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Detection of Orf virus causing contagious ecthyma in goats by PCR

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Abstract
Contagious ecthyma is an acute, highly contagious, debilitating and economically important viral skin disease, commonly occurring in sheep and goats. Contagious ecthyma is light to moderate and self-limiting disease, but its economic impact can be very significant. The disease is zoonotic in nature. In the present study, during the period from June-2021 to December-2021, a total of 30 samples of affected goats were collected. The different genes like B2L, VIR, GIF, viral RNA polymerase and A32L were targeted for molecular detection of ORFV. Molecular detection of ORFV by targeting B2L gene revealed 100% (30/30) positive result by semi-nested PCR. The positivity of ORFV by targeting VIR, RNA polymerase and A32L was 93.33% (28/30). A total 29 (96.67%) goats' scab samples turned positive, while only one (3.33%) sample from Goat 5 was found negative in GIF gene based PCR.

Keywords: Contagious ecthyma, Orf virus, PCR, goat

1. Introduction
T The goat is one of the most reliable economic sources of revenue for small and marginal farmers in India. The major goat diseases are peste des petits of ruminant, goat pox, contagious ecthyma, enterotoxaemia, tetanus, brucellosis, mastitis, contagious caprine pleuropneumonia. The different terms used to describe the contagious ecthyma are orf, contagious pustular dermatitis, infectious labial dermatitis, scabby mouth and sore mouth. Contagious ecthyma is an acute, highly contagious, debilitating, zoonotic and economically important viral skin disease, commonly occurs in sheep and goat (De la Concha-Bermejillo *et al.*, 2003) [1]. The contagious ecthyma is characterised by proliferative lesions on the lips and mouth, which progresses from erythematous macule, papule, vesicle, pustule to scab formation in 4–6 weeks and young animals are at high risk (Haig and McInnes, 2002) [2] and normally disappear in 1–2 months (McKeever *et al.*, 1988) [3]. The morbidity of disease can be as high as 100% and subsequent bacterial infections can cause deaths in approximately 15% cases (Gumbrell and McGregor, 1997) [4].

Contagious ecthyma is caused by dsDNA virus species Orf virus which belongs to genus *Parapoxvirus* and family *Poxviridae*. The members of the genus *Parapoxvirus* have an oval shape (250-300 nm in length and 160-190 nm in diameter) and are covered with long thread-like surface tubules that appear to be arranged in criss-crossed pattern, resembling a ball of yarn. Virions are made up of a dumbbell-shaped core and two lateral bodies surrounded by one or two lipid membranes (Maclachlan and Dubovi, 2010) [5]. Orf virus (ORFV) is a large enveloped virus that replicates in the cytoplasm of the infected host cell. The ORFV has a double-stranded DNA genome (134–139 kbp). The PCR technique has been shown to be quicker and more effective than other tests in diagnosing ORFV infection. Multiplex PCR and PCR targeting major enveloped protein (B2L) gene or virus interferon resistance gene (VIR) gene has been employed to diagnose the parapoxvirus infections (Inoshima *et al.*, 2000; Torfason and Gunadottir, 2002) [6, 7]. The ORFV specific primers targeting a highly conserved granulocyte monocyte colony-stimulating factor and interleukin-2 inhibiting factor (GIF) and A32L gene were shown to be sensitive for rapid diagnosis (Chan *et al.*, 2009) [8]. Semi-nested PCR using the B2L gene has been shown to detect low copy number viral particles in clinical samples.

Considering the importance of contagious ecthyma in goats and paucity of literature on molecular characterization of Orf virus particularly in Gujarat the research work was carried out for molecular detection of Orf virus by targeting various genes.

2. Material and Method

2.1 Collection of Samples

The disease contagious ecthyma was categorized based on the location of lesions on the body of the goat i.e., labial form, genital form and generalized form. A total of 30 scab samples from 30 affected non-descript goats having labial form were collected aseptically in sterile 5 ml microcentrifuge tubes during period from July-2021 to December-2021. Scab samples were stored at -20 ℃ till DNA extraction for detection of virus. The detail information of samples are given in the Table 1. DNA from the scab samples was extracted using QIAamp® DNA Mini kit from following the manufacturer's instructions with some modifications. The NanoDrop spectrophotometer was used to determine the quantity and quality of extracted DNA.

2.2 Molecular detection of Orf virus by targeting different Genes

The ORFV was detected from the extracted DNA using PCR by targeting 5 different genes. A total of 30 scab samples were checked for presence of 5 different genes namely, Major envelope protein (B2L) gene (Inoshima *et al.*, 2000) [6], *RNA polymerase gene* (Torfason and Guonadottir, 2002) [7], granulocyte monocyte - colony stimulating factor /interleukin-2 inhibition factor (GIF) gene (Klein *et al*., 2005) [9], viral interferon resistance (VIR) gene (Guo *et al*., 2003) [10] and viral ATPase protein (A32L) gene (Gelay *et al*., 2016) [11]. The sequences of primers and their product size are according to reference given. The PCR conditions for the reactions is given in Table 2.

3. Results and Discussion

The B2L gene encodes a 45kDa major envelope protein which is an immunogenic in nature and elicits the significant antibody response in the host. The B2L gene is highly conserved and has been targeted for detection of ORFV through PCRs. All the 30 goats' scab samples were screened for presence of B2L gene by semi-nested PCR method. All the goats' samples (100%) were found positive by semi-nested PCR. The first PCR reaction was performed using PPP1 and PPP4 primer set, then second semi-nested PCR reaction (Figure 1) was performed using PPP3 and PPP4 primer set. Out of all 30 samples only two goats' scab samples (Goat 5 and Goat 6) were unable to produce a desired amplicon size of 594 bp in the first PCR reaction. This could be due to some sequence heterogenicity in complementary primer binding sites or low amplification of gene which couldn't be detected in agarose gel electrophoresis. However, those two samples turned positive in the second semi-nested PCR reaction and yielded a 235 bp long amplicon. The positive result found in the present study was in agreement with the findings of Zeedan *et al.* (2015) [12], Venkatesan *et al.* (2018) [13] and Sahu *et al.* (2019) [14]. The other scientists Bora *et al.* (2015) [15] and Babu *et al.* (2018) [16] reported 87.5%, 76.92% and 60% positive results, respectively.

The virus interferon resistance protein encoded by *VIR* gene, binds with dsRNA and prevent the activation of protein kinase R and thus inhibits the host IFN-shutdown of protein translation. A total of 28 (93.33%) goats' scab samples produced an expected product size of 555 bp (Figure 2). In the present study, positive results recorded was nearly similar to Kottaridi *et al*. (2006) [17], who recorded 87.5% (7/8) samples positive, while De la Concha-Bermejillo *et al.* (2003) [1] and Sahu *et al*. (2019) [14] reported 100% positive results.

The viral RNA polymerase gene regulates the replication and transcription of viral genome. In the case of a positive sample, a desired product size of 140 (Figure 3) was produced. Out of 30 samples subjected for detection of *viral RNA polymerase* gene, 28 (93.33%) goats' scab samples were found positive through PCR. Similar type of result was found by Torfason and Guonadottir (2002) [7], in which they got 95.65% (22/23) samples of humans and sheep positive, while Gelaye *et al.* (2016) [11] reported 75% (18/24) positive result.

The *GIF* gene product inhibits the biological activity of GM-CSF an IL-2. The GIF gene based PCR was performed for all the samples of 30 goats. The desired amplicon size of 408 bp (Figure 4) was observed in a positive sample. A total 29 (96.67%) goats' scab samples turned positive, in *GIF* gene based PCR. The result of the study was nearly similar with the study of Hosamani *et al*. (2007) [18], who got 100% (10/10) positive results from contagious ecthyma outbreak in North Indian goats.

The A32L encodes an ATPase protein which involves in viral DNA packaging. A total of 28 (93.33%) goats' samples produced an expected product size of 1096 bp (Figure 5) and two (6.67%) goats' samples were found negative in PCR. The results were similar to Gelaye *et al.* (2016) [11] and Sahu *et al.* (2019) [14] reported 100% positive results.

Table 1: Details of Samples collected from goats and PCR result

	B2L (1st)	B2L (2nd)	VIR	RNA polymerase	GIF	A32L
Total positive goats	28	30	28	28	29	28
Percentage of Positive Results (%)	93.33	100	93.33	93.33	96.67	93.33

Table 2: Steps and conditions of thermal cycling for primer pair used in PCR

Primers (Forward and Reverse)	Cycling conditions				
	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
PPP1 PPP4	95 °C 7 min	95 °C 60 s	55 °C 60 s	72 °C 60 s	72 °C 5 min
		Repeated for 30 cycles			
PPP3 PPP4	95 °C 5 min	95 °C 60 s	55 °C 60 s	72 °C 60 s	72 °C 5 min
		Repeated for 25 cycles			
ORF1 ORF2	95 °C 5 min	95 °C 45 s	63 °C 30 s	72 °C 30 s	72 °C 5 min
		Repeated for 25 cycles			
VIR1 VIR2	95 °C 5 min	95 °C 60 s	55 °C 45 s	72 °C 60 s	72 °C 5 min

		Repeated for 25 cycles			
GIF5 GIF6	95 °C 5 min	95 °C 30 s	58 °C 30 s	72 °C 45 s	72 °C 5 min
		Repeated for 25 cycles			
A32LF A32LR	95 °C 5 min	95 °C 50 s	57 °C 45 s	72 °C 60 s	72 °C 7 min
		Repeated for 35 cycles			

Fig 1: Agarose gel showing amplified product of B2L gene of ORFV in semi-nested PCR reaction (approximately 235 bp)

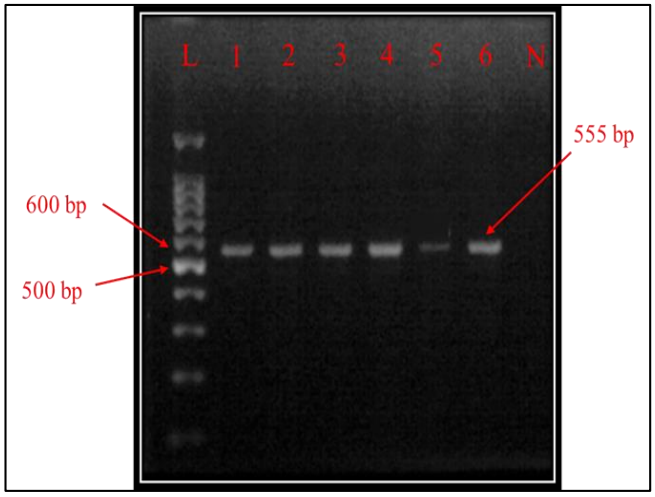

Fig 2: Agarose gel showing amplified product of *VIR* gene of ORFV in semi-nested PCR reaction (approximately 555 bp)

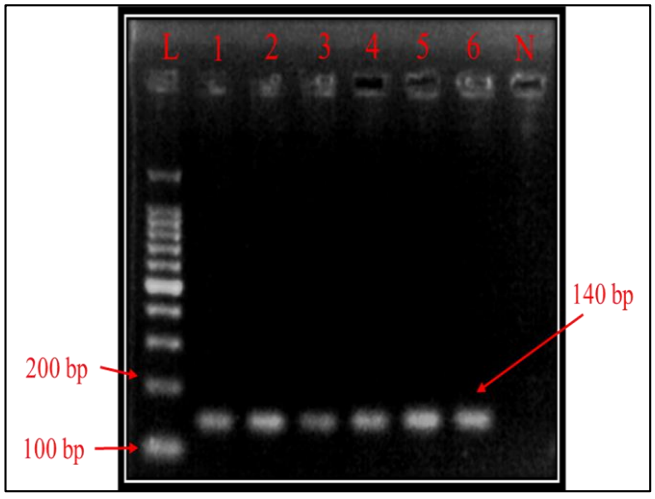

Fig 3: Agarose gel showing amplified product of *viral RNA polymerase* gene of ORFV (approximately 140 bp)

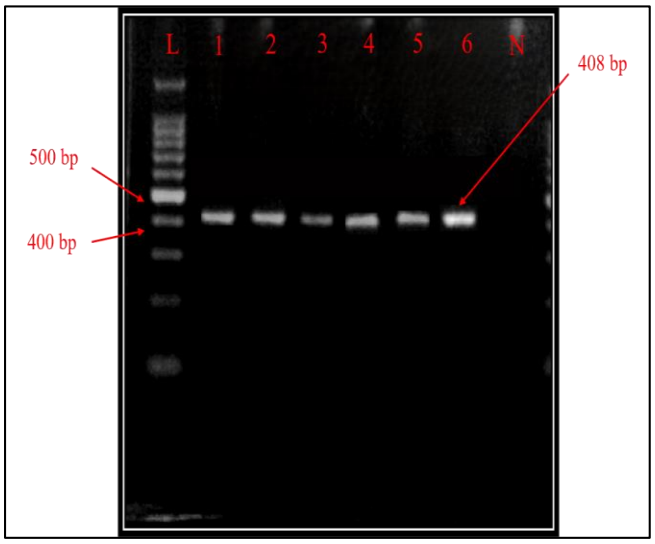

Fig 4: Agarose gel showing amplified product of *GIF* gene of ORFV (approximately 408 bp)

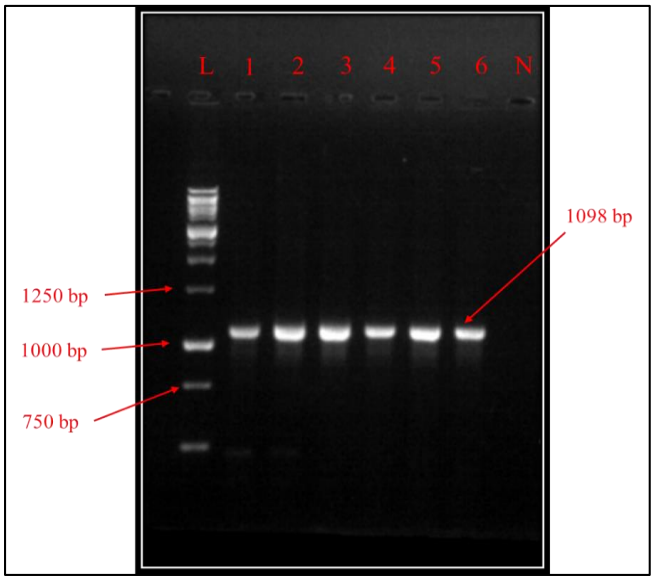

Fig 5: Agarose gel showing amplified product of A32L gene of ORFV (approximately 1098 bp)

4. Conclusions

Overall, scab samples only form Goat 5 and Goat 6 were negative in VIR, viral RNA polymerase, GIF and A32L gene based PCR. However, both the goats were clinically affected with contagious ecthyma. There might be low number of virus in scab samples which couldn't be detected by PCR. The semi-nested PCR targeting B2L gene is highly specific and sensitive which can even detect low copy number of viruses.

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