

CONFIRMATORY DIAGNOSIS OF CONTAGIOUS ECTHYMA BY AMPLIFICATION OF THE GIF / IL-2 GENE BY PCR

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ABSTRACT

A polymerase Chain reaction (PCR) assay for rapid diagnosis of contagious ecthyma (CE) was applied to 55 scab samples obtained from suspected CE outbreaks among sheep and goats. This assay used the CE virus specific primers for GIF/IL-2 gene - a highly conserved gene of parapox genome to diagnose CE and also the assay was evaluated for its sensitivity and specificity so as to be used as a diagnostic tool. This PCR assay was sensitive enough to detect a minimum DNA concentration of 5 ng.

Keywords : Contagious Ecthyma – GIF / IL2 Gene – PCR – Diagnosis

INTRODUCTION

Contagious Ecthyma is a highly contagious, zoonotic, viral disease, which affects sheep, goats and other domesticated and wild ruminants. The disease is caused by the contagious ecthyma virus (CEV), which belongs to the genus Parapoxvirus of the family Poxviridae and is worldwide in distribution (Fenner, 1993). The disease is characterized by proliferative lesions in the lips around the nostrils and in the oral mucosa, which usually resolve in 1-2 months (McKeever *et al.*, 1988). Severe facial and oral lesions in lambs may interfere with suckling. The morbidity of the disease may reach 100% and mortality due to secondary causes may reach 15%. Zoonosis occurs most frequently during lambing, shearing, docking, drenching or slaughtering. Lesions in human proceed from macular through papular to large nodules. The objective of the present study was to employ PCR assay to detect CEV from clinical samples and differentiate it from the closely related sheep and goat pox viruses by amplifying a part of GIF / IL-2 gene of CEV.

MATERIALS AND METHODS

Collection of samples

Fifty five number of scab materials collected from suspected contagious ecthyma outbreaks among the sheep and goat population reported during the period 2004 to August '2008 with the history of papular lesions in oral commissure, muzzle and lower jaw regions were used in this study.

Sample preparation

The field materials were minced using sterile scissors and forceps and then triturated in a sterile pestle and mortar with phosphate buffered saline and made into 10 percent suspension. The mixture was clarified at 1800 rpm for 10 minutes and then transferred to a microcentrifuge tube and subjected to DNA extraction.

DNA extraction by Phenol: Chloroform: Iso-amyl alcohol method

The DNA was extracted from scab materials by using the Phenol: chloroform: iso-amyl

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alcohol method as described by Klein (2004) with slight modifications. The extraction procedure is as follows :

Two hundred microlitre of the suspected scab suspension was taken in a 1.5 ml micro centrifuge tube and twenty microlitre of proteinase K (HiMedia) was added and mixed well. To this, equal volume of Phenol: Chloroform: Iso-amyl alcohol (25:24:1) (Sigma) was added, then gently mixed and centrifuged at 8000 rpm for 2 minutes under refrigerated condition. The upper aqueous phase was transferred to a new micro centrifuge tube and above procedure was repeated. The upper aqueous phase was transferred to a new micro centrifuge tube and equal volume of chloroform (SDL), was added, then gently mixed and centrifuged at 8000 rpm for 2 minutes under refrigerated condition. 3 M Sodium acetate was added to one tenth of the volume of protein free DNA solution (upper aqueous phase) and two volumes of absolute ethanol was added, and kept at -80°C for one hour followed by centrifugation at 13000 rpm for 15 minutes. The DNA pellet was resuspended in 70% ethanol and centrifuged at 13000 rpm for 15 minutes and the DNA pellet was dried till no more ethanol left in the tube and suspended in 50 μl of DNase RNase free water.

Primers and Cycling profile

The extracted DNA was subjected to PCR technique as per the procedure standardized in this laboratory. CEV specific primers GIF/IL-2 gene, the virulence factor found only in Parapox viruses (GIF gene accession number AF192803; Deane *et al.*, 2000) was used in the PCR assay. The primers were synthesized and supplied by Sigma Aldrich Chemicals, India. The primer sequences used for amplification of partial GIF / IL-2 gene were :

Forward primer : 5' gct cta gga aag atg gcg tg-3'
Reverse primer : 5' gta ctc ctg get gaa gag cg 3'

The PCR mix was prepared (DNA template - 2 μl ; Forward primer - 1 μl ; Reverse primer - 1 μl ,

Distilled water - 8.5 μl and Genei red dye Master mix, CAT # kt-78 -12.5 μl) and then subjected to the following PCR cycling conditions in a thermal cycler (Eppendorf Mastercycler). Initial denaturation at 94°C for 5 minutes followed by denaturation at 94°C for 30 seconds annealing at 57°C for 1 minute and extension at 72°C for 1 minute. The 30 cycles were followed by a final extension at 72°C for 10 minutes. The amplified product was electrophoresed on 2% agarose (Himedia) gel in Tris acetate EDTA (TAE) buffer using 8 volts current per cm^2 and viewed with transilluminator.

RESULTS AND DISCUSSION

Out of the 55 samples tested for CEV, 48 scab samples were found positive by PCR showing the predicted PCR amplicon size of 408 bp (Fig. 1). The seven samples that did not show the predicted 408 bp bands were taken as negative for CEV infection.

To evaluate the analytical sensitivity of the PCR, the genomic DNA of the CEV suspected scab samples at different concentrations were amplified. The PCR assay was sensitive enough to detect a minimum DNA concentration of 5 ng total DNA from CE virus from suspected scab samples. The analytical specificity of the PCR reaction was checked using the genomic DNA of both goat pox and sheep pox viruses as known negative control. The negative results with the closely related goat pox and sheep pox viruses showed that the assay is specific and these primer sets can be used routinely for the contagious ecthyma virus diagnosis. Klein and Tryland, (2005) have used PCR to amplify parts of GIF gene with an amplicon size of 408 bp for detection of CE virus in tissue samples and for virus species differentiation. Similarly, many scientists have used the PCR for the detection CEV (Kanou, *et al.*, 2005; Hosamani, *et al.*, 2007).

Since the target selection, primer design and assay optimization was perfect, this PCR assay assured reproducibility and specificity of the results. Hence, this PCR assay using GIF / IL-2 gene primers

system for the diagnosis of contagious ecthyma field outbreaks without using cell culture system or electron microscopy.

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Fig. 1

Agarose gel electrophoresis showing PCR amplicons obtained from CEV suspected scab materials

