

Detection and differentiation of sheeppox virus and goatpox virus from clinical samples using 30 kDa RNA polymerase subunit (*RPO30*) gene based PCR

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Abstract

Aim: To detect and differentiate *Capripox virus* (sheeppox virus and goatpox virus) infections by using 30 kDa RNA polymerase subunit (*RPO30*) gene based PCR.

Materials and Methods: Two capripox viruses' viz., sheep pox virus (SPPV) and goatpox virus (GTPV) from clinical samples of different outbreaks were detected and differentiated using capri pox virus (CaPVs) genotyping PCR targeting the CaPV *RPO30* gene. By using the above PCR assay, a total of 54 scab samples from pox disease outbreaks occurred in goats (n=21) and sheep (n=33) were screened.

Results: Out of 54 clinical samples, 43 [17 out of 21 (80.95%) goat scabs and 26 out of 33 sheep (78.78%)] were found positive for capripox virus infection. All positive samples yielded expected amplicon sizes of 172 bp for goatpox virus and 152 bp for sheep pox virus.

Conclusion: The current study demonstrated that *RPO30* gene based PCR assay could be used for molecular epidemiology of capripox virus infection and differentiation of causative agent viz., sheep pox virus and goatpox virus.

Key words: capripox virus, differentiation PCR, goat pox, *RPO30* gene, sheeppox

Introduction

There are many diseases affecting sheep and goats. Among them, sheeppox and goatpox are the two major diseases which are caused by sheeppox virus (SPPV) and goatpox virus (GTPV), respectively. These viruses belong to the genus *Capripoxvirus* of the family *Poxviridae* [1]. *Capripox* is a transboundary disease [1] and was categorized earlier under notifiable diseases by the World Organization for Animal Health [2]. The genus *Capripoxvirus* (CaPV) also includes lumpy skin disease virus (LSDV), which causes lumpy skin disease/nodular dermatitis in cattle. Sheeppox and goatpox exhibit similar clinical signs characterized by pyrexia and pock lesions in the skin and lungs. Sheeppox and goatpox are endemic in Africa, India, North Equator, Middle East countries including Egypt, Turkey, Iraq, Iran, and Afghanistan, and India [1, 3]. In India, sheeppox and goatpox are have been reported from almost all the states [4–6].

The current criterion used for classifying CaPVs within the genus is based upon the animal species from which the viruses are isolated, viz., SPPV from sheep, GTPV from goats and LSDV from cattle [1]. This tends to suggest that CaPVs are strictly host-specific. However, there are several reports indicating the SPPV could infect goats and GTPV could infect sheep [7].

Therefore, this method of classification is inaccurate [1]. Homologous live attenuated sheeppox and goatpox vaccines [8, 9], which are currently in use, offer only partial or no protection under field conditions [8]. This may be due to the use of vaccine strain or field virus with unknown host specificity. Because of these reasons, we are in need of differentiating SPPV and GTPV as well as their host specificity in order to select appropriate vaccine candidate and challenge virus.

As SPPV and GTPV are antigenically closely related, they cannot be differentiated using serological tests [10]. Currently, differentiation of these pathogens using molecular methods such as restriction endonuclease analysis (REA), whole genome sequencing, P32 gene based PCR-RFLP [11], PCR targeting *RPO30* gene [12], and GPCR gene based quantitative PCR [13] are available. Among them, PCR targeting *RPO30* (RNA polymerase 30 kD subunit gene - ORF036) gene is simple, cheap and quick to differentiate SPPV and GTPV. The present study describes the use of *RPO30* gene based PCR for genotype identification of capripox viruses from clinical samples of pox disease outbreaks.

Materials and Methods

Clinical samples: In the present study, 54 scab samples (33 sheep and 21 goats) which were suspected for pox disease were obtained from repository of Pox Laboratory of Division of Virology, Indian Veterinary Research Institute, Mukteswar, Uttarakhand. These samples were previously collected from various parts of the country



Figure-1. *RPO30* gene based PCR assay for differentiation of SPPV and GTPV. Lanes M1-100 bp plus ladder, M2-50 bp ladder, Lanes 1-4 GTPV positive samples, Lanes 5-7 SPPV positive samples, Lane 8 negative control.

between the years 2004 and 2013.

DNA Extraction: Scab samples were triturated and homogenized with 50% phosphate buffered saline and made into 10% suspension. DNA from scab samples was extracted using FNES (fast, non-enzymatic and simple) technique [14] with necessary modification. Briefly, 500 μ L of processed clinical sample was centrifuged at 10,000 \times g for 3 minutes. The supernatant was discarded and 600 μ L of DNA extraction buffer [100 mM Tris pH 8.0, 50 mM EDTA pH 8.0, 500 mM NaCl, 1.5% SDS, and 0.3% β -mercaptoethanol] was added and incubated at 65 $^{\circ}$ C for 10 min. About 200 μ L of 3 M potassium acetate was added and centrifuged at 20,800 \times g for 1 min. The supernatant was transferred to a new tube and equal amount of chilled isopropanol was added and incubated at 4 $^{\circ}$ C for 10 min. The mixture was centrifuged at 17,000 \times g for 5 min; the pellet was washed by adding 500 μ L of 70% ethanol and was centrifuged at 8000 \times g for 5 min. Finally, the DNA pellet was dried and resuspended in 30 μ L of nuclease free water.

Polymerase chain reaction: The *RPO30* gene based PCR to differentiate GTPV from SPPV [12] was used. The PCR primers used in this study were; SpGp RNA Pol F 5'-tctatgtcttgatgtggtgtag-3' and SpGpRNA Pol R 5'-agtgattagtggtgtattttcc-3' (synthesized by IDT, USA). PCR was carried out in a 25 μ L reaction volume in a 200 μ L capacity PCR tube containing 12.5 μ L Maxima Hot Start Green PCR Master Mix (Fermentas, USA), 0.5 μ L of each primer (10 pmol/ μ L), 1 μ L of extracted DNA and 10.5 μ L of nuclease free water. DNA extracted from known SPPV and GTPV was kept as positive control whereas nuclease free water was used in negative control. The amplification was performed in a thermocycler (Eppendorf AG, Hamburg, Germany) after initial denaturation at 95 $^{\circ}$ C for 4 min, followed by 40 cycles of denaturation at 95 $^{\circ}$ C for 30 sec, annealing at 55 $^{\circ}$ C for 30 sec and extension at 72 $^{\circ}$ C for 30 sec and final extension at 72 $^{\circ}$ C for 5 min. The PCR products were electrophoresed on 3% agarose gel for 1 h at 100 V.

Results

The modified FNES method yielded a DNA

concentration ranging from 13 to 17 μ g/mL with OD260/OD280 ratio >1.8. Out of 54 clinical samples, 43 [17 out of 21 (80.95%) goat scabs and 26 out of 33 sheep (78.78%)] were found positive for the capripox virus infection. All positive samples yielded expected product sizes respective to their host origin i.e. 172 bp for goatpox virus and 152 bp for sheeppox virus (Figure-1).

Discussion

Conventional DNA extraction procedures and commercial spin -column-based kits are time consuming and relatively expensive, [14]. One of the simplest methods of DNA extraction from skin is by boiling the tissue samples [15, 16]. Sufficient DNA was recovered for PCR amplification, but sometimes, boiling produced thick elutes [15] and non-specific amplification [16]. Recently, a rapid non-enzymatic procedure for extracting camelpox viral DNA from dried scabs was developed to overcome some of the limitations of the currently available DNA extraction techniques [14]. In the present study, in order to enhance the purity of the DNA, the protocol was modified by inclusion of precipitation and washing of DNA, with isopropanol, and 70% ethanol, respectively.

RPO30 gene of SPPV has a 21-nucleotide deletion in the 5' end compared to GTPV. Primers used in this study bind and amplify the partial *RPO30* gene including the 21 nucleotide deletion. As a result SPPV yields the product size (152 bp) which is lesser than the GTPV product size (172 bp). This genotyping PCR was successfully used for identifying SPPV and GTPV in Indian scenario. The same PCR was also used by Lamien et al., 2011 and Yan et al., 2012 [12,17] for CaPV species identification. Furthermore, similar results were observed with P32 gene based PCR-RFLP [17]. In the present study, all the scab samples tested yielded the product size respective to their host origin.

Earlier, several PCR assays have been developed for the detection of CaPVs [11,16,18,19,20]. However, most of them were confined to detect up to genus level. Apart from *RPO30* gene based PCR [12] few assays such as P32 gene based PCR-RFLP [11, 21], multiplex

PCR based on ORF016 [20], and GPCR gene based real time PCR [12] are currently available to differentiate SPPV and GTPV. These assays have demerits like requirement of RE digestion, multiplexing of primers, and sophisticated equipments. The PCR assay used in the current study using RPO30 gene based differentiation PCR, which is simple, robust and cost effective as SPPV and GTPV can be differentiated in a single PCR.

Conclusion

RPO30 gene based genotyping PCR was found to be robust and reliable to detect and discriminate SPPV and GTPV without the need of labour intensive techniques like gene sequencing and PCR-RFLP. The current study using a limited number of samples indicated that sheepox and goatpox infections were associated with SPPV and GTPV, respectively. This assay in combination with sequencing can be used for molecular epidemiology of capripox virus infection.

Authors' contributions

RS, RY, ABP and MAR implemented the study design. RS and MAR carried out the work part. RS, GV, SBS and MAR did the analysis part and wrote the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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