Mini Review

Molecular Diagnostic Assays for the Detection of Peste des Petits Ruminants Virus: A Concise Review

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Abstract | Peste des petits ruminants (PPR) is as a transboundary disease whose occurrence hampers the trade and consequently causes an enormous economic losses. It is a disease of sheep and goat, particularly in the regions of Africa, Middle East and Asia. Besides clinical signs and symptoms of PPR various diagnostic assays, both serological and molecular, have been developed and are applied for estimating the current status of the disease. This review article focuses on the development of different molecular diagnostic assays from conventional reverse transcriptase polymerase chain reaction to real time reverse transcriptase polymerase chain reaction and reverse transcriptase loop mediated isothermal amplification over the last decade. These diagnostic assays vary in their sensitivity, specificity, reliability and reproducibility. Although these assays are detecting PPRV with precision but there is need to develop some cost effective diagnostic assays which would be applicable in the field conditions and in the developing countries like Pakistan.

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Introduction

Peste des Petits Ruminants (PPR) is a disease of high economic value which causes extraordinary losses of livestock in the world. It is recognized as a transboundary disease whose occurrence hampers the trade and consequently causes an enormous economic losses. It is a disease of sheep and goat, particularly in the regions of Africa, Middle East and Asia. (Kwiatek et al., 2009). The causal organism, PPR virus (PPRV) is an RNA enveloped virus. It belongs to the genus *Morbillivirus* of family *Paramyxoviridae*under the order *Mononigavirales*. It is a -ve sense single stranded RNA virus. (Balamurugunet al., 2014).

PPRV is inherently classified into four evident lineages (1, 2, 3 and 4) based on the partial order analysis of fusion protein (F) gene. This grouping of PPRV into lineages has extended our interpretation towards molecular epidemiology and worldwide dissemination of PPR viruses. (Kerur et al., 2008). Earlier, the grouping based upon F gene was applied for genetic information and for phylogenetic analysis which was later switched to N gene due to its ability to reproduce improved epidemiological motif (Kwiatek et al., 2007). At present either N gene or both of these genes (F and N) are in use for classification of PPRV strains. Recent research also recommended the use of surface glycoprotein (HN) for epidemiological con-

nection along with F and N gene based analysis (Balamurgan et al., 2010).

PPR is clinically diagnosed by abrasive stomatitis, pyrexia, diarrhea and by oculo-nasal discharges. Morbidity and mortality rates are high both in sheep and goat but sheep experiences less severe clinical disease. (Abubakar et al., 2015). Due to high rate of morbidity and mortality PPR is considered one of the biggest hurdle in subsistence farming. At times, mortality rate may reach upto 90% in a naïve population (Banyard et al., 2010).

Besides clinical signs and symptoms of PPR the development of efficient diagnostic assays (Serological and Molecular Biological) for the virus has contributed enormously in estimating the current status of the disease (Munir et al., 2013). Serological techniques such as Competitive ELISA (c-ELISA) and blocking ELISA (B-ELISA) has been developed on the basis of monoclonal antibodies produced against the HN protein of PPRV (Singh et al., 2004). Different molecular techniques such as RT-PCR, qRT-PCR, multiplex PCR and LAMP has been established to easily recognize the PPRV genome (George et al., 2006).

Reverse transcriptase polymerase Chain reaction (RT-PCR)

RT-PCR is used for detecting nucleic acid of PPRV from clinical samples. It has been applied for the confirmatory and differential diagnosis of PPRV usually by targeting F and N gene of PPRV (Couacy-Hymann et al., 2002). Evaluation of PCR was conducted by designing an experiment on goats in which they were infected with PPRV during a vaccine trial. Ocular samples from the infected animals were detected positive for PPRV through PCR hence it can be used for the detection and characterization of PPRV (Forsyth and Barret, 1995).

The sensitivity of Matrix (M) gene targeted PCR was higher followed by N, F and H gene based PCR respectively. M gene PCR detected virus in sample having 101 TCID50/ml followed by 102, 104 and 105 TCID50/ml respectively (George et al., 2006). Couacy-Hymann et al. (2002) found that RT-PCR assay was almost a thousand times more sensitive than traditional titration methods on Vero cells. They developed this fast and sensitive technique on the basis of NP3/NP4 primers.

RT-PCR was compared with virus isolation for the diagnosis of PPRV and was found many fold more sensitive to detect the virus. The nested PCR was also conducted for the samples found positive by RT-PCR. Although the sensitivity of both assays were similar but nested PCR was found more specific and accurate (Brindha et al., 2001).

A PCR-ELISA was developed for the diagnosis of PPRV by targeting its N gene. This assay can detect PPRV infected cell culture fluid with 0.1 TCID50/ml, so, its sensitivity is 10,000 times more than RT-PCR (Saravanan et al., 2004).Farooq et al., 2008 optimized and validated RT-PCR using phosphoprotein and fusion protein gene limited primer sets to identify and discriminate RPV and PPRV by using conventional and nested PCR. A complete N gene based PCR was developed and standardized which detected 47.06% (16/34) PPR suspected samples (Unpublished data).

Real time RT-PCR

Real time RT-PCR (qRT-PCR) has been used for quantitative analysis and identification of PPRV. Quite recently, qRT-PCR has been developed into the most famous test to detect the viruses of Paramyxoviridae family. This advanced technique of qRT-PCR has dominance on the traditional techniques (Grant et al. 2009). Bao et al. (2008) developed one step real time quantitative reverse transcription PCR (qRT-PCR) based upon TaqMan probe to detect PPRV. He concluded that PPRV identification rate improved from 46.7% to 73.3% by the use of qRT-PCR as compared to traditional RT-PCR.

Among the genes (L, M, N, and P) set to detect PPRV in qRT-PCR L gene lay out provides the highest diagnostic significance as compared to the three other genes. Moreover, the efficiency of qRT-PCR also depends upon the amplicon size as it is inversely proportional to the amplicon size (Dincer and Ozkul, 2015). An M gene real time RT-PCR based upon SYBER Green I was developed for the diagnosis of *Peste des petitsruminants virus* (PPRV). This assay can detect viral RNA in cell culture fluid with 0.0001 TCID50/ml as compared to 1TCID50/ml by conventional RT-PCR. So, this assay was highly specific, sensitive and reproducible for the diagnosis and quantification of PPRV (Abera and Thangavelu, 2014).

Currently, Probe based qRT-PCR and SYBER Green

based qRT-PCR are in practice. The diagnostic sensitivity of probe based qRT-PCR and SYBER Green based qRT-PCR is comparable but the second one is cost effective than the first one (Muthuchelvan et al., 2015).

Loop mediated Isothermal Amplification

Li et al. (2010) established reverse transcriptase loop mediated isothermal amplification (RT-LAMP) assay for the identification of PPRV. They designed a set of six primers on the basis matrix (M) gene sequence of PPRV to amplify viral RNA by incubating it at 63°C for one hour. The lamplicons were observed by the unaided eye. The sensitivity of the assay was found similar to that of qRT-PCR and 10 times more than the conventional RT-PCR. No other assay is applicable in the field as they need thermal cycler and gel electrophoresis equipment for RT-PCR and for qRT-PCR probe or SYBER-Green based assay (Munir et al., 2013).

An N gene RT-LAMP was developed and found to be 100 folds more sensitive as compared with RT-PCR and 1000 times more sensitive as compared to sandwich ELISA (Dadas et al., 2012). The highly sensitive and specific RT-LAMP assay was developed based upon N gene of PPRV. This developed assay is robust, reliable, rapid and applicable molecular diagnostic assay of choice in the field condition. This assay is 10 fold more sensitive as compared with RT-PCR and its sensitivity and specificity is comparable with real time RT-PCR (Ashraf et al., 2017). RT-LAMP developed by Li et al., 2010 was evaluated with field samples. The sensitivity and specificity of the assay was compared with RT-PCR and real time RT-PCR and found that it is many fold sensitive than RT-PCR and its specificity and sensitivity is similar to real time RT-PCR (Unpublished data,).

Conclusion

Reliable and robust diagnostic tools are imperative for the success of any disease control program. Although there are sufficient diagnostic tests are available for the confirmed diagnosis of PPRV but they require sophisticated labs which are difficult to maintain in developing countries like Pakistan. So, need of the hour is to develop some kits or diagnostic assay which would be applicable in the field conditions or which obviate the need of sophisticated equipment like thermal cycler and tube scanner required for the available diagnostic assays.

Authors' Contribution

Aitezaz Ahsan: Wrote initial contents of the article and submitted as corresponding author by the format available on the journal website
Muhammad Usman: Helped in writing up and proof reading
Ihsan Ullah and Adnan Rasheed Malik: Provided the data for the write up
Aamer Bin Zahur: Reviewed the article as peer and suggested different improvements.

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