

Molecular Identification of Capripox in Kanni and Kodi Goat Breed of Virudhunagar Distt. of Tamil Nadu

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ABSTRACT: Investigated a capripox infection occurred in organized native breed goat farm in a village named Chennelkulam, Virudhunagar District. A flock, 240 native breed of male goats aged between 6-8 months rearing for intensively meat purpose. A small number of goats were get affected without any mortality. Scab materials was collected from affected goats and processed for nucleic acid extraction and subjected to PCR and it was confirmed as capripox viral genome. Ailing animals were treated and it gave an event full recovery. In addition this finding clearly highlights the native breed also susceptible to capripox without mortality.

Keywords: capripox, Kanni Goat, Kodi Goat, Tamil Nadu.

INTRODUCTION

Goats serve as a vital income stream for those with limited resources, earning them the moniker "the cow of the poor." However, numerous viral diseases significantly hinder their productivity, leading to substantial economic setbacks for goat farmers globally. Pox viral infections are among the contagious, infectious and economically important diseases in small ruminants. Pox viral infections of goats include Goatpox (GP), Sheep pox (SP), and Orf. The SP, GP and Lumpy Skin Disease (LSD) are caused by Capripoxvirus (CaPV) genus under the family of poxviridae (ICTV, 2019). The virions exhibit enveloped, brick-shaped structures with complex symmetry, measuring approximately $300 \times 270 \times 200$ nm, and containing a double-stranded DNA genome. In SPPV and GTPV, the genomic size is approximately 150 kbps, with terminal repeats present at both ends (Tulman *et al.*, 2002).

The Goatpox virus induces severe illness in goats, yet only elicits mild symptoms in sheep. Nevertheless, certain strains can provoke equally severe disease in both species. Symptoms of CaPV infection typically begin with fever, followed by the emergence of red patches on the skin, which then evolve into blisters, raised bumps, pus-filled lesions, and eventually scabs. CaPV lesions can also manifest on mucous membranes and internal organs, leading to conditions such as pneumonia, diarrhoea, lethargy, weight loss, miscarriage, and, in severe instances, fatality (Bhanuprakash *et al.*, 2010). GPV stands as a primary cause of deterioration of skin quality, resulting in significant economic losses for farmers across the globe (Kenubih *et al.*, 2021). Goat pox initially reported by Hansen in Norway in 1879 (Rafiyi and Ramyar 1959).

In India, infections of CaPV in sheep or goats often go undetected or are misdiagnosed as SP or GP depending on the host species. Therefore, early detection and differentiation of these viruses are crucial to halt the spread of infections through timely vaccination. The morbidity rate of GPV can climb as high as 90%, with mortality reaching up to 10% in endemic regions and 100% in populations lacking prior exposure. In India, the initial GPV outbreak was documented in 1936 at Imperial Institute of Veterinary research (Madhavan *et al.*, 2016). Subsequently, the disease has been recorded in several states including Jammu and Kashmir, Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Kerala, Chhattisgarh, Uttar Pradesh, Madhya Pradesh, Orissa, Haryana, West Bengal and North – eastern regions (Murthy *et al.*, 1971; Sharma *et al.*, 1986; Mondal *et al.*, 2004; Bhanuprakash *et al.*, 2005; Govindarajan *et al.*, 2005; Roy *et al.*, 2008).

The disease has become endemic in Tamil Nadu and, many outbreaks have been reported among sheep and goats between 2005 to 2018 in different districts of the Tamil Nadu state including Chennai, Chengalpattu, Kanchipuram, Salem, Tirunelveli, Uthagamandalam, Madurai (Sivaseelan *et al.*, 2005; Roy *et al.*, 2008; Manimaran *et al.*, 2017; Soundararajan *et al.*, 2014; Pothiappan *et al.*, 2014).

The present investigation describes the occurrence, early detection and management of capripox infection in Kanni and Goat breeds reared at an organized goat farm in the Virudhunagar district of Tamil Nadu.

MATERIALS AND METHODS

Location of the investigation and details of the farm:

The current work hails from Virudhunagar district, Tamil Nadu, located at approximately 9.587209 N and 77° 57' 5.1516" E. The farm is located Chennaekulam,

near Rajapalayam, about 12-15 kilometers away. The Disease investigation was conducted in December 2021 at an organized slatted floor goat farm, which housed 240 bucks, consisting of 120 Kanni and 120 Kodi native breeds of Tamil Nadu. This farm is managed by a young entrepreneur. The purpose of raising these male goats is specifically for fattening and eventual sale during festivals, ensuring a profitable margin. Though, entrepreneurs routinely vaccinate against Peste des pet its ruminants (PPR) and enterotoxemia, but they do not administer vaccinations against goat pox because of their lack of awareness.

Clinical evaluation of animals and sample collection:

In December 2021, a few animals started exhibiting lesions suggestive of pox viral disease. The clinical signs observed initial fever followed by characteristic skin lesions, mainly around the oral commissures and under the tail. Some animals also showed symptoms such as anorexia, fever, rhinitis and dyspnoea. The scab materials were collected from the affected goats for detection of capripox viral genome using following prime sequence described in Table 1.

Molecular detection capripoxgenome: The scab materials were crushed and mixed in sterile PBS with a pH of 7.4 to make 10% w/v suspension. The tissue mixtures were frozen and thawed three times, then spun at 3000 rpm for 10 minutes to remove debris. The supernatant, which holds the virus, was used to isolate genomic DNA with the DNeasy® Blood & Tissue Kit as per manufacturer’s protocol and finally DNA was eluted in 50µl elution buffer and stored at -20°C. In a reaction volume of 25 µl, Taq DNA polymerase 2x Master Mix RED (Ampliqon, Denmark) was combined with 1.5mM MgCl₂ (12.5 µl), forward and reverse primers (each at 10 pmol), template DNA (2µl), and nuclease-free water (8.5 µl). The amplification reaction was carried out using a thermal cycler (Eppendorf Mastercycler® nexus GX2, Hamburg, Germany) which amplification conditions described in Table 1 and the PCR products were subsequently analyzed via agarose gel electrophoresis (using a 1.5% gel containing 1 µg/ml of ethidium bromide).

Table 1: Details of Primers and PCR cycling conditions used in the study.

Gene Target	Name of Primer	Orientation	Primer Sequence (5'.....3')	Amplification conditions	Amplicon Size	Reference
CaPV attachment protein	CaPV P32	Forward	TCCGAGCTCTTTCCTGATTT TTCTTACTAT	95°C for 5 min, 34 cycles of 95°C 45 sec, 50°C 50 sec, 72°C 1 min; 72°C 10 min	192	Ireland & Binepal (1998)
		Reverse	TATGGTACCTAAATTATATAC GTAAATAAC			

RESULT AND DISCUSSIONS

Goat pox and sheep pox are categorized by the OIE as notifiable diseases due to potential for rapid spread and substantial economic impact (WOAH, 2008). Capripox is an acute viral infection with incubation period ranging from 1 to 2 weeks depending upon the strain of the virus and host susceptibility. Mortality and morbidity depend upon factors such as breed, immune status, previous history of exposure, age of animal and strain of virus. In the ongoing investigation, clinical manifestations such as early fever, nasal discharge, loss of appetite, and difficulty breathing/coughing were noted. Additionally, the affected animals exhibited distinctive pox lesions indicative of capripox infection. The initial papules developed into vesicles and pustules, which subsequently ulcerated, leading to scab formation.

Capripox infection is consistently reported in India throughout the year, with outbreaks peaking during both winter and summer months (Bhanuprakash *et al.*, 2006). We have also encountered the capripox infection during winter month of December. The affected goats (n=15) showed proliferative skin lesions confined to commissure of lips (Fig. 1-2) and under the tail (Fig. 3). However, in the present investigation though total morbidity was reported to be 6.25%, in fattening bucks and there was no mortality recorded. The scab materials were subjected for PCR reaction to found positive for

capripox viral genome of P32 gene and yields an amplicon of 196 base pairs (Fig. 4).

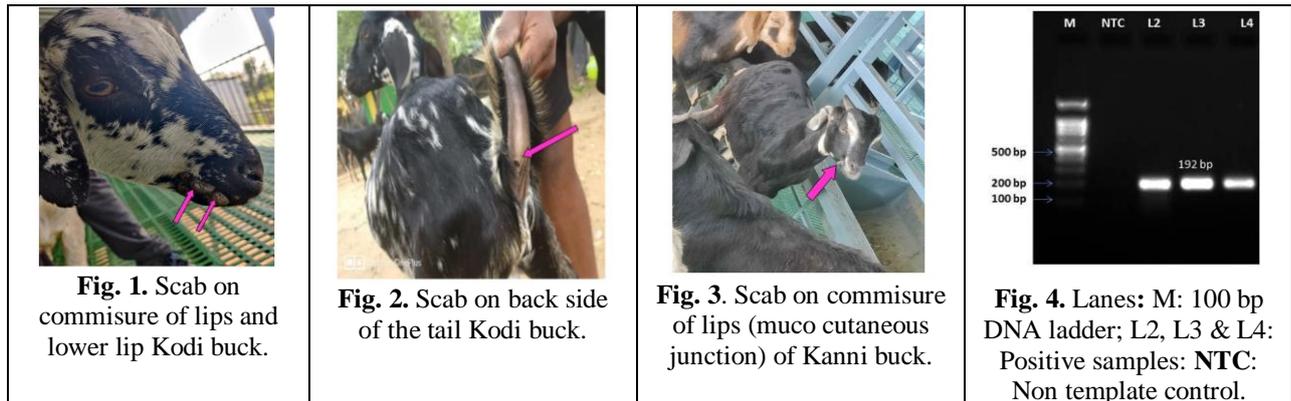
This is the first recorded report in Kanni and Kodi breed of goat. Kanni and Kodi goats are breed of Tamil Nadu especially from Virudhunagar, Tirunelveli and Tuticorin District of Tamil Nadu(<https://nbagr.icar.gov.in/en/registered-goat/>). In addition, this finding clearly highlights the native breed also susceptible to pox infection without mortality.

The PCR assay is quick, genus-specific, and sensitive assay for detecting the pox virus before or after the formation of antibody responses is the capripoxviral DNA amplification method. The common amplification techniques include the conventional polymerase chain reaction (PCR), real-time PCR, and the loop-mediated isothermal amplification (OIE, 2013). For obtaining sufficient genetic material for identification, using conventional PCR methods is very useful for species identification during subsequent sequence and phylogenetic analysis (Leo Goff *et al.*, 2009).

The outbreak underscores the significance of this disease as an emerging concern within the southern district of Tamil Nadu. In this study, a significantly lower mortality rate was observed compared to the typically reported high mortality rates from various sources. This difference may be attributed to the significant role played by native breeds in combating capripox infection. Local breeds such as Kanni and

Kodi demonstrated relative resistance to capripox infection. However, indigenous breeds are more adopted to sudden environmental fluctuations and diseases outbreaks than other exotic breeds of goats

(Ramachandran and Sejian 2022). Additionally, early disease detection and prompt segregation of affected animals likely contributed to the lower mortality rate observed.



SUMMARY

The investigation focused on a capripox infection in a native breed goat farm Virdhunagar District. The farm housed a flock of 240 bucks, aged between 6-8 months, primarily reared for meat. A small portion of the goat population showed signs of infection, although there were no reported fatalities. Scab material were collected and found positive for capripox viral genome by PCR assay. Affected animals were treated, resulting in full recovery without complications. This finding underscores the susceptibility of native goat breeds to capripox infection, even in the absence of mortality.

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Conflict of Interest. None.

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