

Original Research Article

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Molecular Confirmation of Contagious Ecthyma Among Goats in Dharmapuri District of Tamil Nadu, India

P.V. Sowmiya^{1*}, K. Ramya¹, K. Sukmar¹ and R. Madheswaran²

¹Department of Veterinary Microbiology, ²Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal - 637 001, Tamil Nadu, India

*Corresponding author

ABSTRACT

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Contagious ecthyma is one of the most important viral diseases of goats with zoonotic implications and causes economic losses to the farmers. The present study was undertaken to investigate the Contagious ecthyma infections in goats during the month of September and October 2017 in different places of Dharmapuri district. The goats were affected irrespective of their age group and sex. Affected goats had pyrexia, anorexia, nasal discharge and proliferative scab lesions around the mouth suggestive of Contagious ecthyma. The disease was confirmed by polymerase chain reaction targeting B2L gene. It is also screened for *Pestedes petiti* ruminants by polymerase chain reaction targeting N-gene. Affected animals were treated with antibiotic, anti-inflammatory and vitamin B complex injections parenterally. Topically glycerin and boric acid paste or neem oil and turmeric paste was applied to alleviate the lesions. Affected animals were recovered in 2-3 weeks.

Introduction

Contagious ecthyma is a viral disease primarily of sheep and goats caused by *Parapoxvirus* belonging to family *Poxviridae*. The disease has been considered benign but it will take serious course if secondary infections with other viruses and bacteria which increase the mortality rate from 10 to 20 percent. The disease has a significant economic impact on rural communities where the marginal farmers are predominantly dependent upon livestock farming as a source of livelihood. The synonyms of disease used in various outbreaks

included contagious pustular stomatitis, *Contagious ecthyma*, ecthyma contagiosum, infectious pustular dermatitis, infectious labial dermatitis, sore mouth, scabby mouth and Orf (De Wet and Murie, 2011). The etiology of *Contagious ecthyma* disease is type species of the genus *Parapoxvirus* of subfamily *Chordopoxvirinae* of family *Poxviridae* (Nandi *et al.*, 2011; ICTV, 2014). It is an epitheliotropic *parapoxvirus* that induces proliferative lesions in the skin of sheep, goats, wild ruminants, humans, and very rarely, dogs (Bassioukas *et al.*, 1993; Haig and Mercer, 1998). The virus is ellipsoidal in

shape (Ishii *et al.*, 1953). The virus in dried scabs were viable from eight months to more than a year at room temperature (Manley, 1934; Newsom and Cross, 1934), but crusts exposed to external conditions lost their infectivity rapidly during summer (Manley, 1934; Aptow, 2010). However, during winter infectivity persisted for at least six months (Boughton and Hardy, 1935).

In sheep and goats, *Contagious ecthyma* is recognized by the appearance of vesicles, papules and crusty, rapidly growing scabs in the skin of lips and nose of affected animals. In more severe cases, the skin of other areas, such as the eyes, feet, vulva, or udder, may be affected (De la Concha-Bermejillo *et al.*, 1999). The disease usually runs its course in 3-4 weeks. However, prolonged infections and secondary bacterial infections or myiasis of affected areas may occur (Abu and Housawi, 1997; Greig *et al.*, 1984; Zamri-Saad *et al.*, 1993). Occasionally, mortality as high as 10 per cent have been reported (Gumbrell and McGregor, 1997). The differential diagnosis of *Contagious ecthyma* from similar vesicular diseases like Pestedes petits ruminants (PPR), blue tongue, foot and mouth disease and other vesicular diseases is perplexed by the similar clinical signs. Hence, the prompt diagnosis of the disease will help to contain the spread of infections within and the nearby herds.

The B2L gene encodes for a highly immunogenic major envelope protein and able to induce both antibody and cell-mediated immune responses. This gene has been used for detection of *Contagious ecthyma* virus by PCR (Inoshima *et al.*, 2000).

Primers for amplification of N- gene of PPR virus genome with 351 bp fragment was used for detection of PPR virus. The present study was undertaken to confirm the etiology of the vesicular disease, *Contagious ecthyma* in sheep and goats.

Materials and Methods

Collection of samples

Ten number of scab samples were collected from *Contagious ecthyma* suspected goat in different places of Dharmapuri district during the month of September and October with the history of pyrexia, anorexia, proliferative scab lesions around the month. The scab samples were collected in a sterile container and stored at 4 °C.

Sample preparation

The collected scab samples were triturated in a sterile mortar and pestle with sterile normal saline and made into 10 per cent tissue suspension. The mixture was centrifuged at 1800 rpm for 10 min. The supernatant was transferred into a sterile microcentrifuge tube and subjected for DNA extraction.

DNA extraction

The DNA was extracted from the collected scab materials by Phenol: Chloroform: Isoamyl alcohol method as described by Klein (2004) with slight modifications. The extraction procedure is as follows:

Five hundred microlitre of tissue suspension was taken in a sterile microcentrifuge tube and five hundred microlitre of Phenol: Chloroform: Isoamyl alcohol was added to the tissue suspension. The mixture was centrifuged at 4000rpm for five minutes. The supernatant (aqueous phase) was collected (approximately 300 µl) in a new microcentrifuge tube and equal volume of chloroform was added and gently mixed and centrifuged at 4000rpm for five minutes. The supernatant was collected in another microcentrifuge tube and two volumes of ice cold ethanol were added. The mixture was centrifuged at 12,000 rpm for ten minutes. The

upper phase ethanol was discarded. Five hundred microlitre of 70 per cent ethanol was added to the precipitate and centrifuged at 14000rpm for 10 min. The supernatant was removed and the pellet was air dried. The pellet was resuspended in 50 µl of Tris EDTA (TE) buffer.

Polymerase Chain Reaction

The extracted DNA was subjected to PCR for molecular conformation. For the molecular confirmation, DNA extracts from tissue suspension were used.

The PCR mixture was prepared in total volume of 20 µl containing 1 µl DNA template, 1 µl forward primer, 1 µl reverse primer, 7 µl nuclease free water and 10 µl amplicon master mix.

The B2L gene approximately 592bp was amplified according to the methods of Inoshima *et al.*, (2001) using partial B2L gene specific primers:

Forward: 5'-GTCGTCCACGATGAGCAGCT-3'

Reverse: 5'-TACGTGGGAAGCGCCTCGCT-3'

The cycling conditions for the partial B2L gene were: initial denaturation at 95°C for 5 minutes, followed by 35 cycles at 95°C for 50 seconds, 56°C for 60 seconds and 72°C for 90seconds, and final extension at 72°C for 7 minutes.

The extracted DNA was also screened for PPR to rule out mixed infections and to differentiate the vesicular diseases. N-gene of PPR virus was targeted to amplify 351bp fragment from the genome using the following primers (Jhala *et al.*, 2009; Couacy Hymann *et al.*, 2002).

Forward primer: 5'-TCTCGGAAATCGCCTCACAGACTG-3'

Reverse primer: 5'-CCTCCTCTGGTCTCCAGAATCT-3'.

The thermal cycling conditions were initial denaturation at 94°C for 4 minutes followed by denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 30 seconds. The 35 cycles were followed by a final extension at 72°C for 10 min.

The amplified products were electrophoresed on 1.5 per cent agarose (HiMedia) gel in Tris acetate EDTA (TAE) buffer and visualized in the UV transilluminator.

Results and Discussion

In the present study, the clinical signs in all the cases reported in different herds from Dharmapuri district of Tamil Nadu were typically descriptive of *Contagious ecthyma* viz., lesions ranged from nodular lesions on the muzzle to diffuse proliferative scabs, dry crusts with heavy vascularization leading to inanition (Fig. 1).

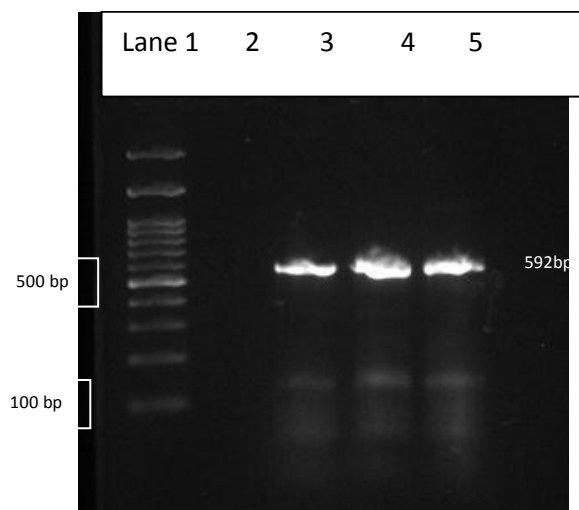
They were reported to be anorexic, hyperthermic, dull and depressed. The scabs were often friable and bled easily. The lesions around the mouth were severe in lactating kids feeding on the infected dam. Moreover, the dams had proliferative lesions on their teat and udder due to the infection contracted while feeding by the ailing kids. The occurrence of the disease was reported to be high immediately after the onset of monsoon in the region. The morbidity rate of the disease in the infected herds was 80 per cent and there were no mortality reported in that area.

DNA could be positively extracted from all the clinical samples.

Fig.1 Affected goat showing typical proliferative scabs and crusts around the mouth and nostrils



Fig.2 PCR amplification of B2L gene (592 bp) of ORF virus using specific primers. Lane-1: 100 bp DNA ladder; Lane-2: Non template control; Lane- 3-5 positive samples from Dharmapuri district of Tamil Nadu



All the ten scab samples collected during the outbreak were subjected to PCR for B2L gene specific for *Contagious ecthyma* virus.

The likely product of 592 bp fragment of B2L gene of *Contagious ecthyma* virus could be successfully amplified by PCR from all the samples collected in Dharmapuri district. (Fig. 2). The PCR targeting N gene of PPR virus confirmed that all the samples were negative for PPR from the affected flocks.

The affected animals recovered after ten days of continuous treatment with antibiotics to prevent secondary bacterial infection and palliative care with boroglycerine paste (Ali *et al.*, 2013).

Orf infection is one among the vesicular diseases universally encountered in sheep and goats. The impact of the disease is considered less significant, since it is a self-limiting disease, though it causes high mortality in

young lambs and adult animals due to secondary bacterial infection. In the present study, the disease was reported in more number of goats and less in sheep. Goats are generally more susceptible and develop more severe cases of *Contagious ecthyma* than sheep (Nandi *et al.*, 2011).

The conventional method for diagnosis of *Contagious ecthyma* by clinical signs and pathological lesions are always confounded by other diseases with similar clinical signs like, pox, PPR, Foot and Mouth Disease (FMD) and vesicular stomatitis etc., Virus isolation from the infected animals is regarded as the confirmatory test for the diagnosis of *Contagious ecthyma* which is laborious and time consuming (Chan *et al.*, 2007). With advent of molecular biology, PCR technique is being widely used to amplify the desired genomic fragments from tissue specimens of affected animals and it has become a powerful tool in molecular diagnosis (Inoshima *et al.*, 2000). Recently, PCR targeting B2L gene has been developed for confirmatory diagnosis in sheep. In the present study, the PCR technique could amplify the desired gene product as low as 16ng/µl. The efficacy of PCR was comparable to the cell culture/neutralization assays (Zheng *et al.*, 2007).

The analytical specificity of the PCR reaction was checked to rule out the presence of closely related disease like PPR in all the ten clinical samples. The samples were negative for PPR in PCR techniques. The clinical picture together with the molecular confirmation by PCR assured that the outbreak was due to *Contagious ecthyma* virus infection. Hence, the PCR assay is proved to be very rapid, accurate and cost effective for the diagnosis of *Contagious ecthyma* without using the cell culture system or electron microscopy during field outbreak.

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