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Molecular identification of contagious ecthyma in Kanni and Kodi goats breeds of Virudhunagar district, Tamil Nadu

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Abstract

Goat farming plays a pivotal role in income generation for rural households in Virudhunagar district, providing a reliable source of livelihood and economic stability for many families. However, the present study highlights a potential threat to this vital sector, as it investigated and confirmed the outbreak of contagious ecthyma (orf) in Kanni and Kodi goat breeds in the region. The outbreak was reported from six different blocks of Virudhunagar District, Tamil Nadu. A total of 139 clinical cases of contagious ecthyma were reported during various outbreaks from 2022 to 2023. The results confirmed the outbreak of contagious ecthyma virus through PCR assay. This assay employed CE virus-specific primers for the GIF/IL-2 gene, a highly conserved region of the parapoxvirus genome, to diagnose contagious ecthyma (CE) virus in the kanni and kodi goat breeds of Virudhunagar district.

Keywords: Virudhunagar district, contagious ecthyma, Kanni and Kodi

Introduction

Orf, also known as contagious ecthyma (CE), contagious pustular dermatitis (CPD), or ovine pustular dermatitis (OPD), is a specific dermal infection affecting sheep and goats. It is caused by a Parapoxvirus, either the contagious pustular dermatitis virus (CPDV) or orf virus (ORFV), which belongs to the subfamily Chordopoxvirinae in the family Poxviridae (Matthews 1979) [15]. This non-systemic, eruptive skin disease has significance for public health. The classical clinical presentation typically features scabby lesions around the mouth. (Gameel *et al.*, 1995; Robinson, 1982) [7, 19].

The disease is widespread globally, leading to high morbidity (80%) and low mortality (5-10%), and results in significant economic losses for sheep and goat farming. (Gokce *et al.*, 2005; Vikoren *et al.*, 2008) [8, 24].

This disease is enzootic in India, resulting in high morbidity among sheep and goats. Although it is relatively easy to diagnose, the lesions and symptoms can be confused with those of other skin diseases, necessitating laboratory confirmation. (Venkatesan *et al.*, 2012) [23]. The disease usually appears in a mild form, but its global significance lies in its ability to cause repeated infections, high morbidity in adults, mortality in young lambs and kids, its zoonotic nature, and its potential to cross-infect other species

The disease poses a zoonotic risk and can induce contagious pustular dermatitis in humans (Tryland *et al.*, 2013) [22]. Mostly people who work closely with animals, such as farmers, shepherds, veterinarians, livestock managers, and others who frequently handle or come into contact with animals, are at a higher risk. (Centre for Food Security and Public Health, 2007) [4]. The orf outbreaks have been documented in several Indian states, including Uttarakhand (Venkatesan *et al.*, 2011) [23], Assam, Meghalaya (Bora *et al.*, 2012) [3], Haryana (Kumar *et al.*, 2013) [12], Uttar Pradesh (Kumar *et al.*, 2014) [13], Rajasthan (Maan *et al.*, 2014) [14], Tripura (Venkatesan *et al.*, 2018) [23], Kashmir (Ahanger *et al.*, 2018) [1], Tamil Nadu (Nagarajan *et al.*, 2019) [16], and Odisha (Sahu *et al.*, 2022) [20]. This study describes an outbreak of contagious ecthyma among Kanni and Kodi goats at Virudhunagar Districts of Tamil Nadu.

Materials and Methods

The outbreak occurred during the year of 2022-2023 in Virudhunagar district native goat breed of Kanni and kodi in different blocks viz., Virudhunagr (18), Arupukottai (22), Sattur (26), Sivakasi (27), Kariyapatti (29) and Narikudi (17). Goats of all ages were affected and exhibited the typical lesions of contagious ecthyma (Fig.1-3). A total of 16 outbreaks were reported wherein 139 goats were affected and showed the typical clinical signs of contagious ecthyma. The disease mainly observed during the drier period of the year mostly in the month of August, September, October and February. Highest cases were recorded in in the month of August. The clinical signs observed mainly progressive erythematous swelling followed by formation papule and then scab in and around mouth and gum (Fig.3).

Molecular detection of orf virus genome GIF/ IL2

CEV specific primers GIF/IL-2 gene, the virulence factor found only in Parapox viruses was used in the PCR assay (Deane *et al.*, 2000) [6]. Klein and Tryland (2005) [11] employed PCR to amplify segments of the GIF gene, producing a 408 bp amplicon for CE virus detection in tissue samples and species differentiation. Similarly, several researchers have utilized PCR for detecting CEV (Kanou *et al.*, 2005; Hosamani *et al.*, 2007) [10, 9].

Sample details and processing

The scab samples from goats were thawed at room temperature and they were cut with a sterile scalpel blade into small pieces. The small pieces were grounded with sterile sand using a sterile mortar and pestle and suspended in 1ml of sterile 1X phosphate-buffered saline (PBS). Each homogenate was frozen at -20 °C, thawed three times, and centrifuged at 3000 rpm for 15 min at 4 °C. The 200 ul of clear supernatant fluid was used for DNA extraction. The remaining homogenate was stored at -20 °C until used.

DNA Extraction

Total DNA was extracted from swab samples by EZ-10 Spin Column Genomic DNA Kit as per the instructions of the manufacturer (BioBasic, Canada). Briefly, 200ul of clear supernatant fluid was taken into a 1.5 ml of micro-centrifuge tube and then added 0.6 ml of Lysis-Buffer-V Reagent into the tube. The mixture was vortexed vigorously for 30 seconds and incubated at 65 °C for 10 min. The mixture was transferred into the EZ-10 spin column and Kept at room temperature (23-25 °C) for 2 minutes. The EZ-10 spin column was centrifuged at 10,000 g for 1 minute after that the flow-through was discarded. Wash solution (0.5ml) was added to the spin column and spin column was centrifuged at 10,000 g for 1 minute after that the flow-through was discarded. Wash step was repeated one more time. The column was transferred to a new 1.5 ml micro-centrifuge tube, 30 µl of TE Buffer was added onto the centre of the column and kept at room temperature (23-25 °C) for 2 minutes. Finally, the column was centrifuged at 10,000 g for 1 minute. The purified viral DNA was used for PCR and real time PCR. The purity and concentration of DNA in the extract was estimated using Nanodrop.

Polymerase chain reaction

The PCR procedures were performed according to Sambrook *et al.* (1989) [21]. The primer sets developed from

the gene of highly conserved sequence within ORFV (IL-2) gene using forward primer 5-GCT CTA GGA AAG ATG GCG TG-3 & reverse primer 5-GTA CTC CTG GCT GAA GAG CG-3) (Ramesh *et al.*, 2008) [17]. Reactions volumes of 25 µl containing 0.5 µl each of forward and reverse primers at a final concentration of 100 pmol of each primer, 12.5 µl master mix, 3 µl of DNA template (contains 200 ng) and nuclease-free sterile double distilled water up to 25 µl. Negative and positive controls were included for each reaction. Amplification was done under the following conditions: Initial denaturation cycle at 95 °C for 5 min, 35 cycles (denaturation at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 45 s), followed by a final extension cycle at 72 °C for 10 min. Amplicons (5 µl) were separated on a 1.4% agarose gel at 90 V for 30 min and the 100 bp DNA ladder (GeneRuler, ThermoScientific) was used as a size standard. The PCR Products were visualized with a gel documentation system (Gel Doc TM XR+ with image LabTM Software, BIO RAD).

Table 1: Percentage - wise distributions of contagious ecthyma lesions

Site of lesions	No. of cases	Percentage (%)
Oral commissure	88	63.3
Lips	64	46.04
Muzzle	55	39.56
Nostril	24	17.26
Gum	9	6.47
Tongue	2	1.43

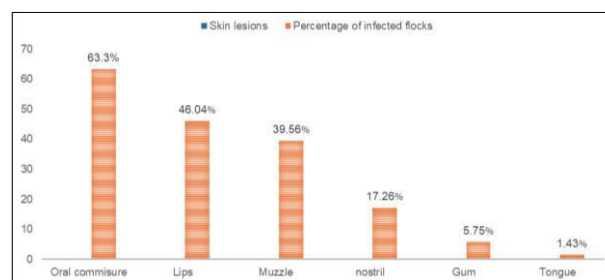


Fig 1: Location of skin lesions in suspected flocks

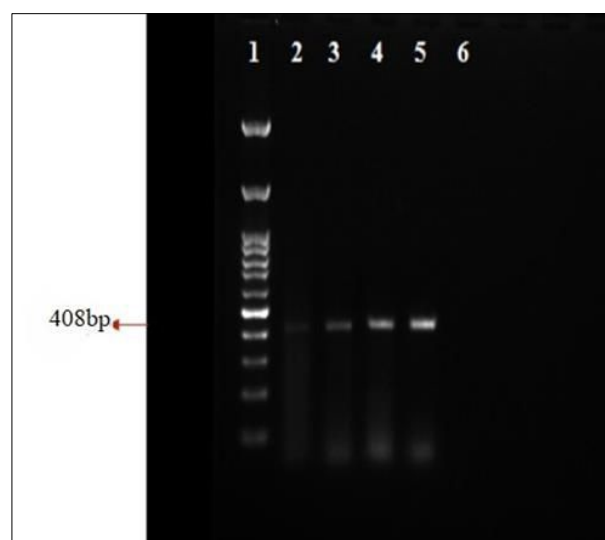


Fig 2: Lane 1: Marker 100 bp, Lanes 2, 3 and 4: Test samples; 5 positive sample; 6 - Negative samples



Fig 3: A. Thick scabby lesions on the muzzle and around the nostril of kid, B-C. Thick scabby lesions on the muzzle and around the nostril of adult goat, D. Lesion on buccal cavity of kodi goat, E. Dull and depressed kid due orf virus, F. Proliferative skin lesions on oral commissure, lips, nostrils and muzzle

Result and Discussion

Orf is an acute, highly contagious and economically important viral diseases of small ruminants. In this study 16 outbreak of contagious ecthyma were investigated by the Veterinary University Training and Research Centre, Rajapalayam during 2022-2023 (Table.1 & Fig map Virudhunagar). During the outbreak, 139 goats were get affected irrespective of their age and sex with high 82% morbidity and without any mortality (Table.1. Percentage – wise distributions of contagious ecthyma lesions) with symptoms of fever, anorexia, depression, proliferative scab lesions on the skin around the mouth nostril (Fig.1). All the outbreaks occurred in the current study started in rainy season, during late summer. Contagious Ecthyma virus primarily spreads and thrives in late summer due to the hot, humid, and dry conditions (Coradduzza *et al.*, 2021) [5].

Contagious ecthyma infections are regularly reported year-round in India, with heightened occurrences during the rainy season or late summer. The current investigations also finding the same season (Balakrishnan *et al.*, 2014) [2]. CEV is highly resistant to inactivation in the pen environment (Kumar *et al.* 2014) [13], allowing it to persist and reinfect the flock. This characteristic makes the virus challenging to eliminate, potentially posing public health risks for both animals and humans in the future. The affected goats exhibited proliferative skin lesions confined to face, oral commissure, nostrils and lips. (Fig.1). However, in the present investigation though total morbidity was reported to be 82% and there was no mortality recorded. 82% morbidity is recorded in this study were more or less agreement with those reported by Ramesh Kumar, *et al.* 2022 [18].

Symptomatic treatment combined with effective stick management practices has completely ceases mortality. The scab materials were subjected for PCR reaction to found positive for targeted gene of ORFV (IL-2) and visualized with a gel documentation system (Gel Doc TM XR+ with image LabTM Software, BIO RAD) with a band size of 408bp (Fig.2).

The diagnosis of the disease is typically based on the appearance of clinical lesions, followed by laboratory confirmation. Nevertheless, with the development of biotechnology techniques, especially PCR for the amplification of the virulence gene, confirmatory diagnosis has become more reliable and accurate. In the current investigation, the outbreak of the contagious ecthyma has been confirmed by the amplification of the ORFV IL-2 gene by PCR assay which targets the final amplification product of 408 bp (Ramesh *et al.*, 2008) [17].

The current study employed is the prime report of the confirmatory diagnosis of contagious ecthyma outbreak in Kanni and kodi goat breeds of Virudhunagr District, Tamil Nadu. Nonetheless, a detailed analysis is needed to establish the epidemiology and virus lineage.

Timely diagnosis of contagious ecthyma disease is crucial for enabling the implementation of control regimens to minimise the losses suffered by small and marginal farmers of this regions.

Most uncomplicated infections resolve within 1 to 4 weeks. In this outbreak, all age groups of goats were affected, but no deaths occurred because timely symptomatic treatment was administered, including antibiotics, anti-pyretic, anti-inflammatory, and vitamin B injections.

Uneventful recovery was achieved within 30 days. The spread of the infection was controlled by segregating healthy goats from the infected ones and administering symptomatic treatment to those already infected.

Conclusion

The goats, from kids to older ones of both sexes, exhibited symptoms such as fever (40°C), anorexia, depression, and scab-like lesions around the mouth and nostrils, with 82% of the herd affected. Secondary bacterial infections were prevented through symptomatic treatment with anti-pyretic, anti-inflammatory, and vitamin B injections. Neem and turmeric paste were applied to the lesions to aid in skin recovery. The goats fully recovered within four weeks, and the infection was confirmed using PCR, which targeted a 408 bp sequence of the CEV gene.

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