus from diseased goat tissues and secretions (Saliki *et al.*, 1994).

An Immunocapture ELISA was described for rapid differential diagnosis of RP virus and PPR virus (Libeau *et al.*, 1994).

Dot immunoassay was used by Obi and Ojeh (1989) for the visual detection of PPRV antigen from infected caprine tissues and 83.1% of the samples tested were positive by this test but could not differentiate PPR virus from RP virus. This test could detect PPR virus with a titre of $10^{4.5}$ tissue culture infective dose / ml ($TCID_{50}$).

Rao *et al.* (1998b) and Rajeswari *et al.* (2000) employed Immunocapture ELISA for the diagnosis of an outbreak of PPR in small ruminants in Andhra Pradesh.

A sandwich ELISA for the diagnosis of PPR infection in small ruminants using anti nucleocapsid protein monoclonal antibody was developed (Singh *et al.*, 2004b).

Karunakaran *et al.* (2006) reported the use of sandwich ELISA for detecting PPR antigen in suspected clinical samples.

2. 10. 1. 6 Nucleic acid hybridization techniques using c DNA probes

Diallo *et al.* (1989) described a rapid method for differential diagnosis of RP virus and PPR virus using radiolabelled c DNA probes.

Shaila *et al.* (1989) differentiated PPR virus from RP virus by dot blotting the RNA into nylon membrane and hybridized to c DNA probes specific for the nucleocapsid gene DNA probe.

Pandey *et al.* (1992) prepared nonradioactively biotinylated c DNA probes from N gene of RP virus and PPR virus for differentiating them.

2. 10. 1. 7 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

Forsyth and Barrett (1995) developed a RT-PCR test, using P and F protein gene specific primer to detect and differentiate RP and PPR viruses. This RT-PCR technique had been used to confirm PPR virus outbreaks by many wokers (Nanda *et al*., 1996; Shaila *et al.*, 1996)

Brindha *et al.* (2001) reported that RT-PCR was more sensitive than virus isolation.

Couacy- Hymann *et al.* (2002) developed a rapid , sensitive method for the detection of PPR virus. This assay was based on the rapid purification of RNA on glass beads followed by RT-PCR.

Detection of virus genetic material by RT- PCR is one of the tests used most frequently in reference centers, together with ELISA because it is rapid, accurate, highly sensitive and can discriminate between PPR and RP virus (OIE., 2004).

2. 10. 1. 8 Immunohistochemical staining

Yener *et al.* (2004) carried out immunohistochemical detection of PPR viral antigens in tissues from cases of naturally occurring pneumonia. Immunohistochemical staining was carried out by the Avidin Biotin Peroxidase complex. The presence of PPR viral antigen was detected in 17 out of 42 pneumonic lungs.

2. 10. 2 Detection of Viral antibodies

2.10. 2.1 Agar gel Immunodiffusion

Agar gel immunodiffusion test can be used for the detection of PPR antibodies (OIE, 2004).

2.10. 2. 2 Serum neutralization Test

Microneutralization system for PPR virus was developed and the titre of PPR virus and neutralizing antibodies were assayed in tubes and microplates (Rossiter *et al.*, 1985).

Serum neutralization Test was later used by many scientists for detecting the neutralizing antibodies against PPR virus (Furley *et al.*,1987; Taylor *et al.*, 1990; Lefevre *et al.*, 1991; Libeau *et al.*, 1995 ;Afaleq *et al.*, 2004).

2.10.2. 3 Enzyme Linked Immunosorbent Assay

Saliki *et al.* (1993) developed a monoclonal antibody based blocking ELISA (B-ELISA) for specific detection of PPR virus antibody in caprine and ovine sera. The sensitivity and specificity of B-ELISA relative to the VNT were 90.4 and 98.9% respectively.

A competitive ELISA (c-ELISA) using monoclonal antibodies (MAbs) and a recombinant nucleioprotein of PPR was developed (Libeau *et al.*, 1995).

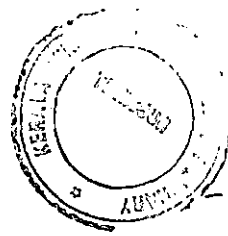
A c-ELIZA using a recombinant N antigen (RP Virus N) expressed in a baculovirus and a ruminant Morbilli virus (RP Virus and PPR) specific monoclonal antibody was developed for simultaneous detection of RP Virus and PPR virus antibodies (Choi *et al.*, 2003).

Singh *et al.* (2004c) developed a monoclonal antibody based c ELISA for detection and titration of antibodies to PPR virus in the sera samples of goats and sheep. The test uses monoclonal antibody to a neutralizing epitope of haemagglutinin protein of the virus. A total of 1668 sera samples from goats and sheep and 32 sera sample from cattle were screened by c ELISA and VNT. Efficacy of cELISA compared well with VNT having high relative specificity (98.4%) and sensitivity (92.4%). Findings suggest that cELISA test developed can easily replace VNT for serosurveillance, seromonitoring, diagnosis from paired sera sample and end point titration of PPR virus antibodies.

2.10. 2. 4 Haemagglutination (HA) and hamagglutination inhibition (HI) test

The PPR hamagglutinin was demonstrated in ocular and nasal discharges in PPR affected live goats and it was observed that HA titre was not a reflection of the concentration of the virus in secretions but a reflection of the degree of dilution of virus in the secretion or with the diluents (Wosu, 1991).

Shaila *et al.* (1996) confirmed the identity as PPR virus using the sera from recovered animals by HA test using chicken erythrocytes as well as from eye swabs from ailing animals.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A simplified and standardized assay based on haemagglutination of infected culture supernatants was developed to quantify PPR neutralizing antibody (Raj *et al.*, 2000).

Sivaseelan *et al.* (2005) carried out a serosurveillance of *peste des petits ruminants* in sheep and goats in Tamilnadu using Haemagglutination Inhibition Test. Forty one sera samples were screened and no antibodies could be detected.

2. 11 CONTROL

The policy is to eradicate PPR in the shortest possible period, using a combination of strategies including stamping out, quarantine and movement controls, decontamination of facilities and products, tracing and surveillance, zoning to define infected and disease free areas and an awareness campaign (Ausvet plan, 1996).

The principal means suggested for controlling PPR was by vaccination (OIE, 2004).

2.11.1 Vaccines

Goats vaccinated with attenuated rinderpest were protected from *peste des petits ruminants* virus for at least 12 months. Before challenge neutralizing antibodies were detected primarily against rinderpest but following exposure to *pest des petits ruminants* high antibody level to both viruses was found (Taylor, 1979a).

The safety of tissue culture RP vaccine in pregnant goats was assessed and all vaccinated goats produced RP antibodies and retested against PPR virus challenge (Adu and Nawathe,1981).

Ata *et al.* (1989) revealed that maternally derived antibodies remained for 3 to 4 months after birth and then decreased.

The thermostable vero cell adapted rinderpest vaccine was evaluated in terms of immunogenicity as a heterologous vaccine against PPR and it was found to be a suitable immunogen for the protection of goats against PPR (Mariner *et al.*,1993).

Jones *et al.* (1993)developed an effective vaccinea virus double recombinant expressing the haemagglutinin and fusion gene of RPV. Vaccinated animals developed antibodies to RPV but they were completely protected against challenge inoculation with virulent PPRV.

Couacy Hymann *et al.* (1995) reported the ability of attenuated PPR vaccine to protect small ruminants against virulent rinderpest virus.

The attenuated virus has been used to control PPR outbreaks (Choudhari *et al.*, 1995; Joshi *et al.,* 1996; Shaila *et al.*, 1996; Singh *et al.*, 1996; Nayak *et al.*, 1997; Sreeramulu, 2000).

Matrenchar *et al.* (1999) studied the cost effectiveness of a homologous PPR vaccine in Northern Cameroon. The results demonstrated that the mortality rates were significantly decreased in the vaccinated flocks.

Rashwan *et al.* (2000) prapared a freeze dried live attenuated vaccine against PPR, by growing an attenuated PPR strain 75/1 in vero cell culture.

Goats were vaccinated with a vaccinea virus double recombinant expressing the haemagglutinin and fusion genes of RPV. A chimeric rinderpest virus expressing the F and H genes of PPRV was recovered and characterized and goats infected with the chimera showed no adverse reaction and were protected from challenge with wild type PPR virus (Das *et al.*, 2000).

A recombinant capripox virus containing a cDNA of PPRV fusion protein gene was constructed (Berhe *et al.*, 2003) and a trial showed that a dose of this recombinant as low as 0.1 PFU protected goats against challenge with a virulent PPRV strain.

Sarkar *et al.* (2003) studied the thermostability of a live attenuated PPR vaccine recently developed at IVRI. The study revealed that the PPR vaccine lyophilized with either lactalbumin hydrolysate- sucrose (LS) or Trehalose dehydrate (TD) was more stable than rest of the stabilizers.

Materials and methods

3. MATERIALS AND METHODS

The study was carried out in the department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy during 2006-2007.

3.1 MATERIALS

3. 1. 1 Glass wares and plastic wares and reagents.

The glass and plastic wares were of Tarson®, Laxbro ® or Corning brand®. Chemicals were of analytical or guaranteed reagent grade. The materials were processed using standard methods and sterilized either in hot air oven or autoclave depending upon the material to be sterilized.

3. 1. 2 Serum samples

A total of 412 serum samples were collected randomly from goats of all districts of Kerala. Samples were collected from apparently healthy animals and animals with symptoms suggestive of PPR such as fever, discharges from the eyes and nose, sores in the mouth, distended breathing and cough and foul smelling diarrhoea. Samples were collected from different farms, veterinary hospitals and from the field.

3. 1.3 Competitive ELISA

Competitive ELISA was performed using the kit supplied by IVRI, Izatnagar as per the method of Singh *et al.* (2004c).

3. 1. 3. 1. Antigen

Reconstituted the freeze dried contents of the vial with one ml of sterile distilled water. Stored the antigen stock at -20°c.

3. 1. 3. 2 Monoclonal antibody

Reconstituted monoclonal antibody in the vial with one ml of sterile distilled water and stored at -20°c.

3. 1. 3. 3 Serum controls

a) Strong positive serum
b) Weak positive serum
c) Negative serum

All are caprine sera, freeze dried and stored at -20°c. Reconstituted the freeze dried contents of a vial each of control serum separately with one ml of sterile distilled water

3. 1. 3. 4 Antimouse HRPO conjugate

Stored Antimouse HRPO conjugate at 4°c .

3. 1. 3. 5 Phosphate buffered saline

Dissoved the contents of the one litre phosphate buffered saline (PBS) pouch in 100 ml of fresh glass distilled water to make 10 X PBS. Diluted the 10 X stock 1 in 10 and stored at +4°c.

3. 1. 3. 6 Blocking buffer

PBS containing 0.1% Tween 20 and 0.2% negative serum.

PBS: 20 ml

Tween 20 : 20 µl

Negative serum: 20 µl

3. 1. 3. 7 Chromogen substrate solution

Dissolved one tablet of Orthophenyline Diamine (OPD -30 mg) in 75 ml distilled water and stored at -20 °c.

3. 1. 3. 8 Stopping solution

One molar suphuric acid- Added 5.45 ml of concentrated Sulphuric acid to 94.5 ml of distilled water, mixed well and stored in amber coloured tight stoppered vessel.

3. 2 METHODS

3. 2. 1 Collection of test sera

Collected five ml of blood aseptically through the jugular vein puncture using a 20 G sterile hypodermic needle in test tubes having a capacity of 15 ml. After labelling kept the tubes at an angle for clotting. After one hour breaking of clots was done with the help of a long needle. Kept the tubes at 37°c for 30 minutes. Transferred the tubes to refrigerator having a temperature of + 4°c. After 12-18 hours separated the serum and centrifuged at 1000 G for 10 minutes. Inactivated at 56°c for 30 minutes to destroy the nonspecific factors. After that kept the serum in cryovials of two ml capacity and labelled. Stored the samples at -20°c(deep freezer) until tested.

3. 2. 2 Assay procedure

Competitive ELISA was performed as per the method described in the laboratory manual provided by IVRI.

3. 2. 2. 1 Coating of microplates

Diluted the reconstituted stock antigen in a fresh container at the ratio of 1:100. For coating one plate added 60 µl of antigen to six ml of PBS. Mixed well and dispensed 50 µl of diluted antigen to all the wells of 96 well ELISA plate. Tapped the plates gently to ensure that the fluid is settled at the bottom of the well. Covered the plate with a lid or wrap and incubated the plate for one hour at 37°c in an ordinary incubator under continuous shaking.

3. 2. 2. 2 Addition of test sera and control sera

Dispensing of reagents

1. Wash buffer- PBS diluted four times with distilled water.
2. Blocking buffer-@ 20 ml per plate.
3. Test sera samples thawed from freezer and properly mixed by gentle tilting
4. Control sera samples reconstituted from the kit
5. Monoclonal antibody- Diluted 50 µl of monoclonal antibody with 5 ml of blocking buffer.

3. 2. 2. 3 Washing the plate and addition of sera

At the end of the incubation period discarded the antigen from the plate by inverting the plate over the sink and tapping it down with a single motion of the hand. Washed the plates three times with wash buffer with the help of a wash bottle. After each washing discarded the buffer by inverting the plate over the sink and tapped it with a piece of filter paper. Added the following reagents very carefully

- 40 µl of blocking buffer in all the wells.
- 20 µl of additional blocking buffer to monoclonal antibody control wells.
- 60 µl of additional blocking buffer to each of the conjugate control wells.
- 20 µl of each test serum samples in a set of two wells using separate tips for each sample.
- 20 µl of strong positive serum control (C++) in each of the four designated wells in the plate.
- 20 µl of weak positive serum control (C+) in each of the four designated wells in the plate.

- 20 µl of negative serum controls (C-) in each of the two designated wells in the plate.
- 40 µl of diluted monoclonal antibody in each well of the plate except the conjugate control wells.

Mixed the contents of the wells by gently tapping the sides of the plates. Covered the plates and incubated at 37°c for one hour on an orbital shaker with continuous shaking at moderate speed.

3. 2. 2. 4 Addition of antimouse conjugate

Working solution of antimouse conjugate was prepared by adding six µl of antimouse conjugate to six ml of blocking buffer.

Washing the plate and addition of conjugate

After one hour of incubation repeated the discarding and washing procedures three times. Added 50 µl of diluted antimouse conjugate in all the wells of the plate. Covered the plates, incubated for one hour at 37 °c on an orbital shaker.

3. 2. 2. 5 Addition of OPD solution

Preparation of working solution

Dissolved one tablet of OPD in 75 ml glass distilled water. Added 24 µl of 3 per cent Hydrogen peroxide (H2O2) solution to six µl of OPD solution. Took the plates out of the incubator and discarded the contents. Repeated the discarding and washing procedures three times.

Added 50 µl of freshly prepared OPD substrate mixture in each well of the plate. Also added 50 µl of the same in each well of the blank eight well module. Incubated the plates and the blank module for about 10-20 minutes at 37°c without shaking.

3. 2. 2. 6 Addition of stopping solution

Added 50 µl of stopping solution (1M Sulphuric acid) to each well of the plate and the blank module and gently tapped the plates for thorough mixing.

3. 2. 2. 7 Measurement of colour development

The microplate ELISA reader is turned on and allowed to warm up for 15 minutes before reading to ensure uniformity of reading for all plates. Put the blanking plate in the ELISA plate reader followed by the plate containing the test proper.

3. 2. 2. 8 Interpretation of the test results

The test sera samples showing more than 40 % inhibition of mean OD values of the monoclonal antibody wells (Cm) are taken as positive for PPR antibodies provided other control wells fall within the range. The percentage inhibition can also be calculated manually as follows.

PI = 100-{(OD of test sample/OD of Cm) × 100}.

The plate reading should normally be rejected if the PI in the control panel do not fall with in the expected range as below.

The range of PI in the control panel

Conjugate control	:	91-105%
Strong positive (C++)	:	81-100%
weak positive (C+)	:	45-80%

Negative control (C-) : -25- 25%

Acceptable OD range of Cm wells : 0 .3- 1.00.

Acceptance of control data

The data expressed in OD values and PI values for the controls are used to determine whether or not the test has performed with in the acceptance limit of variability.

Acceptance of test sera data

Test sera having mean PI values greater than 40% are taken as positive.

3. 2. 3 Statistical analysis

The results of prevalence of PPR antibodies in goats were subjected to statistical analysis (Chi square test) as per the procedures of Snedecor and Cochran (1994).

Results

4. RESULTS

A total number of 412 serum samples were collected from goats of different districts of Kerala comprising of different age groups, breeds, managemental practices and health status and were subjected to competitive ELISA for detection of antibodies to PPR. Of these 64 samples (15.5 per cent) gave positive result.

4. 1 Seroprevalence of PPR in different managemental practices.

Seroprevalence of PPR under various managemental practices are given in table1 and figure 1.

One hundred and fifty two samples were collected from organized farms, out of which 38 (25 per cent) samples were found positive.

Two hundred and sixty samples were from goats reared under rural farming system among which 26 (10 per cent) samples were found positive.

Statistical analysis of the results showed highly significant difference at 1 per cent level between the above two groups.

4. 2 Seroprevalence of PPR in animals of different health status

Seroprevalence of PPR in animals of different health status are given in table 2 and figure 2.

Out of 412 serum samples collected, 337 were from apparently healthy animals while 75 were from animals with history of disease. Among apparently healthy animals, 51 (15. 1 per cent) animals showed positive result and 13 (17. 3 per cent) animals of diseased group showed positive reaction.

No statistical difference was observed between the above two groups.

4.3 Seroprevalence of PPR among different breeds of goats

Seroprevalence of PPR among different breeds of goats are given in table 3 and figure 3.

Samples were collected from Malabari, Jamunapari and cross bred goats. One hundred and thirty four samples were collected from Malabari breed of goats, out of which 39 (29.1per cent) were found positive. Thirty samples were collected from Jamunapari breed of goats out of which 3 (10 per cent) samples were positive. Two hundred and fourty eight samples were collected from crossbred animals out of which 22 (8.87) samples were positive.

Stastical analysis showed highly significant difference between animals of Malabari breed and cross bred animals and a significant difference between animals of Malabari breed and Jamunapari breed. No significance difference existed between Jamunapari and cross bred animals.

4.4 Seroprevalence of PPR among goats of different age groups

Seroprevalence of PPR among various age groups are given in Table 4 and figure 4.

Seroprevalence was observed among goats from six months of age to five years of age . Highest prevalence rate was observed among animals of 6 months to one year of age (30. 1 per cent) followed by animals of one to three years of age (13. 16 per cent) and animals above three years of age(11.9 per cent). No positive reaction was observed in 16 samples of kids below six months of age.

On statistical analysis the animals of the age group of six months to one year and one to three years showed highly significance difference at one per cent level. A significant difference was observed between the animals of the age group of 6months to one year and above three years of age. No significant difference was observed between animals of the age group of one to three year and animals above three years.

4.5 Sex wise seroprevalence of PPR

Seroprevalence of PPR in male and female animals are presented in table 5 and figure 5.

Thirty eight male animals were tested for PPR antibodies, out of which 5 (13. 15 per cent) animals were found positive. Out of 374 female animals 59 (15. 77 per cent) animals were found positive.

No significant difference was observed in sex wise prevalence of PPR.

4.6 Seroprevalence of PPR in different farms.

Seroprevalence of PPR in various farms are presented in table 6 and figure 6.

The highest seroprevalence was observed in KLDB goat farm, Dhoni, where nine out of 15 samples (60 per cent) were positive. A seroprevalence of 36.1 per cent was

observed in Jersey farm, Vithura where17 samples out of 47 were positive. The disease was not found to be prevalent in K. A. U. goat farm, Pookott, Wayanad.

The highest seroprevalence of 16 per cent was observed in a private goat farm, Kollam (4/25) followed by a seroprevalence of 10 per cent in a private goat farm, Manjapra, Ernakulam district (3/30) and a seroprevalence of 5 per cent (1/20) in a private goat farm, Vallikunnam, Alappuzha district.

Highly significant difference was observed between the different farms.

4.7 Seroprevalence of PPR in animals with different clinical manifestations.

Different clinical manifestations in PPR seropositive animals are given in table 7 and figure 7.

The highest prevalence was observed in animals with a history of abortion. Seven samples were from cases of abortion among which 3 animals showed positive results (42.8 per cent). Among 28 animals with a history of ocular lesions, nine animals tested positive(32.14 per cent). Among 41 animals with a history of oral lesions nine animals showed antibodies to PPR (21.95 per cent). Thirty four animals had a history of respiratory disorders , among these nine animals showed positive reaction (26.4 per cent). Among 43 animals with a history of diarrhoea 13 showed positive result (30.2 per cent).

Statistical analysis revealed no significant difference between the animals with different clinical manifestations.

4.8 Seroprevalence of PPR in different districts of Kerala.

Number of serum samples collected from each district and number of seropositive animals are given in table 8 and fig 8.

Highest seropositivity was observed in Kozhikkode district (66.6per cent) followed by Trivandrum (36.1 per cent), Trichur (23.9 per cent) and Palakkad (18.6 per cent) . Positive reaction was not observed in samples collected from Kottayam, Idukki, Wayanad, Kannur and Kasaragode districts.

Statistical analysis revealed highly significant difference between the different districts of Kerala.

Table1 Seroprevalence of PPR among goats reared under different managemental practices.

Managemental practice	Number of animals tested	Number of positive animals	Percentage
1.Organized farming	152	38	25
2.Rural farming	260	26	10

Highly significant difference between 1 and 2 ($P \leq 0.01$).

Table2 Seroprevalence of PPR in animals of different health status

Health status	Number of animal tested	Number of positive animals	Percentage
1. Healthy animals	337	51	15.1
2. Diseased anima	75	13	17.3

NS- No significant difference between 1 and 2.

Table 3 Breed wise seroprevalence of PPR among goats

Breed	Number of animals tested	Number of positive animals	Percentage
1.Malabari	134	39	29.1
2. Jamunapari	30	3	10
3. Crossbreds	248	22	8.87

Highly significant difference between 1 and 3.(P≤ 0.01).

Significant difference between 1 and 2 . (P≤0.05).

No significant difference between Jamunapari breed and cross bred.

Table 4 Age wise seroprevalence of PPR among goats

Age group	Number of animals tested	Number of positive animals	Percentage
1.0-6 months	16	0	0
2. 6m -1 year	73	22	30.1
3. 1-3 year	281	37	13.16
4. Above 3 year	42	5	11.9

Highly significant differnce between 2 and 3. (P≤ 0.01).

Significant difference between 2 and 4. (P≤ 0.05).

No significant difference between 3 and 4.

Table5 Sex wise seroprevalence of PPR in goats

Sex	Number of animals tested	Number of positive animals	Percentage
1. Male	38	5	13.15
2. Female	374	59	15.77

No significant difference between 1 and 2.

Table 6 Seroprevalence of PPR among goats of different farms

Farm	Number of animals tested	Number of positive animals	Percentage
K. L. D. B goat farm Dhoni	15	9	60
Jersy farm, Vithura	47	17	36. 1
K. A. U farm, Pookott	15	0	0
Private farm, Manjapra	30	3	10
Private farm, Vallikunnam	20	1	5
Private farm, Kollam	25	4	16

Highly significant difference between the above groups ($P \leq 0.01$).

Table7 Seroprevalence of PPR among goats with different clinical manifestations

Form of infection	Number of animals tested	Number of positive animals	Percentage
Oral lesions	41	9	21.95
Occular lesions	28	9	32.14
Diarrhoea	43	13	30.2
Abortion	7	3	42.8
Respiratory signs	34	9	26.4

No significant difference between the above groups.

Table 8 Seroprevalence of PPR in different districts of Kerala

Districts	Number of animals tested	Number of positive animals	Percentage
Thiruvanathapuram	47	17	36. 1
Kollam	25	4	16
Alappuzha	40	1	2. 5
Pathanamthitta	30	3	10
Kottayam	20	-	0
Idukki	20	-	0
Ernakulam	30	3	10
Thrissur	45	11	23.9
Palakkad	75	14	18. 6
Malappuram	15	1	6. 6
Kozhikkode	15	10	66.6
Wayanad	20	-	0
Kannur	15	-	0
Kasaragode	15	-	0

Highly significant difference between the above groups.

Fig. 1 Seroprevalence of PPR among goats reared under different managemental practices

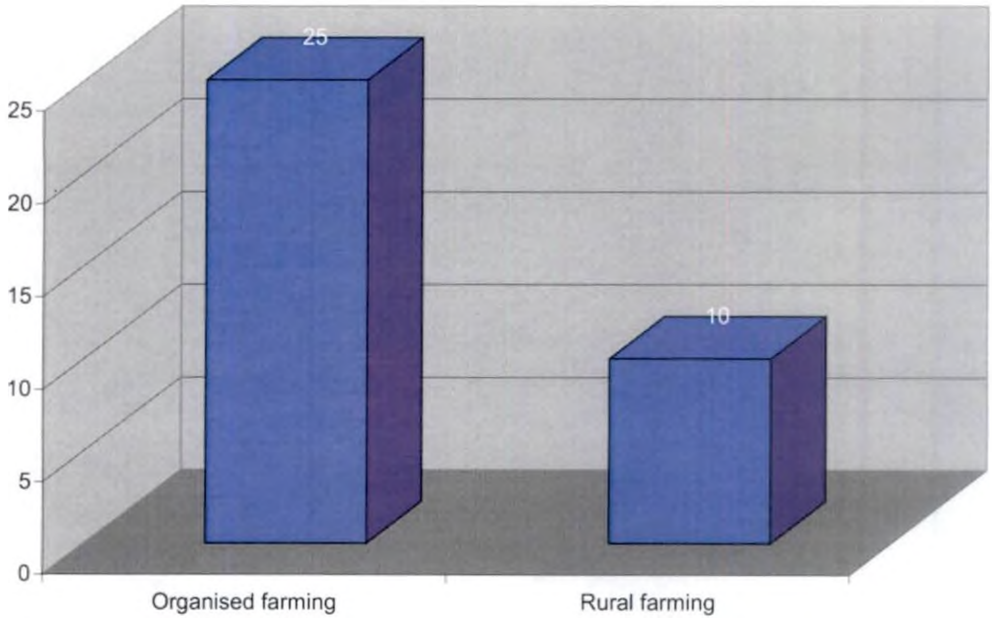


Fig. 2 Seroprevalence of PPR in animals of different health status

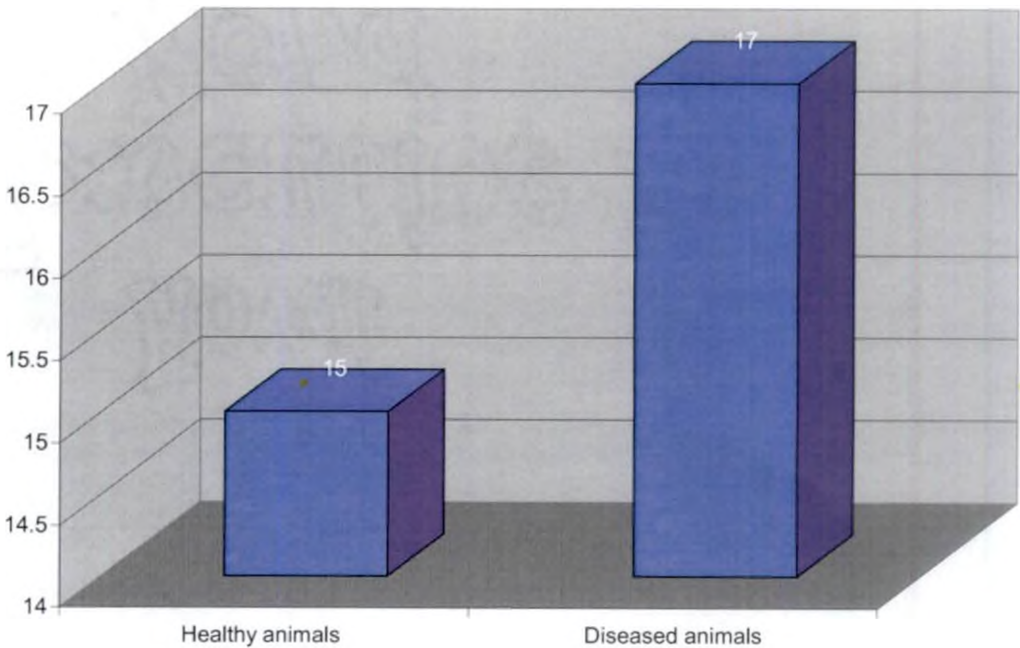


Fig. 3 Breed wise seroprevalence of PPR among goats

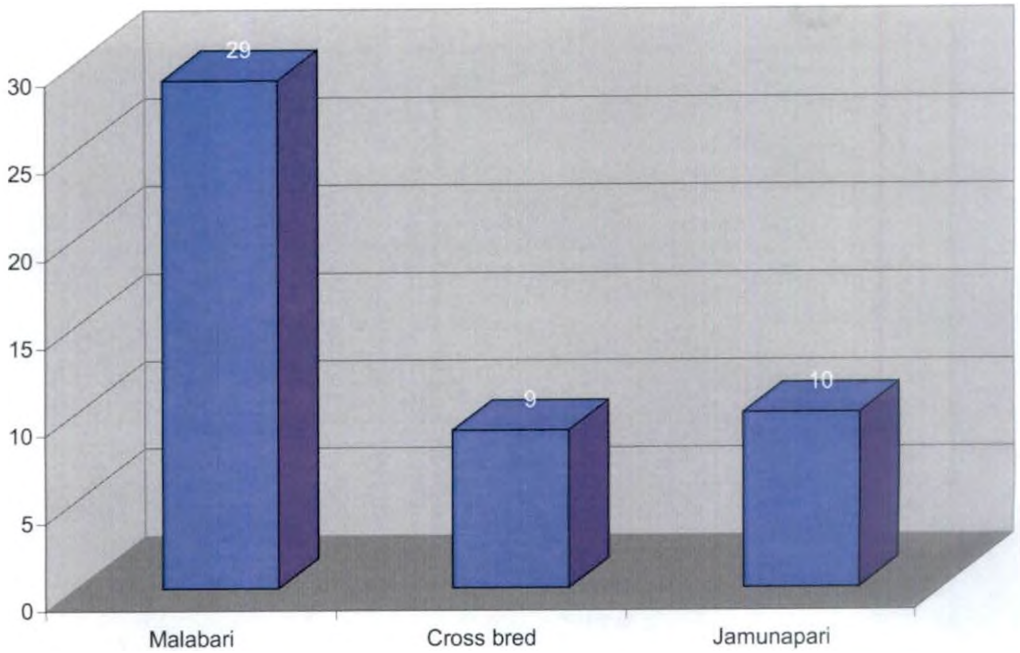


Fig.4 Seroprevalence of PPR among goats of different age groups

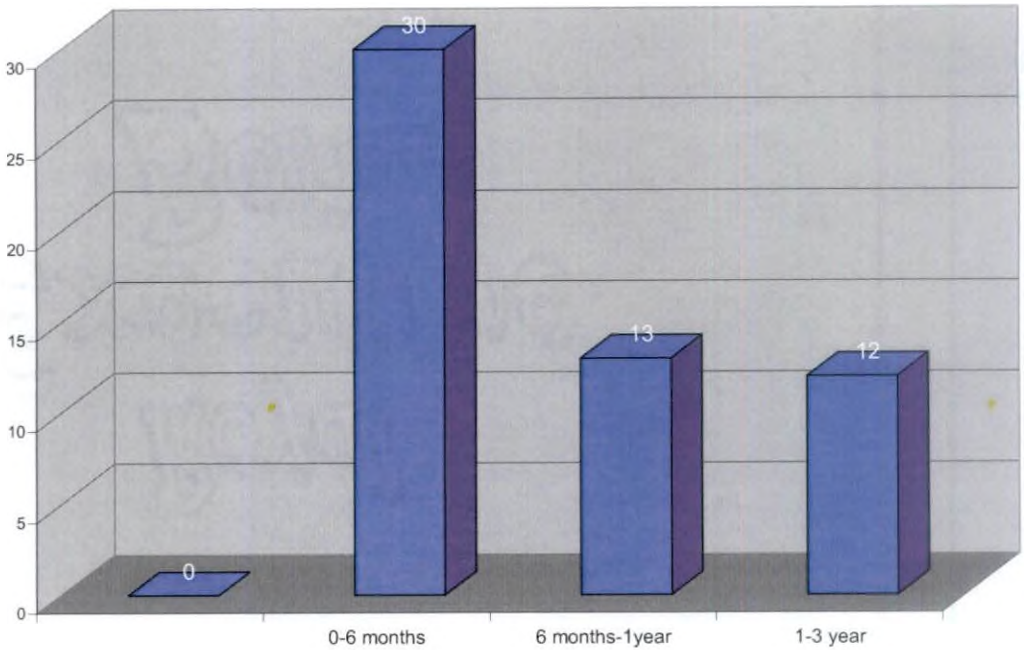


Fig. 5 Sex wise seroprevalence of PPR among goats

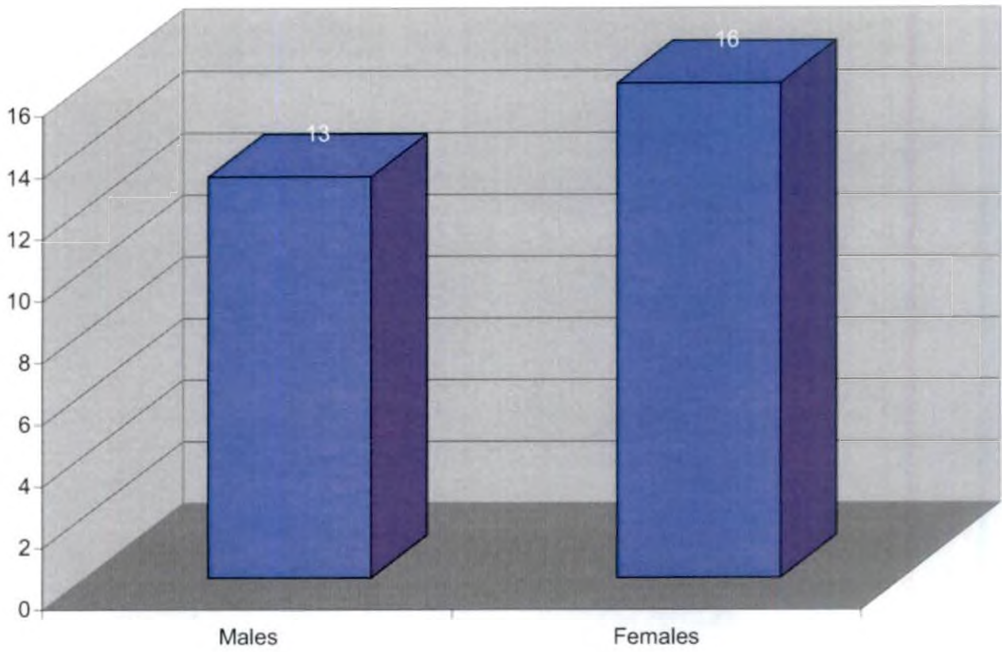
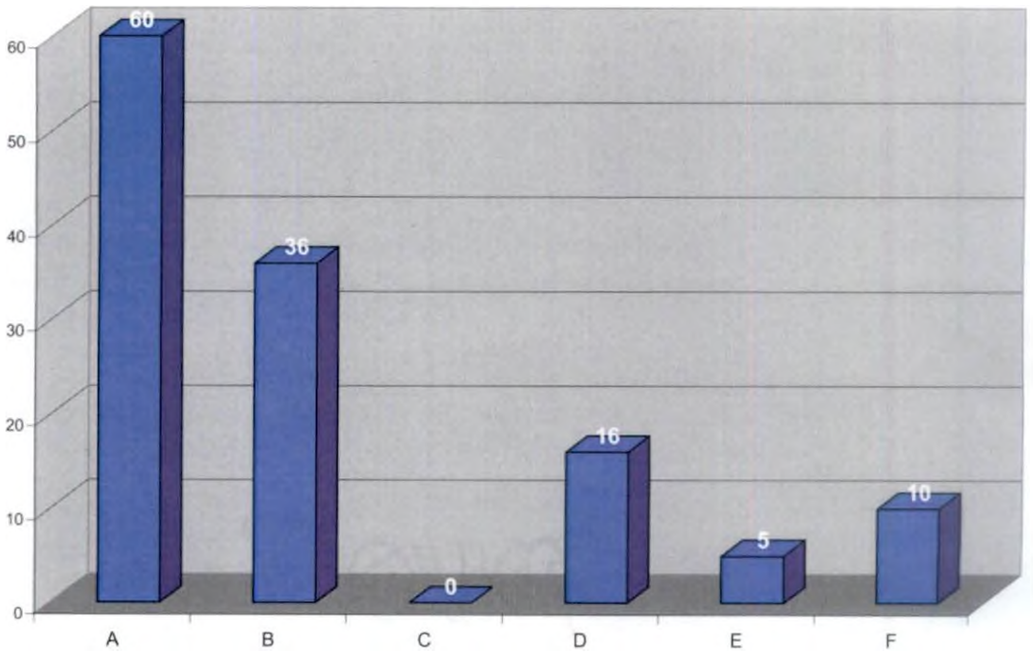


Fig. 6 Seroprevalence of PPR in different farms



A- KLDB goat farm, Dhoni

B- Jersey farm, Vithura

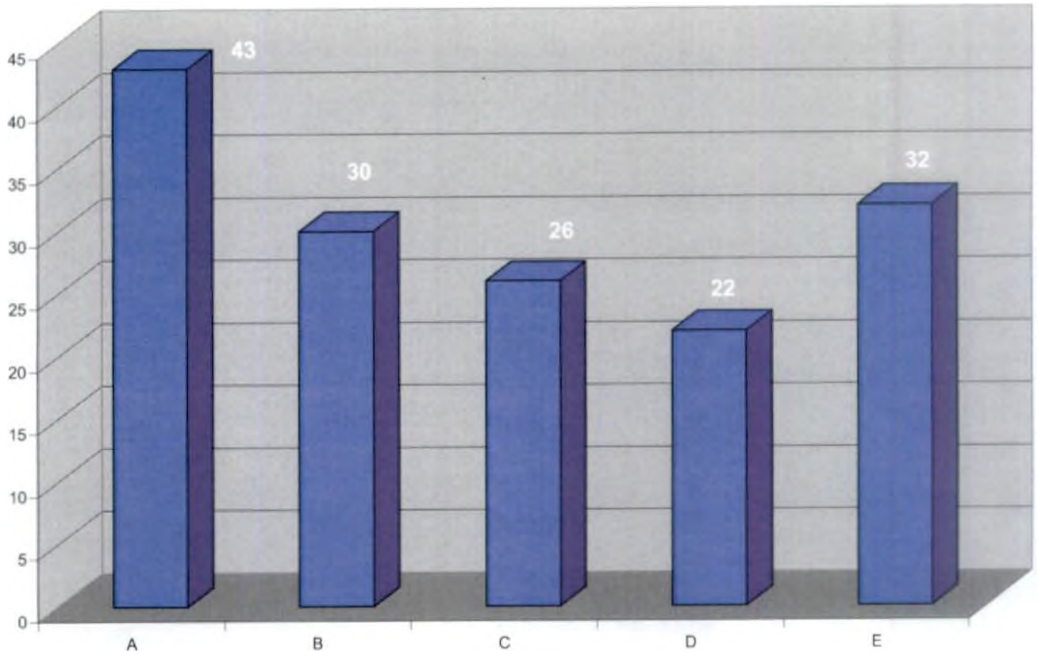
C- KAU farm, Pookkot

D- Private farm, Kollam

E – Private farm, Vallikunnam

F- Private farm, Manjapra

Fig. 7 Seroprevalence of PPR in animals with different clinical signs



A- Abortion

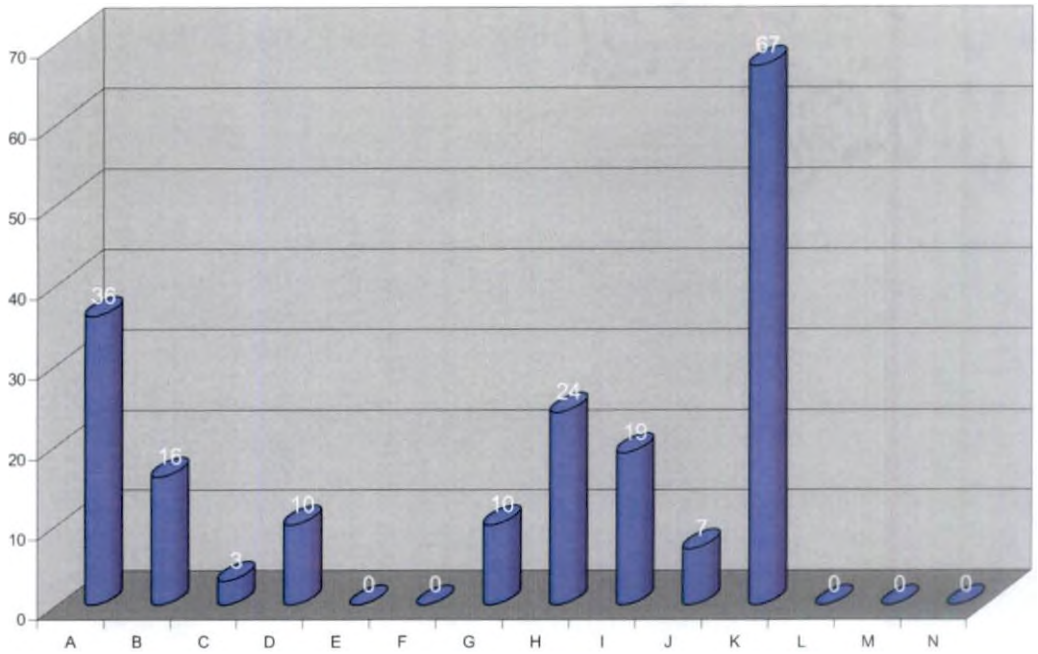
B- Diarrhoea

C- Respiratory infection

D- Oral lesions

E- Occular signs

Fig. 8 Seroprevalence of PPR in different districts of Kerala



A-TRIVANDRUM
B-KOLLAM
C- ALAPPUZHA
D- PATHANAMTHITTA
E-KOTTAYAM
F- IDUKKI
G- ERNAKULAM
H- TRICHUR
I-PALAKKAD
J- MALAPPURAM
K- KOZHIKKODE
L- WAYANAD
M- KANNUR
N-KAZARAGODE

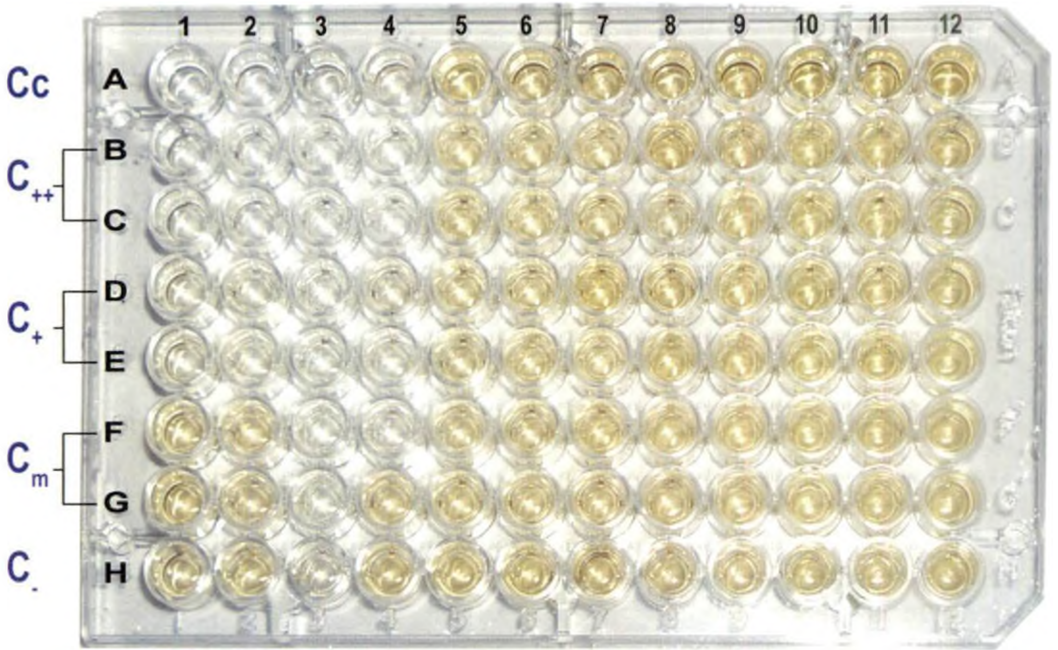


Plate 1. **Competitive Enzyme Linked Immunosorbent Assay (c ELISA) Test Plate**

Conjugate Control-	A1, A2
Strong Positive Control-	B1, B2, C1, C2
Weak Positive Control-	D1, D2, E1, E2
Monoclonal Antibody Control-	F1, F2, G1, G2
Negative Control-	H1, H2
Test Serum Samples-	A3 – H12
Positive Serum Samples –	1 (A3, B3) 2 (C3, D3) 3 (E3, F3) 4 (G3, H3) 5 (A4, B4) 6 (C4, D4) 7 (E4, F4)

Plate 2. **Seroprevalence of PPR in goats of Kerala**

Discussion

5. DISCUSSION

In the present study seroprevalence of *Peste des petits ruminants* among goats of Kerala was assessed using competitive ELISA. Results revealed a prevalence rate of 15.5 per cent antibodies. The preliminary serological study of PPR in Kerala by Sunilkumar *et al.* (2005) revealed a prevalence rate of 0. 93 per cent among 536 goat sera samples tested. The higher prevalence rate reported in the present study could be attributed to the increased animal movement from the neighbouring states .

Constant and uncontrolled movement of animals have been reported to be the cause of PPR outbreaks in sheep and goats.(Shaila *et al.*, 1989). Kumar *et al* . (1999) also suggested that introduction of infection into clean or virgin areas occurred whenever infected animals are introduced due to unchecked movement or due to lack of quarantine. Krishna *et al.* (2001) and Dorairajan *et al.* (2006) have reported significant seropositivity of PPR in small ruminants of Andhra Pradesh and Tamil Nadu respectively.

5. 1 Seroprevalence of PPR among goats reared under different managemental practices

In the present study serological evidence of infection was observed both in organised herds and in goats maintained by rural farmers. (Table 1 and figure1).

Percentage of seroprevalence was more in animals reared under organised farming (25 per cent) than in animals reared under rural farming (10 per cent).

In organized farming, as the number of animals reared is more the chance for the diseae to spread is more. Roeder and Obi (1999) reported that the discharges from eyes, nose, mouth and loose faeces contain large amount of the virus and when the fine infective

droplets released into the air are inhaled by other animals they become infected. Barua *et al.* (2004) also reported that the disease spread through animal to animal while on grazing land and through indirect contact via persons involved in feeding and grazing of these animals.

In organized farming contact of the animal with infected fodder while grazing or introduction of infection into the farm premises by transport vehicles or unrestricted movement of farm workers might have been the possible sources of infection as suggested by Kumar *et al.* (1999).

Taylor *et al.* (1990) reported a higher rate of infection among animals belonged to small holder farmers as they could not prevent contact between their own animals or those of their neighbours.

In rural farming practice, movement of goats for grazing could be the source of infection (Anjaneyalu and James, 1999).

5. 2 Seroprevalence of PPR among goats with different health status

Peste des petits ruminants antibodies were detected from healthy animals and from animals having a history of disease such as oral lesions, respiratory infections, abortion and diarrhoea (Table 2 and Figure 2).

Higher percentage of positive reaction among animals with a history of oral lesions, respiratory infection, abortion and diarrhoea suggests the possible association of PPR virus with such conditions or a mild form of the disease. All these symptoms were reported to be present in PPR infections by many workers (Bundza *et al.*, 1988; Scott *et al.*, 1990; Brown *et al.*, 1991). Similar observations were also made by Ozkul *et al.* (2002) in a

seroprevalence study conducted in Turkey, where he reported a higher level of seroprevalence (51. 6 per cent) in sheep and goats with clinical signs of PPR.

Appearance of PPR in apparently healthy animals could be associated with herding of animals from different locations, varying degrees of stresses and sudden changes in the enviornment and feeding habit. Enviornmental stress, particularly hot and humid climate also favour precipitation of disease (Wosu, 1995).

5. 3 Breed wise seroprevalence of PPR

Present study revealed a higher seroprevalence of PPR in goats of Malabari breed (Table 3 and figure3).

Malabari goats showed a seroprevalence of 29. 1 per cent where as Jamunapari goats had a seroprevalence of 10 per cent. . In the present study 134 samples were from Malabari breed . The higher percentage of positive reaction may be because of the higher proportion of younger, more susceptible animals of Malabari breed in the total goat population than Jamunapari breed.

This is contradictory to the findings of Saha *et al.* (2005) who reported a higher prevalence in Jamunapari goats than other breeds. Jamunapari being a free grazing breed of goats helped in predisposing of animals to infection (Kumar *et al.*, 2001)

5. 4 Age wise seroprevalence of PPR

Seroprevalence of PPR was highest in animals of the age group of 6 months to one year. Similiar finding was also reported by Saha *et al.* (2005) who reported a highest prevalence of the disease in the age group of five to 12 months. Higher susceptibility of

prevalence of the disease in the age group of five to 12 months. Higher susceptibility of kids to PPR infection is attributed to the concurrent intestinal infections due to coccidia, *E. coli*, enteroviruses and gastrointestinal parasites which further enhances the susceptibility of younger animals to PPR infections. A lower seroprevalence was observed in adults because adult goats are less susceptible to PPR due to strong and developed immune system in adults (Kumar *et al.*, 2001).

In contradictory to the findings, Agrawal *et al.* (2006) recorded a highest seroprevalence among goats of three to five years age.

Absence of seroprevalence in goats below 6 months of age may be due to the presence of colostral antibodies in the kids (Mondal *et al.*, 1995; Saha *et al.*, 2005; Agrawal *et al.*, 2006).

5. 5 Sex wise prevalence of PPR

Even though a higher seroprevalence was observed in females (15. 77 per cent) when compared to males (13. 15 per cent) no significant difference was observed statistically. This is in agreement with the studies of Agrawal *et al.* (2006), where sex wise seroprevalence revealed a higher prevalence among females (9. 8%) than males (7. 7%). He also suggested that it may be attributed to the variation in sample size, more over the males are sold at a much earlier age while females are kept for breeding purpose of getting milk and there by the chances of the disease in females may be more .

These findings are in contrary to the observation by Shankar *et al.* (1998) who reported a higher attack rate and case fatality rate in males (66. 6 per cent) than in females (39. 6 per cent).

No sex variation for prevalence of PPR was reported by Mondal *et al.* (1995) and Saha *et al.* (2005).

5. 6 Seroprevalence among goats of different farms.

Seroprevalence of PPR was more in KLDB goat farm, Dhoni, and Jersey farm, Vithura. The variation in the seroprevalence between different farms may be due to differences in the managemental practices. PPR is an airborne virus and so there is more chance of the spread of infection in organized farms affecting more number of animals. In KLDB goat farm there was an outbreak of PPR prior to the blood collection which might have led to the higher positive reaction. In Jersey farm, Vithura, there is a history of introduction of new stock.

Taylor (1984) and Barua *et al.*(2004) reported a close link between the appearance of the disease and introduction of new stock. The higher seroprevalence may be due to the introduction of new stock

5. 7 Seroprevalence of goats with different clinical manifestations

Seroprevalence was observed in samples collected from animals with respiratory infections, oral lesions, abortion and diarrhoea (Table7 and figure 7). Higher level of seropositivity was also observed by Ozkul *et al.* (2002) among animals with clinical disease.

Highest seropositivity to PPR was (42. 8%) was observed in samples collected from animals having abortion. Samples collected from diarrhoeic animals showed a

seropositivity of 30. 9 per cent. Taylor (1984) and Bundza *et al.* (1988) associated symptoms of abortion and diarrhoea with PPR..

Twenty six per cent seropositivity was observed in samples collected from animals with respiratory infection. Ability of PPR virus to cause respiratory infection was reported by many workers (Bundza *et al.*, 1988; Scott., 1990 and Brown et al., 1991).

A seropositivity of 21. 95 per cent was observed in animals having oral lesion. A seropositivity of 32. 14 per cent was observed in animals having a history of ocular lesions. Brown *et al* (1991) associated erosive stomatitis and occulonasal discharges with PPR.

5. 8 Seroprevalence of PPR in different districts of Kerala

Seroprevalence of PPR was recorded from almost all districts of Kerala (Table 8 and Fig 8). Seroprevalence ranged from zero per cent to 66. 6 per cent in different districts.

In Kozhikkode district the number of samples collected was comparatively less and the highest positive reaction may be due to a recent outbreak , either in test animals or in incontact animals.

The higher prevalence rates were observed in boarder districts such as Trivandrum, Trichur, Palakkad, which may be due to increased animal movement from our neighbouring state, Tamil Nadu, Andhra Pradesh and Karnataka.

Constant and uncontrolled movement of animals have been reported to be the cause of PPR outbreaks in sheep and goats.(Shaila *et al.*, 1989). Dorairajan *et al.* (2006) has reported significant seropositivity of PPR in small ruminants of Tamil Nadu .

Summary

6. SUMMARY

PPR is caused by morbilli virus of paramyxoviridae family. The disease is characterized by the sudden onset of depression, fever, discharges from the eyes and nose, sores in the mouth, distended breathing and cough, foul smelling diarrhoea and death.

The disease was first reported in India in 1987 by Shaila *et al* (1989).

Seroprevalence studies have shown that the disease is prevalent throughout the country.

Seroprevalence of PPR in goats of Kerala was studied in this work. Four hundred and twelve samples were collected from all districts of Kerala and were subjected to competitive ELISA for detecting antibodies against PPR infection . Sixty four samples (15. 5per cent) were found positive for PPR antibodies and results were subjected to statistical analysis.

Seroprevalence of PPR was more in animals reared under organized farming system than in rural farming system and there is a highly significant difference between them.

Animals with a history of disease showed more seroprevalence to PPR.

Seropositivity of PPR was more in Malabari breed of goats than Jamunapari breed and cross breds.

Seroprevalence of PPR was more in animals of the age group of 6 months-one year.

Female animals showed a higher seropositivity than male animals.

High percentage of seroprevalence of PPR was detected in animals with a history of abortion.

Percentage of seroprevalence of PPR in cattle of Kerala was observed as 15.5 per cent.

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ANNEXURE I

Competitive ELISA plate lay out

Controls	Serum samples in duplicate										
	1	2	3	4	5	6	7	8	9	10	11	12
A	Cc	Cc	1	5	9	13	17	21	25	29	33	37
B	C++	C++	1	5	9	13	17	21	25	29	33	37
C	C++	C++	2	6	10	14	18	22	26	30	34	38
D	C+	C+	2	6	10	14	18	22	26	30	34	38
E	C+	C+	3	7	11	15	19	23	27	31	35	39
F	Cm	Cm	3	7	11	15	19	23	27	31	35	39
G	Cm	Cm	4	8	12	16	20	24	28	32	36	40
H	C-	C-	4	8	12	16	20	24	28	32	36	40

Cc : conjugate control

C++ : Strong positive

C+ : Weak positive

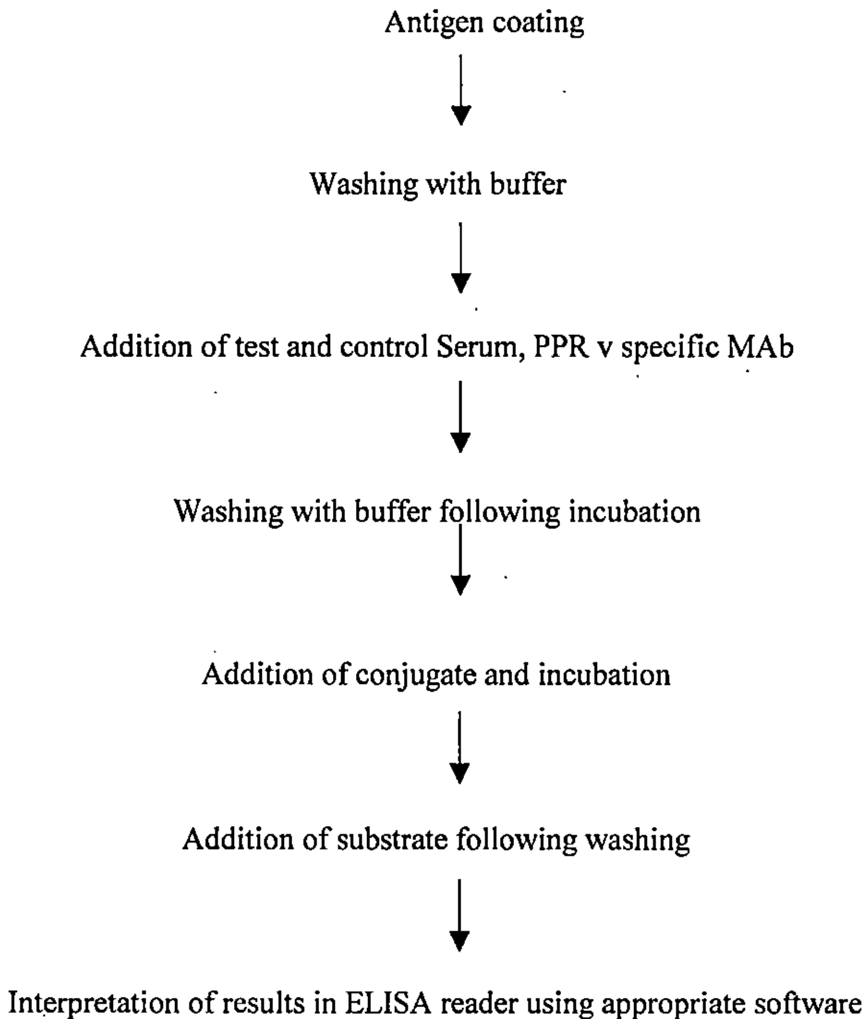
Cm : monoclonal antibody control

C- : Negative

Numbers – From 1-40 : Serum samples in duplicate

ANNEXURE II

Competitive ELISA



ANNEXURE III

Competitive ELISA

Result sheet lay – out

Plate status

Test name — Test date

Plate Name — Test Time

Filter — Technician

Blanking Value — Kit batch

Controls: Acceptable OD range: 0.300-1.00

Threshold: PI.>=40 % Outside Control Limits: Od(#), PI(*)

ID	STATUS	OD1	OD2	OD3	OD4	PI1	PI2	PI3	PI4	LCL	UCL
C++	in									81	100
C+	in									45	80
C-	in									-25	25
Cc	in									91	105
Cm	in									-19	20

SEROPREVALENCE OF *PESTE DES PETITS RUMINANTS* IN GOATS OF KERALA

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ABSTRACT

Seroprevalence of PPR in goats of Kerala was studied using competitive ELISA. Four hundred and twelve sera samples were collected from goats belonging to different places of Kerala covering all the districts.

Samples were collected randomly from goats of different age, managemental practice, breed, sex, different health status and also from different farms.

Samples were subjected to c ELISA for detecting antibodies against PPR infection. Out of 412 sera samples tested 15.5 per cent gave positive result for PPR antibodies.

Seroprevalence of PPR antibodies was more in animals reared under organized farming system.

Seroprevalence of PPR antibodies was more in animals with a history of disease than apparently healthy animals.

Prevalence of PPR antibodies is found to be more in malabari breeds of goats when compared to jamunapari and cross bred animals.

Seroprevalence of PPR in various age group revealed that percentage of positivity was more in animals of the age group of 6 months- one year.

Animals from different farms were tested for antibodies against PPR infection. Seroprevalence of PPR was more in KLDB goat farm, Dhoni, and Jersey farm, Vithura.

Seroprevalence of PPR among goats with different clinical manifestations was recorded. High percentage of seroprevalence was noted in animals with abortion.

Seroprevalence of PPR in different districts of Kerala was assessed. The highest seroprevalence was recorded in Kozhikkode district.

Seroprevalence of PPR in goats of Kerala was detected as 15.5 per cent

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