A Polymerase Chain Reaction (PCR) Based Diagnostic Assay for Identification of Contagious Ecthyma Virus From Isolates Collected From the North-Eastern State of Tripura

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Abstract - Contagious ecthyma results from infection by the orf virus, a member of the genus Parapox virus belonging to the family Poxviridae. A polymerase chain reaction (PCR) assay for rapid diagnosis of Contagious ecthyma was applied to the 26 scab samples collected from the suspected contagious ecthyma outbreaks among the Black Bengal goats in the north-eastern hilly state of Tripura. The scab materials were collected from popular lesions in oral commissure; lower jaw as well as muzzle. The PCR assay in the present study used the contagious ecthyma virus specific primers for GIF/IL-2 gene (having a size of 408 bp) which is a highly conserved gene of Parapox genome to diagnose Contagious ecthyma. This PCR assay was found to be sensitive enough for detecting a minimum DNA concentration which is as small as 5 nano gram (ng). The reproducibility as well as specificity of the results had been assured by this particular PCR assay.

Keywords:- Contagious ecthyma, GIF/IL-2 gene, PCR, Gel Electrophoresis apparatus.

I. INTRODUCTION

Contagious ecthyma is a highly contagious, zoonic, viral disease, which affects sheep, goats, and other ruminants also. It is an acute as well as both contagious and debilitating disease. This disease is caused by the contagious ecthyma virus which belongs to the genus Parapoxvirus of the family Poxviridae and is worldwide in distribution [1]. The virus is highly resistant to desiccation in the environment. This disease is characterized by proliferative lesions in the lips around the nostrils and in the oral mucosa, which usually resolve in 1-2 months [2]. The disease is more severe in goats than in sheep. People are occasionally affected through direct contact. The mortality due to secondary cause may reach 15%. Zoonosis occurs frequently during lambing, shearing, docking, drenching or slaughtering.

The aim of this study is to detect contagious ecthyma viruses by amplifying a part of GIF/IL-2 gene, collected from the Black Bengal goat samples in North eastern state of Tripura. This particular fragment is of size 408 bp [3].

II. MATERIALS AND METHODS

Collection of samples:- The size of the sample was 26 ranging in age group between 3 month to 5.2 years (Mean=2.4 years), collected from suspected Contagious ecthyma outbreaks among the Black Bengal goats population from West Tripura district during the year 2015. The scab materials were collected from popular lesions in oral commissure; lower jaw as well as muzzle. Out of 26 number of scab samples, 20 samples were found positive by PCR technique. The samples are mainly collected from muzzle and lower jaw part of the affected black Bengal goats.

Sample preparation:- The scab samples are collected by using sterile forceps and then triturated in a sterile pestle and made into 10% suspension. The mixture was clarified at 15000 rpm for 10 minutes and then transferred to a micro centrifuge tube and subjected to DNA extraction.

DNA extraction by Phenol: Chloroform:- Isoamylalcohol method- The DNA was extracted from scab alcohol method as described by Klein [4-5] with slight modifications. The extraction procedure is as follows.

200 µl of sample was mixed with 200 µl of reaction buffer and 20 µl of proteinase K in 1.5 ml of micro centrifuge tube heated in water bath. Then equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added and gently mixed, centrifuge at 8000 rpm for 2 minutes. The upper aqueous phase was transferred to the new tube and add again equal volume of Phenol: Chloroform: Isoamylalcohol (25:24:1), centrifuge at 8000 rpm for 2 minutes. Supernatant phase was transferred to new tube and add equal volume of cold chloroform, gently mixed and centrifuge at 8000 rpm for 2 minutes. 3 M sodium acetate was added to one tenth of the volume of protein free DNA solution and add double volume of absolute ethanol , kept at -80^0C for one hour followed by centrifuge at 13000 rpm for 15 minutes. The DNA pellet was resuspended in 70% ethanol and again centrifuged at

13000 rpm for 15 minutes. DNA pellet was dried till no more ethanol left in the tube. At last DNase RNase free water add into the DNA containing tube and store them at 4^0C.

DNA purity test by Spectrophotometer:- The extracted DNA samples by Phenol: Chloroform: Isoamylalcohol method was subjected to spectrophotometer as per the standard laboratory procedure. The average DNA purity was obtained- 1.74 (260nm/ 280nm) from the extracted DNA samples by Phenol: Chloroform: Isoamylalcohol method.

Primer amplification by PCR method:- The extracted DNA was subjected to PCR as per the procedure standardized in the laboratory. Contagious ecthyma specific primers GIF/IL-2 gene, the virulence factor found only in Parapox viruses [6] was used in PCR assay. The primer sequences used for amplification of GIF/IL-2 gene were:

Forward primer: 5´-GCTCTAGGAAAGATGGCGTG-3´

Reverse primer: 5´-GTACTCCTGGCTGAAGAGCG-3´

The PCR mix (25 µl) was prepared with DNA template and then subjected to the PCR cycling in following steps. Initial denaturation at 94^0C for 5 minutes, denaturation at 94^0C for 30 seconds, annealing at 60^0C for 1 minute and extension at 72^0C for 10 minutes. The PCR cycling amplified DNA product was electrophoresed on 2% agarose gel.

III. RESULTS AND DISCUSSION

The twenty suspected scab samples age range (Figure-1) were positive by PCR showing. The isolated DNA samples were electrophoresed on 0.8% agarose gel. To evaluate the analytical sensitivity of the PCR, The genomic DNA of the Contagious ecthyma virus 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 Contagious ecthyma virus from suspected scab samples. Many scientists have used the PCR for the detection of Contagious ecthyma virus [7-8]. The PCR cycling amplified DNA product was electrophoresed on 2% agarose gel (Figure-2) for the detection of Contagious ecthyma virus by a specific primers for GIF/IL-2 gene [9].

Since the target selection, primer design and assay of optimization was perfect, this PCR assay assured reproducibility as well as specificity of the results [3, 10].

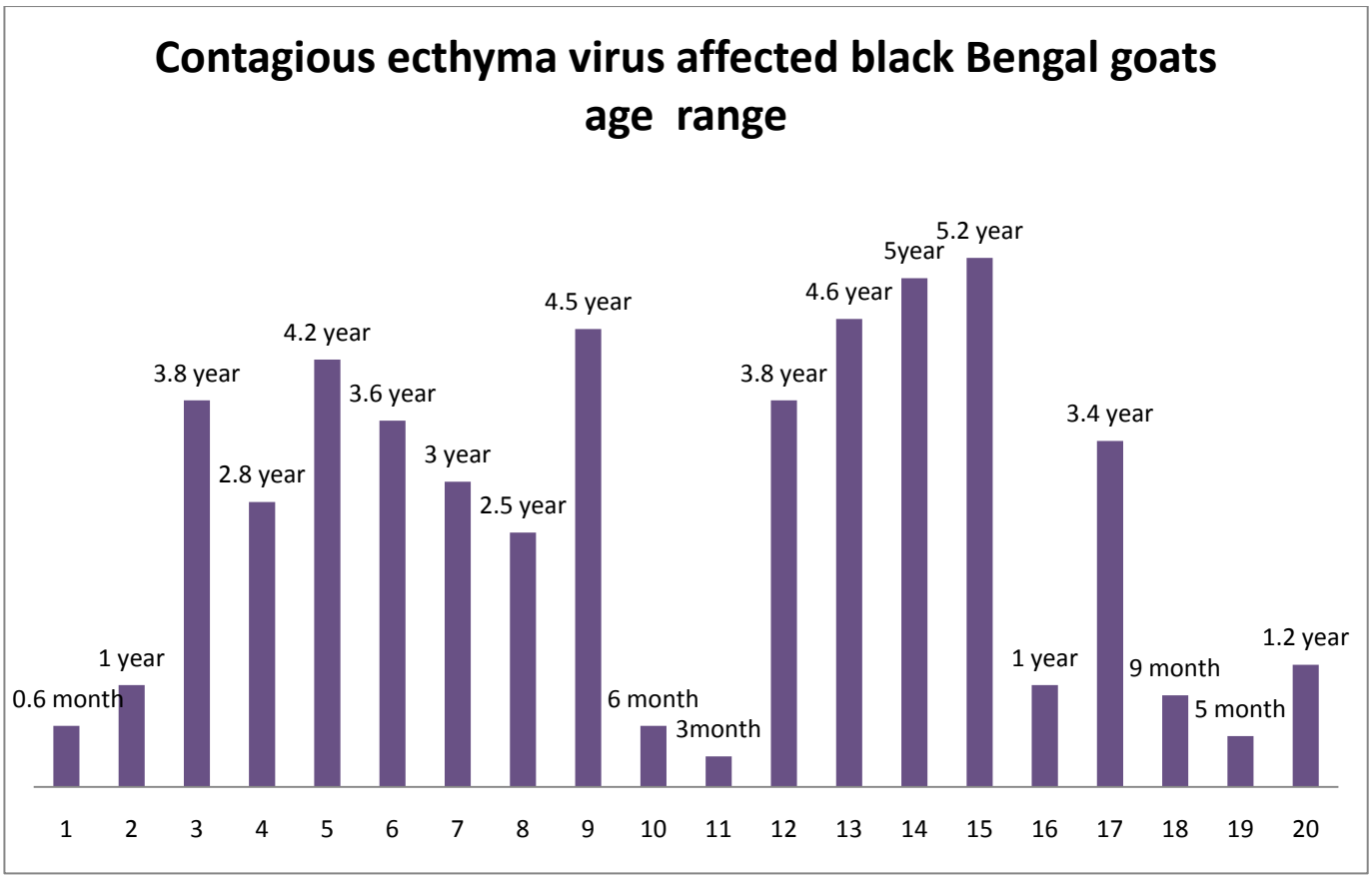

Figure 3.1. Age range (Mean=2.4 years) of Black Bengal goats

408 bp

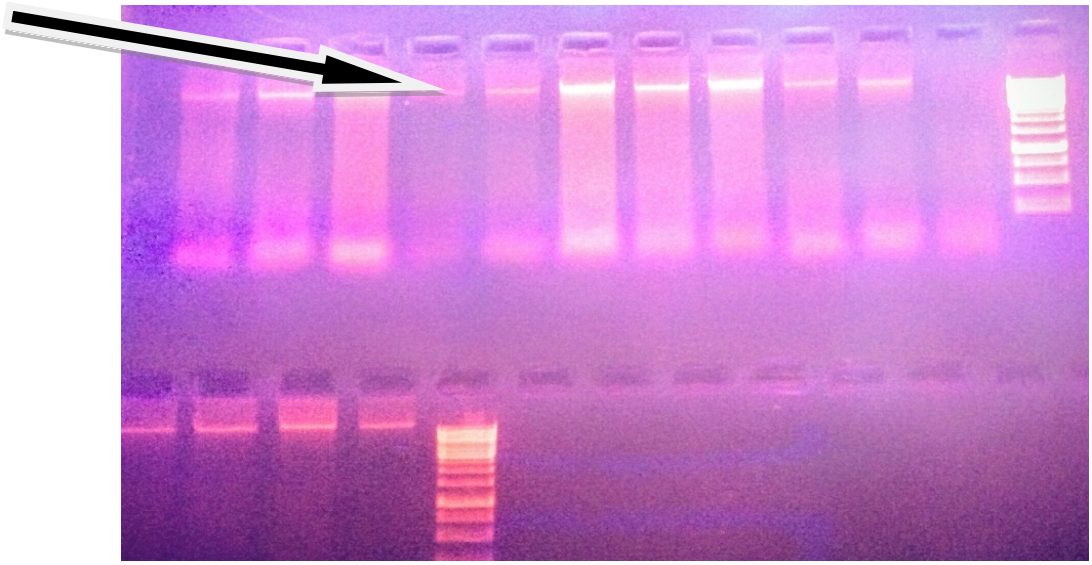

Figure 3.2. PCR Amplified GIF/IL-2 gene (408 bp) on 2% Agarose gel

IV. CONCLUSION

The expression of the PCR amplification study by GIF/IL-2 gene a highly conserved gene of Parapox genome to diagnose Contagious ecthyma. The results from this study would be used to help developing diagnostic and treatment strategies that are based on the risk factors of the individual patient among the population of the Black Bengal goats. DNA amplification by PCR technique using specific primer is a widely used technique for different type of disease diagnosis and it is reliable technique in the modern day medicinal field.

V. ACKNOWLEDGEMENT

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