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# Application of PCR in Diagnosis of Peste des Petits Ruminants Virus (PPRV)

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## 1. Introduction

a. Global perspective of PPRV

A Peste des petits ruminant (PPR) is a viral disease of sheep, goats and wild ruminants. It is acute disease which is endemic in many countries of Africa, Arabian Peninsula, Middle east and India. [7, 12, 13]

It was first reported in Côte d'Ivoire in West Africa [14] and was named as Kata, psuedorinderpest, pneumoenterititis complex and stomatitis-pneumenteritis syndrome [15] . Then in 1972 a sort of disease in goats in Sudan was identified to be PPR [16] . In recent years either the presence of antibodies to the virus or viral nucleic acid has been confirmed from the countries like Burkina Faso (2008), Ghana (2010), Nigeria (2007) and Senegal (2010) [17] .

Recently detection of PPRV in East Africa countries is shown by the detection of Antibodies in Kenya (1999 and 2009) and Uganda (2005 and 2007) [18]. It has also been detected in North Africa (Egypt) in 1987 and 1990.

In Saudi Arabia, an outbreak of PPRV has been reported in April, 2002 in Sheeps and Goats [1]. In Pakistan PPRV has been reported since 1991 which was confirmed by PCR in 1994. [19] In India the was first reported in 1987 [11]. In Iran the disease was reported in 1995 [20] while in Iraq it was first detected in 2000 [21] .

b. Disease picture of PPRV

Peste des petits ruminants (PPR) represents one of the most economically important animal diseases in areas that rely on small ruminants. Outbreaks tend to be associated with contact of immuno-naïve animals with animals from endemic areas. In addition to occurring in extensive-migratory populations, PPR can occur in village and urban settings though the number of animals is usually too small to maintain the virus in these situations.

- Morbidity rate in susceptible populations can reach 90–100%
- Mortality rates vary among susceptible animals but can reach 50–100% in more severe instances

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- Both morbidity and mortality rates are lower in endemic areas and in adult animals when compared to young ones.

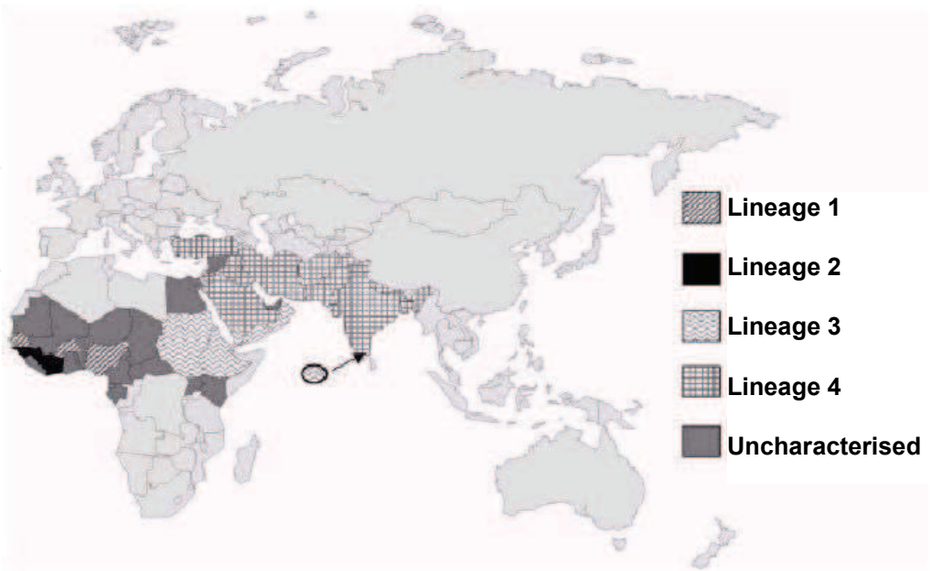


Fig. 1. Geographic distribution of PPRV lineages (Dhar *et al*., 2002)

c. Hosts Range

- Goats (predominantly) and sheep
  - Breed-linked predisposition in goats

Fig. 2. Clinical Picture and Severity of the Disease

Wildlife host range not fully understood

  - documented disease in captive wild ungulates: Dorcas gazelle (Gazelle dorcas), Thomson's gazelles (Gazella thomsoni), Nubian ibex (Capra ibex nubiana), Laristan sheep (Ovis gmelini laristanica) and gemsbok (Oryx gazella)
- Experimentally the American white-tailed deer (Odocoileus virginianus) is fully susceptible

- Cattle and pigs develop in-apparent infections and do not transmit disease
- May be associated with limited disease events in camels

## 2. Molecular epidemiology of PPRV

A close contact between the infected animals which is in the febrile stage and susceptible animals is a source of transmission of the disease [15]. During sneezing and coughing the virus spread from animal to animal [22]. Indirect transmission seems to be unlikely in view of the low resistance of the virus in the environment and its sensitivity to lipid solvent. [4]

Epidemiology pattern vary from area to area, for example in the humid Guinean zone where PPR occurs in an epizootic form can cause mortality between 50-80% while in arid and semi-arid regions, PPR is seldomly fatal but usually occurs as a subclinical or inapparent infection opening the door for other infections such as Pasteurellosis [4] . In Saudi Arabia a high morbidity of 90% was reported, [2] 3-8 months animal are more susceptible to disease than either of adults or unweaned animals [23].

a. Genome Organization of PPRV:

PPRV belong to *Morbillivirus* genus. For a long time it was thought to be a variant of RP that was adapted to sheeps and goats and had lost its virulence for cattles. [3] The causative agent of PPR is RNA virus which is single strand and non-segmented. It belongs to the family *Paramyxoviridae* and *genus Morbillivirus* which also includes measles virus, rinderpest virus (RPV), canine-distemper virus, phocinedistemper virus, and dolphin and porpoise morbilliviruses[24]. All the viruses belonging to the genus morbilli are serologically related. Phylogenetic analysis also shows that there is high degree of homology.

The genome contains six tandemly arranged transcription units which encodes six structural proteins i.e the surface glycoproteins F and H, the nucleocapsid (N), the matrix (M), the polymerase or large (L) and the polymerase-associated (P) proteins. The cistron directing the synthesis of this later protein is encoding the virus non-structural proteins C and V by the use of two other open reading frames (ORF) of the messengers. The gene order is 3′N-P-M-F-H-L5′, as determined by transcriptional mapping. [25] The genome is flanked by extragenic sequences at the 3′ ((52 nucleotides, leader) and 5′ ends (37 nucleotides, trailer).

For viruses of the family *Paramyxoviridae*, the genome promoter (GP) contains 107 nucleotides comprising the leader sequence and the adjacent non-coding region of the N gene at the 3′ end of the negative-strand. While antigenome promoter (AGP) contain 109 nucleotides that encompass the trailer sequence and the proximal untranslated region of the L gene. Both GP and the AGP contains the polymerase binding sites and the RNA encapsidation signals for the replication of the full genome while the production of messengers (m-RNA) is a function of the GP [26]. So GP and AGP have an impact on the virulence of virus.

Genes and promoters of *Morbillivirus*; the protein coding regions (N, P, V, C, M, F, H, and L), noncoding intergenic regions and the leader and trailer regions along with the specialized sequence motifs are shown. The genome promoter includes the leader sequence and the non coding regions N at the 3' end of the genomic RNA. The antigenome promoter includes the trailer sequence and the untranslated regions of the L gene at 5′ end. Gene start (GS) and gene end (GE), enclosing the intergenic trinucleotide motifs are also shown.

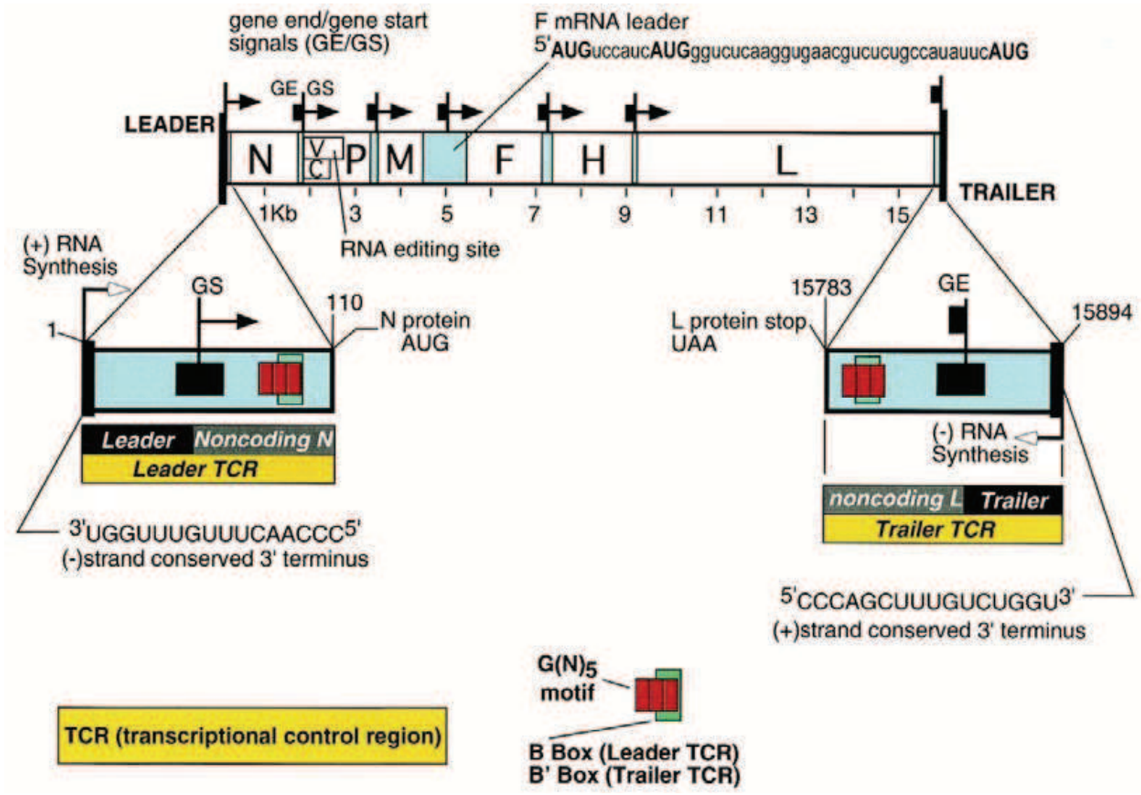


Fig. 3. Genome of PPR virus

b. Antigenic and Immunogenic Epitopes:

Surface glycoproteins hemagglutinin (H) and fusion protein (F) of morbilliviruses are highly immmunogenic and helps in providing the immunity. PPRV is closely related to rinderpest virus (RPV). Antibodies against PPRV are both cross neutralizing and Cross protective. A vaccinia virus double recombinant expressing H and F glycoproteins of RPV has been shown to protect goats against PPR disease though the animals developed virus-neutralizing antibodies only against the RPV and not against PPRV. Capripox recombinants expressing the H protein or the F protein of RPV or the F protein of PPRV conferred protection against PPR disease in goats, but without production of PPRV-neutralizing antibodies[27] or PPRV antibodies detectable by ELISA (Berhe *et al*, 2003). These results suggested that cell-mediated immune responses could play a crucial role in protection. Goats immunized with a recombinant baculovirus expressing the H glycoprotein generated both humoral and cell-mediated immune responses.[28] The responses generated against PPRV-H protein in the experimental goats are also RPV crossreactive suggesting that the H protein presented by the baculovirus recombinant 'resembles' the native protein present on PPRV.[28]

Lymphoproliferative responses were demonstrated in these animals against PPRV-H and RPV-H antigens [28]. N-terminal T cell determinant and a C-terminal domain harboring potential T cell determinant(s) in goats were mapped. Though the sub-set of T cells (CD4+ and CD8+ T cells) in PBMC that responded to the recombinant protein fragments and the synthetic peptide could not be determined, this could potentially be a CD4+ helper T cell epitope, which has been shown to harbor an immunodominant H restricted epitope in

mice[28] .Identification of B- and T-cell epitopes on the protective antigens of PPRV would open up avenues to design novel epitope based vaccines against PPR.

Sheep and goats are unlikely to be infected more than once in their economic life[12]. Lambs or kids receiving colostrum from previously exposed or vaccinated with RP tissue culture vaccine were found to acquire a high level of maternal antibodies that persist for 3-4 months. The maternal antibodies were detectable up to 4 months using virus neutralization test compared to 3 month with competitive ELISA[29]. Measles vaccine did not protect against PPR, but a degree of cross protection existed between PPR and canine distemper. [30]

## 3. Specimen collection, processing and shipment

Before collecting or sending any samples from animals with a suspected foreign animal disease, the proper authorities should be contacted. Samples should only be sent under secure conditions and to authorized laboratories to prevent the spread of the disease. In live animals, swabs of ocular and nasal discharges, and debris from oral lesions should be collected; a spatula can be rubbed across the gum and inside the lips to collect samples from oral lesions. Whole, unclotted blood (in heparin or EDTA) should be taken for virus isolation and PCR. Biopsy samples of lymph nodes or spleen may also be useful. Samples for virus isolation should be collected during the acute stage of the disease, when clinical signs are present; whenever possible, these samples should be taken from animals with high fever and before the onset of diarrhea. At necropsy, samples can be collected from lymph nodes (particularly the mesenteric and mediastinal nodes), lungs, spleen, tonsils and affected sections of the intestinal tract (e.g. ileum and large intestine). These samples should be taken from euthanized or freshly dead animals. Samples for virus isolation should be transported chilled on ice. Similar samples should be collected in formalin for histopathology. Whenever possible, paired sera should be taken rather than single samples. However, in countries that are PPR-free, a single serum sample (taken at least a week after the onset of clinical signs) may be diagnostic.

## 4. Laboratory diagnosis of PPR

a. Conventional Methods of PPRV Diagnosis

Conventional techniques such as the Agar Gel Immuno Diffusion (AGID) test are not routinely used for standard diagnosis as they lack sensitivity when compared to other assays. However, Haemagglutination tests (HA) and Haemagglutination Inhibition tests (HI) tests can be used for routine screening purposes in control programmes as they display comparative sensitivity alongside being simple to perform and cheap to produce.

Virus isolation in cell culture can be attempted with several different cell lines where samples permit. Although Vero cells have been the choice for isolation and propagation of PPRV, it is reported that B95a, an adherent cell line derived from Epstein-Barr virus-transformed marmoset B-lymphoblastoid cells, is more sensitive and support better growth of PPRV lineage IV as compared to Vero cells. More recently, Vero cells expressing the SLAM receptor have been used as an effective alternative for isolation in cell culture. The fragility of morbillivirus virions generally renders techniques such as virus isolation redundant for routine diagnostic use, especially where sample quality is poor. Such

techniques are also considered to be time-consuming and cumbersome. Virus isolation does, however, play an important role from a research perspective.

ELISA tests using monoclonal antibodies are often used for serological diagnosis and antigen detection for diagnostic and screening purposes. For PPR antibodies detection, the competitive ELISA is the most suitable choice as it is sensitive, specific, reliable, and has a high diagnostic specificity (99.8%) and sensitivity (90.5%). Immunocapture ELISA (ICE) is a rapid, sensitive and virus specific test for PPRV antigen detection and it can differentiate between RPV and PPRV and has been reported to be more sensitive than the AGID test.

For rapid diagnosis to enable a swift implementation of control measures, further development and validation of pen-side tests such as the chromatographic strip test and the dot ELISA that can be performed without the need for equipments or technical expertise are highly desirable.

| **Sr #** | **Test Name** | **Acronym** | **Application (Lab or Field)** | **Feature Detected (Antigen or Antibody)** |
|---|---|---|---|---|
| 1 | Agar gel immuno-diffusion | AGID | Both | Both |
| 2 | Counter Immuno-electrophoresis | CIEP | Both | Both |
| 3 | Dot enzyme immunoassay | -- | Lab | Antigen |
| 4 | Differential immuno-histo-chemical staining of tissue sections | IH staining | Lab | Antigen |
| 5 | Haemagglutination and Haemagglutination inhibition tests | HA and HI | Both | Both |
| 6 | Immuno-filtration | IF | Lab | Antigen |
| 7 | Latex agglutination tests | LA | Field | Antigen |
| 8 | Virus isolation | VI | Lab | Antigen |
| 9 | Competitive enzyme-linked Immuno-sorbent assay (c-ELISA) | cELISA | Lab | Antibody |
| 10 | Novel sandwich ELISA | sELISA | Lab | Antigen |
| 11 | Immuno-capture enzyme-linked immunosorbent assay | Ic-ELISA | Lab | Antigen |

Table 1. Detail of conventional methods for the detection and confirmation of PPR

b. Molecular Methods for PPRV Diagnosis

Molecular techniques such as reverse transcription polymerase chain reaction (RT- PCR) and nucleic acid hybridization are generally used. These genome based techniques are largely used because of their high specificity and sensitivity. However, modern one step real-time RT-PCR assays specific for PPRV and loop-mediated isothermal amplification techniques are more sensitive techniques for PPRV detection but do not allow genetic typing of positive samples. RT-PCR coupled with ELISA have also been used to increase the analytical sensitivity of visualization of RT-PCR products and to overcome the drawbacks of electrophoresis-based detection such as use of ethidium bromide, exposure to UV light etc. The assay is reported to detect viral RNA in infected tissue culture fluid with a virus titre as low as 0.01 TCID50/100 µL and has been reported as being 100 and 10,000 times more sensitive than the sandwich ELISA and RT-PCR, respectively. [31]

## 5. Potential and application of PCR technique for future advances in diagnosis of PPR

Among the various techniques developed for the detection of PPRV, PCR technique has been the most popular and highly sensitive tool so far for diagnosis of PPR. The routine serological techniques and virus isolation are normally used to diagnose morbillivirus infection in samples submitted for laboratory diagnosis. However, such techniques are not suitable for use on decomposed tissue samples, the polymerase chain reaction (PCR), has proved invaluable for analysis of such poorly preserved field samples. The PCR test consists of repetitive cycles of DNA denaturation, primer annealing and extension by a DNA polymerase effectively doubling the target with each cycle leading, theoretically, to an exponential rise in DNA product. There placement of the polymerase now fragment by thermo-stable polymerase derived from Thermus aquaticus (Taq) has greatly improved the usefulness of PCR. These qualities have made the PCR one of the essential techniques in molecular biology today and it is starting to have a wide use in laboratory disease diagnosis. Since the genome of all Morbilliviruses consists of a single strand of RNA, it must be first copied into DNA, using reverse transcriptase, in a two-step reaction known as reverse transcription polymerase chain reaction (RT-PCR).Among the various techniques developed for the detection of PPRV, however, polymerase chain reaction (PCR) technique developed using F-gene primers has been the most popular tool so far, for diagnosis as well as molecular epidemiological studies. RT-PCR using phospho-protein (P) universal primer and fusion (F) protein gene specific primer sets to detect and differentiate between PPR and RP are described by [8, 24, 32] developed a RT-PCR test, using phosphoprotein (P) gene and fusion protein(F) gene specific primer sets to detect and differentiate RPV and PPRV. They observed that RT-PCR was able to detect virus secretion in ocular swabs at four days post infection (PI) in experimentally infected goats, as compared to eight days PI by IcELISA. RT-PCR assay preclude the need for virus isolation and, because of the rapidity with which completely specific results could be obtained, the assay appeared to be the test of choice for PPRV detection. Relative specificity and sensitivity of F-gene based RT-PCR with sandwich-ELISA was 100 and 12.5 percent, respectively [31] .

## 6. Conclusion

The conventional techniques are largely replaced by genome-based detection techniques for the diagnosis and confirmation of PPR virus. Molecular-biological techniques such as RT-PCR and nucleic acid hybridization are now in use. These genome based techniques are largely used because of their high specificity and sensitivity. However one step real-time RT-PCR assays specific for PPRV and loop-mediated isothermal amplification techniques are more sensitive techniques for PPRV detection.

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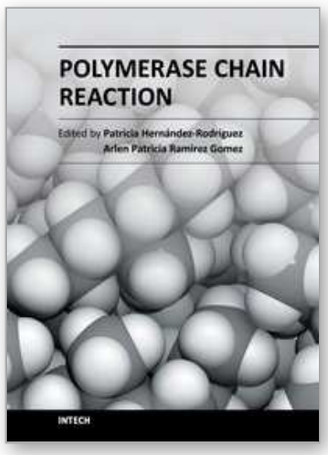


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