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RNA Extraction of Peste Des Petits Ruminants Virus (PPRV) from Clinical Samples using Tri-Reagent and Acid Guanidinium Thiocyanate–Phenol–Chloroform Methods

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Abstract

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants such as goats and sheep. It causes economic losses of Rs. 20.5 billion annually in Pakistan. The diagnosis of PPR infection in sheep and goats populations can be synergistically strengthened by detection of antigen in clinical samples of susceptible population. In present study, two RNA extraction methods, i.e., Tri-reagent and Acid guanidinium thiocyanate–phenol–chloroform (AGPC) method were investigated for the PPR virus antigen detection. Both Tri-reagent and Acid guanidinium thiocyanate–phenol–chloroform methods used for RNA extraction of PPRV have equal importance. The RNA of PPRV was extracted from ten tissue samples that were found positive by Immuno-capture ELISA. RNA extracted from these samples was quantified by spectrophotometric analysis. The rapid detection by such suitable and appropriate methods of nucleic acid detection of PPR virus in infected animals will help in early diagnosis of infection and subsequent control of the PPR disease in Pakistan.

Keywords: RNA extraction, Peste des petits ruminants (PPR), Tri-reagent and AGPC methods.

Introduction

Peste des petits ruminants (PPR) is an acute and highly contagious viral disease of small ruminants such as goats and sheep. Abubakar et al., (2008) have reported dramatic consequences with morbidity of 80-90% and mortality between 50 and 80% due to infection of PPR virus in small ruminants. PPR is endemic in Pakistan and has been reported from all the four provinces. In Pakistan, it causes economic losses of Rs. 20.5 billion annually. Clinical findings are fever, anorexia, ocular and nasal discharges, sores in mouth, diarrhea and pneumonia. The main routes of PPR virus (PPRV) transmission are oral and respiratory tracts and oral, nasal and ocular excretions are the key sources (Couacy-Hymann et al., 2009).

PPR virus (PPRV) is classified as a member of the genus Morbillivirus in the family Paramyxoviridae. It has single-strand negative sense RNA genome of ~16kb (15,948 nucleotides) in length that encodes eight proteins including six structural proteins namely: nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), haemagglutinin protein (H) and large polymerase protein (L), and two nonstructural proteins V and C (Chard et al., 2008).

The small ruminants infected with PPR are routinely diagnosed on the basis of clinical examination, gross pathology, histologic findings and laboratory confirmation. A number of serological and molecular diagnostic tests are used for the detection of PPR virus, including isolation on cell culture, agar gel immunodiffusion (AGID), haemagglutination (HA) test, haemagglutination inhibition (HI), competitive enzyme-linked immunosorbent assay (c-ELISA), immunocapture enzyme-linked immunosorbent assay (IC-ELISA) and reverse transcriptase polymerase chain reaction (RT-PCR) (Nussieba et al., 2008; Forsyth and Barrett, 1995; Libeau et al.,1994).

The main objective of the study was to evaluate two RNA extraction methods, i.e., Tri-reagent and Acid guanidinium thiocyanate–phenol–chloroform method for the PPR virus antigen detection.

Materials and Methods

A total of ten different tissues samples (lung, liver, spleen, heart and lymph nodes) were collected from PPR suspected outbreaks in sheep and goats from different locations of Pakistan (Rawalpindi, Faisalabad and Lahore).

These samples were tested for antigen detection of PPRV using Immuno-capture ELISA. IC-ELISA was

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performed using the kits made from BDSL (Pirbright, UK) as described by Libeau et al., (1994). RNA of PPRV was extracted from the positive samples by Tri-reagent and Acid guanidinium thiocyanate–phenol–chloroform **(**AGPC**)** methods.

RNA Extraction

(i) Trireagent Method:

Five samples that were found positive for PPR virus by IC-ELISA were further processed for isolation of RNA by Trireagent (Molecular Research Center Inc.).

(ii) Acid guanidinium thiocyanate–phenol–chloroform (AGPC) Method:

The same five samples were used for isolation of RNA by AGPC method as described by Chomczynski and Sacchi (2006).

RNA Quantification by Spectrophotometry

RNA was quantified by spectrophotometric analysis using the convention that one absorbance unit at 260 nm wavelength equals 40 µg RNA per ml. The ultra violet absorbance was checked at 260 and 280 nm for determination of RNA concentration and purity. Purity of RNA was judged on the basis of optical density ratio at 260:280 nm. The following formula was used to determine RNA concentration of the original sample:

$$\text{Concentration of RNA } (\mu g/\mu l) = \frac{A_{260} \times \text{Dilution factor} \times 40}{1000}$$

Results

The RNA of PPRV was extracted from five samples that were found positive by IC-ELISA, by Trireagent and AGPC methods. Same samples were extracted by Tri-reagent and AGPC methods. RNA extracted from all samples was quantified by spectrophotometric analysis. Purity of the extracted RNA (i.e. ratio 1.7-2.0) was judged on the basis of optical density ratio at 260:280 nm as shown in tables 1 and 2.

Both Tri-reagent and AGPC methods showed equal compassion in RNA extraction although the AGPC is standardized in the laboratory while the Tri-reagent is the commercially standard method. All the samples were then confirmed for the F-gene of PPRV using Polymerase Chain Reaction (PCR) (Forsyth and Barrett, 1995).

Discussion

In this study, the PPRV antigen detection and RNA extraction was investigated and compared in clinical samples of small ruminants. The RNA of PPRV was extracted by Tri-reagent and AGPC methods. Both Tri-reagent and AGPC methods are recommended as initial steps in molecular diagnosis of PPRV in various studies. Farooq et al. (2008) also utilized AGPC method for RNA extraction in a study on molecular based diagnosis of Rinderpest and Peste Des Petits Ruminants Virus in Pakistan.

Balamurugan et al., (2006) utilized Tri-reagent method in a study while using one-step multiplex RT-PCR Assay for the detection of Peste des petits ruminants virus in clinical samples. Results of this study showed that both RNA extraction methods

Table 1 Optical density ratio at 260:280 nm of RNA samples extracted by Tri-reagent method

Sr #	Samples	Dilution factor (500) RNA µg/µl			Dilution factor (200) RNA µg/µl		
		260nm	280nm	260/ 280	260nm	280nm	260/ 280
1.	Sample 1	1.68	1.72	0.97	0.728	0.68	1.07
2.	Sample 2	3.38	2.7	1.25	2.656	1.528	1.73
3.	Sample 3	1.74	1.64	1.04	0.688	0.648	1.06
4.	Sample 4	1.76	1.66	1.06	0.712	0.664	1.07
5.	Sample 5	1.78	1.68	1.05	0.64	0.616	1.03

Table 2 Optical density ratio at 260:280 nm of RNA samples extracted by AGPC method

Sr #	Samples	Dilution factor (500) RNA µg/µl			Dilution factor (200) RNA µg/µl		
		260	280	260/280	260	280	260/280
1.	Sample 1	0.188	1.74	1.08	0.608	0.576	1.05
2.	Sample 2	1.86	1.88	0.98	0.88	0.8	1.1
3.	Sample 3	1.76	1.76	1.00	0.832	0.752	1.106
4.	Sample 4	1.48	1.46	1.01	0.776	0.72	1.07
5.	Sample 5	1.26	1.32	0.95	0.648	0.6	1.08

have equal importance and can be utilized in extraction of RNA of PPRV. In this study we carried out the initial steps for molecular diagnosis of PPRV by Trireagent and AGPC methods and efficiency of both methods was also compared.

IC-ELISA is used as standard since it has the best sensitivity and specificity and can be utilized for samples which are not kept under ideal conditions. RT-PCR is considered the most precise and sensitive technique for diagnosis in clinical samples of PPRV as compared to IC-ELISA, AGID and HA for increased sensitivity and reduced false positivity (Farooq et al., 2008).

The diagnosis of PPR infection in sheep and goats populations can be synergistically strengthened with detection of antigen in clinical samples of susceptible population. Thus rapid detection by suitable and appropriate methods of antigen and nucleic acid detection of PPRV in infected animals will help in early diagnosis of infection and subsequently control of the PPR disease in Pakistan.

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