DETECTION OF SHEEP AND GOAT POX VIRUSES BY POLYMERASE CHAIN REACTION

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ABSTRACT

Sheep pox and goat pox is a highly contagious viral disease of small ruminants and causes significant economic loss. Hence, the present study was undertaken to design PCR assay for the laboratory diagnosis of Sheep pox virus (SPV) and goat pox virus (GPV) from clinical samples.

Thirteen scab samples from sheep and goat collected from suspected sheep pox and goat pox outbreaks reported at different parts of Maharashtra were used in this study. All the 13 scab samples were processed and inoculated through chorioallantoic membrane (CAM) route in embryonated chicken egg. Death of the embryo's was observed between 9th to 11th day post inoculation with sheep pox suspected inoculum, where as death of the embryo's occurred between 6th and 9th day post inoculation with goat pox suspected inoculum showing characteristics pock lesions in the form of grey white necrotic foci on CAM.

The DNA was extracted from all the scab materials by phenol chloroform method with modifications. The purity and quantity of DNA was evaluated by nanodrop. The PCR was standardized for detection of SPV and GPV directly from the DNA isolated from scab material. All the 13 DNA samples of suspected cases were analyzed by PCR assay using published primer B68 and B69 of P32 gene. Of 13 clinical scab materials, 11 (84.62%) scabs showed the expected amplification of 390 bp represented by single band indicated the positivity for sheep pox and goat pox. The present PCR assay has considerable potential as a rapid and accurate diagnostic method for detection of sheep pox and goat pox from clinical samples over conventional techniques and can be employed in the phase of the outbreak for rapid confirmation.

INTRODUCTION

Among the several infectious agents, pox viruses, especially the capripoxviruses are one of the major threats to small ruminants. Sheep pox and goat pox is a highly contagious viral disease of small ruminants and causes significant economic loss. Sheep pox and Goat pox is usually identified on the basis of the clinical signs that constitute skin lesions, the gross pathology and the host species affected. However, it is very difficult to declare a disease of goatpox based solely on clinical signs alone as clinical signs vary and may lead to wrong diagnosis. Finally, laboratory confirmation of the causative agent in the clinical specimens and isolation of the virus will only lead to confirmatory diagnosis of the disease. In India recent outbreaks of capripoxvirus disease occurred in Himachal Pradesh (Verma *et al.* 2011), goat pox virus isolated from outbreak at Akola in Maharashtra (Venkatesan *et al.* 2010) and sheep pox virus outbreak in Tamilnadu (Roy *et al.,* 2008).

Although, many laboratory tests like agar-gel precipitation (Pandey and Singh, 1972), CIE (Sharma *et al.,* 1988), latex agglutination (Rao *et al.* 1997) and spot agglutination (Tiwari *et al.* 1996) have been utilized for diagnosis and confirmation of sheep pox and goat pox, agar gel immunodiffusion and fluorescent antibody methods are difficult to interpret because of the existence of a common antigen between the Capripoxviruses and Parapoxviruses (Kitching *et al*. 1986) and the lack of monoclonal antibodies against a capripoxvirus-specific antigen.

The use of PCR assays for identification of infectious agent has been proved to be a simple, rapid and specific diagnostic method. Several groups have reported using conventional PCR (Heine *et al.,* 1999; Mangana-Vougiouka *et al.* 2000) or real-time PCR (Balinsky *et al.* 2008; Bowden *et al.* 2008) for detection of capripoxvirus genetic material and hence, the present study was undertaken to isolate Sheep and Goat pox virus and design PCR assay for the laboratory diagnosis of Sheep pox virus (SPV) and goat pox virus (GPV) from clinical samples.

MATERIALS AND METHODS

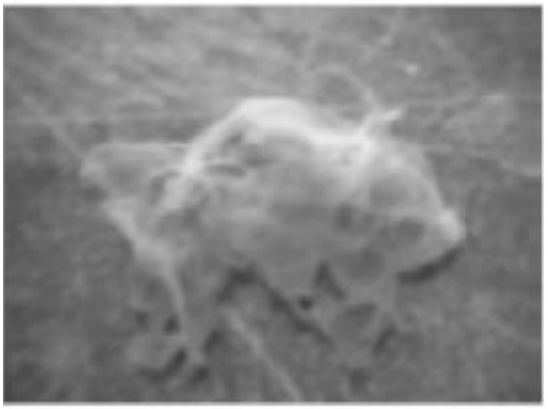
Thirteen scab samples from sheep and goat collected from suspected sheep pox and goat pox outbreaks reported at different parts of Maharashtra were used in this study. All the 13 scab samples i.e, 6 from sheep and 7 from goats were processed and inoculated through chorioallantoic membrane (CAM) route in embryonated chicken egg of 6 to 11 days old.

The DNA was extracted from all the scab materials by phenol chloroform method with modifications. The purity and quantity of DNA was evaluated by NANODROP spectrophotometer (Thermo scientific, USA) instrument. The PCR was standardized for detection of SPV and GPV directly from the DNA isolated from scab material using the following PCR parameters: 94^{0}C for 5mins, followed by 30 cycles of 94^{0}C for 1 min, 53^{0}C for 1 min, 72^{0}C for 1 min and finally at 72^{0}C for 10 min as final extension. All the 13 DNA samples of suspected cases were analyzed by PCR assay using published primer:For-B68-5'CTAAAATTAGAGAGCTATACTTCTT3'and Rev-B69-5'CGATTTCCATAAACTAAAGTG3' of P32 gene (Heine *et al*., 1999).

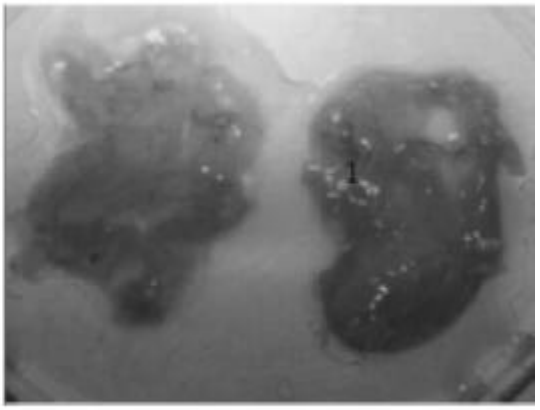
RESULTS AND DISCUSSIONS

Propagation of GPV and SPV in embryonated chicken egg is considered as preliminary test for the detection of Sheep and goat pox viruses as death of the embryo's was observed between 9th to 11th day post inoculation with sheep pox suspected inoculum, whereas death of the embryo's occurred between 6th and 9^{h} day post inoculation with goat pox suspected inoculum showing characteristics pock lesions in the form of grey white necrotic foci on CAM (Fig.1). Similar type of observations was made by Davis and Otema (1975), Soad *et al.* (1996) and Tawfik *et al.* (2001).

Figure. 1: White pock lesions on CAM



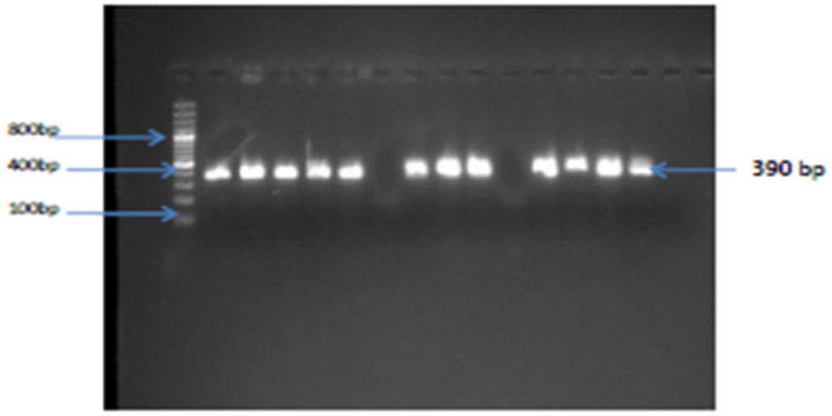
Normal CAM of Eggs



CPE on CAM of Eggs showing white pock Lesions

Further PCR analysis was carried out using the DNA extracted from all the clinical scab samples with OD ranging from 1.76 to 1.82. Out of 13 clinical scab samples, 11 (84.62%) scabs showed the expected amplification of 390 bp represented by single band indicated the positivity for sheep

Figure. 2: PCR amplicons of specific p32 gene of Capripox virus



Lane 1- Molecular marker (100bp)
Lane 2 to 7 – SPV
Lane 8 to 15 – GPV
Lane 16 – Positive control
Lane 6 – Negative SPV
Lane 10 – Negative GPV

pox and goat pox (Fig.2). Two samples did not show the predicted band of 390 bp in gel electrophoresis indicating negativity for sheep pox and goat pox. No amplified product was observed when DNA of negative control was examined in PCR, whereas positive amplified product was observed when DNA of positive control was examined (Fig.2). Similar findings were reported by Ireland and Binepal (1998) with 34.78% positivity out of 23 samples, whereas Mondal *et al. (*2004) and Varshovi *et al.* (2009) observed single amplicons size 390 bp of P32 gene suspected to goatpox and sheep pox infection indicating the PCR assay has tremendous potential for detection of pathogens and mostly used as specific, sensitive and confirmatory diagnosis of the identity of the disease.

CONCLUSIONS

From the present investigation it is concluded that propagation of GPV and SPV in embryonated chicken egg is considered as preliminary test for the detection of sheep and goat pox viruses and the present PCR assay designed based on detection of fusion gene with B68 and B69 primers has considerable potential as a rapid and accurate diagnostic method for detection of sheep pox and goat pox from clinical samples over conventional techniques and can be employed in the phase of the outbreak for rapid confirmation. Further for differentiating GPV from SPV, molecular techniques such as PCR-RFLP of the fusion gene have to be carried out.

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