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Molecular detection of goat pox virus in goats from Kerala, India

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Abstract
Small ruminants are the primary livestock resource of many poor rural families around the globe. Many viral infectious diseases can affect these animals and in turn, can cause economic loss to farmers by causing mortality and production loss. In the present study outbreaks of goatpox viral (GPV) infections in goats were investigated in Kerala, India. Detection of the agents from these outbreaks was carried out employing molecular methods. In this study, the P32 gene for the GPV was targeted for molecular diagnosis. On BLAST analysis of the GPV isolate of the present outbreak, it showed 99.89 to 100% nucleotide similarity with other Indian isolates, with more similarity to the Puducherry isolate (MK805069).

Keywords: Goatpox virus, P32 gene, capripoxvirus, goatpox

Introduction
Goats are an important source of income for low-income people, so they are known as the poor man's cow. Several viral diseases seriously harm optimal productivity and result in economic loss to goat farmers worldwide. Pox viral infections are one among them. Pox viral infections of goats include Goatpox (GP), Sheep pox (SP), and Orf. The causative agents of SP and GP are members of the Capripoxvirus (CaPV) genus under the family of poxviridae (ICTV, 2019) [9]. According to the world organization for animal health, SP and GP are notifiable diseases of sheep and goats. Hansen first described goat pox in Norway in 1879 (Rafyi and Ramyar, 1959) [13]. Goatpox viruses are enveloped viruses having double standed DNA as genetic material and the size of the genome varies from 143 to 147 Kbp (Gershon and black, 1988) [4]. Goatpox virus causes severe disease in goats but only mild disease in sheep; however, some strains cause equally severe disease in both species (Babiuk *et al.*, 2008) [1]. The clinical signs of CaPV infection start with fever, and the development of erythematous macules which progresses to form vesicles, papules, pustules, and scab on the skin. Lesions in CaPV may also form on the mucous membranes and internal organs, resulting in pneumonia, diarrhoea, depression, emaciation, abortion, and, in severe cases death (Bhanuprakash *et al.*, 2010.) [3]. GPV is one of the main causes of loss of skin quality and thereby cause large economic loss to the farmers worldwide (Kenubih *et al.*, 2021) [11]. In India, CaPV infections in sheep/goats are either undiagnosed or diagnosed as SP or GP based on the host species involved. It is therefore critical to detect and differentiate these viruses early to prevent the spread of infections through timely vaccination. The morbidity of GPV can reach up to 90% and mortality can be up to 10% in an endemic area and 100% in naïve populations (Hurisa *et al.*, 2018) [7]. In India, GPV outbreak was first reported in 1936 and further, the disease outbreaks have been reported in many states such as Jammu and Kashmir, Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh, Kerala, Chhattisgarh, Uttar Pradesh, Madhya Pradesh, Orissa, Haryana, and West Bengal (Imperial Institute of Veterinary Research, 1936–37; Bandyopadhyay *et al.*, 1984; Sumana *et al.*, 2020) [8, 2, 14].

Rapid and accurate diagnosis is critical for the prevention and control of GPV infections in goats. Usually, the disease diagnosis was based on clinical, serological, and nucleic acid techniques such as PCR and real-time PCR (Ireland and Binepal, 1998) [10]. Because the clinical signs of GPV infections can be confused with other viral diseases such as SP and Orf, most often it is difficult to distinguish these two diseases solely based on clinical observation. So molecular techniques will serve as a valuable tool for differential diagnosis of pox viral infections in goats. The present study describes the investigation of GPV outbreaks in Kerala and its molecular confirmation.

Materials and Method

Samples were collected from two different goat farms in the Trissur district of Kerala. In the first farm, among the 22 goats, all showed signs such as fever, and pneumonia, and had nodules all over their body. Six among the 22 animals died. In the second farm, all ten goats showed the same clinical signs as described above. Four of the ten goats died.

On necropsy, it was observed that the superficial lymph nodes were enlarged, as well as large nodules were present in the trachea and lungs in some of the goats. Tissue samples of the scab, liver, lungs, and spleen were collected from the carcasses. All the samples for molecular diagnosis were kept at -80 °C until further processing.

Extraction of total DNA from clinical samples

Total DNA was extracted from tissue samples by Qiagen DNeasy Blood and Tissue Kit as per manufacturers protocol. The extracted DNA was kept at -20 °C until further processing.

Detection of GPV virus by PCR

Details of the primers used for the detection of the GPV is given in table 1. Amplification of the P32 gene of GPV was carried out using PCR. 25 µL reaction mix consisted of 12.5 µL of 2X EmeraldAmp GT PCR Master Mix containing Taq polymerase (TaKaRa), 1µL each of 10 pmol forward and reverse primers, 2 µL of DNA, and 8.5 µL NFW. The cycling conditions for GPV were 94°C for 5 min (initial denaturation), and 35 cycles of 94°C for 1 min. (denaturation), 55°C for 1 min. (annealing) and 72°C for 1 min. (final extension) followed by a single cycle at 72°C for 10 min. (final extension).

Table 1: Details of the primers used for the detection of the GPV

	Target Gene	Primers	Product Size (bp)
1	P32	EP32F: 5′-CCCGAATTCATGGCAGATATCCCATTATATG-3′	969 (Pham *et al.*, 2021) [12]
		EP32R: 5′-CCGAAGCTTCTAACTATATACGTAAATAAC-3′	

Visualization of the PCR product

Agarose gel of 1.25 per cent concentration was prepared. 10 µL of the PCR product was loaded into the wells. Electrophoresis was carried out and the gel was visualized under UV illumination.

Sequencing of the PCR Products and Analysis of the Nucleotide Sequences

The PCR products were purified using a PCR gel extraction kit (Gene JET Gel Extraction Kit, Thermo Scientific) and sent to AgriGenome Lab Private Limited, Cochin, India for sequencing. With the help of Chromas Lite v2.01 software (http://www.technelysium.com.au) the chromatograms of the sequences were analyzed. BLAST was conducted to confirm the identity of virus (http://www.ncbi.nlm.nih.gov/BLAST).

Result

Gross pathology

Clinical signs and Post-mortem findings suggest the probable involvement of pox virus infection which includes pustules, nodules, and scabs in hairless areas of the body including lips.\

Molecular detection

PCR targeting the genes *P32* of GPV was able to generate amplicons of size 969 from tissue samples as well as from the positive control. No amplification was observed in the negative control. The sequences obtained after sequencing the PCR products were subjected to chromatogram analysis for correcting the errors and trimmed to get clean 927 bp partial P32 gene. The corrected sequences of these isolates were confirmed by BLAST analysis. The sequences of these isolates were submitted to NCBI GenBank and accession numbers were obtained. The accession number for GPV1/PKD/2021 and GPV2/PKD/2021 were ON650728 and ON650729 respectively.

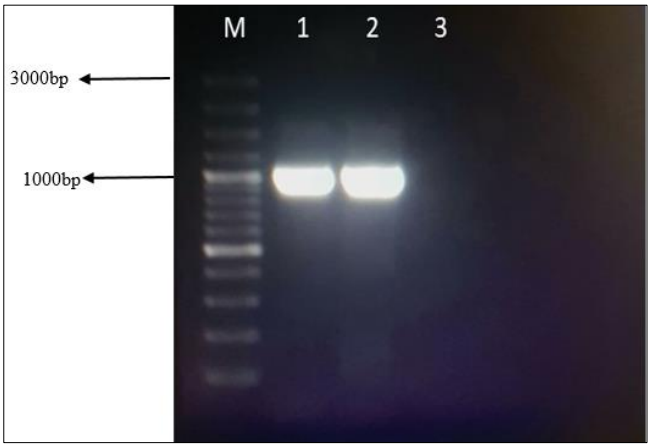

Fig 1: PCR amplification of P32 gene having product size of 969 bp from two GPV field isolates (lane 1 and 2); Negative control (lane 3); M: 3 kbp DNA ladder

On genetic analysis of the two sequences of GPV showed 100% identity with each other. It also showed 99.89% similarity with the previously reported Indian isolate from Kerala and 100% similarity with the Puducherry isolate.

Discussion

Capripoxvirus infections, including SP and GP, are highly contagious diseases that cause fever, generalized papules or nodules, vesicles, internal lesions, and severe mortality in sheep and goats. The affected animals in the present outbreak had nodules and papules all over their skin surfaces and many of them had profuse nasal discharge too. A similar observation was also observed by Bhanuprakash *et al.* (2010) [3]. For the molecular detection of GPV, the preferred tissue samples are skin papules, lung lesions, and lymph nodes (WOAH, 2018) [15].

The PCR technique has become widely used in molecular biology to amplify the desired genomic fragments from tissue specimens, and it has become a powerful tool in molecular diagnosis. In the present study, the identity of GPV was confirmed by the amplification of specific fragments of viral DNA by PCR, and gene sequencing.

Sheeppox and GP are endemic in India and cross infection of GPV and SPV were also reported. GPV was identified as the etiological agent by P32 gene-based PCR and sequencing. P32 is a major envelope structural protein that is found in all CaPV (Heine *et al*., 1999) [5]. Based on the P32 genomic sequence, CaPV members could be divided into three distinct clusters: GPV, SPV, and LSDV. However, comparative nucleotide sequence alignment of GPV isolates including the present isolates showed the GPV signature residues such as G at 77 and 867; T at 275, 403, and 552. Similarly, a comparison of the deduced amino acid sequence of the present isolate also showed unique GPV amino acid substitutions *viz*. G26D, V92A, Y135H, M289I, and Y322I in comparison to other CaPV (Hosamani *et al.*, 2004) [6].

Conclusion

The results of this investigation indicated the occurrence of GPV in Kerala. The present GPV isolates were closely related genetically to isolate from Puducherry, suggesting that the same virus was circulating in South India. Continuous surveillance and vaccination are required to control the goatpox viral outbreaks in goats.

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