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Investigation sheeppox outbreak in migratory flock of Mouli sheep in Karnataka

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Abstract
The present study describes a detailed investigation of the sheeppox outbreak in a migratory flock of Mouli sheep breed in the Vijayapura district of Karnataka. The clinical symptoms during the outbreak investigation recorded were fever, nasal discharge and generalized typical pox lesions on the entire body in clinically ailing sheep. The post-mortem examination revealed typical gunshot wounds on the lungs and small white spots on the liver and intestine. The clinical and postmortem samples include scabs, nasal swabs, blood, and the lung and liver collected were subjected to Polymerase Chain Reaction (PCR) for confirming the Capripox virus. Later in, the same set of samples were subjected to sheeppox species-specific PCR and was confirmed as sheeppox virus. The migratory flocks are subjected to lot of stress and regular vaccination is required to minimize the sheeppox outbreaks.

Keywords: Capripox, Mouli, PCR and sheeppox

Introduction
Sheeppox is a highly contagious disease, that spreads mostly by direct contact with lesions or contaminated feed, fodder, water and wool (Manjunatha Reddy *et al.*, 2024a) [4]. Virus is excreted in the nasal, ocular secretions, milk and possibly through urine. The common practice of migration in search of water and fodder for long routes leads to wading of sheep to nibble leaves through bushes and thorny plants damages the skin and facilitating the disease transmission from the infected to the susceptible animals. Furthermore, these areas are easily accessible to biting flies (mechanical carriers), which can easily bite, suck blood and transmit the virus. *Stomoxys calcitrans* is an efficient mechanical vector of Capripox virus (Kitching and Taylor, 1985) [3].
The incubation period of sheeppox and goatpox is between 8-13 days. Exotic breeds and young animals are more susceptible and may die of acute infection before the development of skin lesions than indigenous and adult animals, which show different stages of disease manifestations such as a rise in rectal temperature to more than 40 °C, followed by development of macules, small circumscribed areas of hyperemia, which are most obvious on unpigmented skin in the initial 2-5 days, followed by appearance of papules, hard swellings of 0.5 and 1 cm in diameter which may cover the body or be restricted to the groin, axilla and perineum. Rarely papules may be covered by fluid-filled vesicles, but this is rare (Zro *et al*., 2014; Manjunatha Reddy *et al.*, 2024a) [12, 4]. Diagnosis of the disease is mainly by the clinical signs and post mortem lesions. Confirmatory diagnosis is done by partial Polymerase Chain Reaction (Sumana *et al.*, 2020a) [9]. Present paper reports the outbreak of sheeppox occurred in the border region of Kalaburagi and Vijayapura districts of Karnataka state.

Materials and Methods
History of outbreak and sample collection
Sheeppox outbreak reported in May 2024 involved Mouli sheep breed commonly found in the northern Karnataka region. It was a migratory flock of 35 in size moved from Vijayapura to Gulbarga in search of fodder and the flock was not vaccinated against sheeppox. The clinical samples such as scab in sterile container, nasal swab in viral transport medium, blood in EDTA coated vials and postmortem samples in 50 percent glycerol for virus identification

were collected and transported to laboratory.

Processing of samples

Tissue/scab

Tissue was sliced into small pieces using a sterile BP blade and triturated with the help of mortar and pestle with 2 ml of chilled 1X PBS. About 200 µl of each tissue suspension was added to the 2 ml microcentrifuge tubes having 200 µl of buffer ATL and 20 µl of proteinase K. The samples were vortexed and incubated at 56 °C for 10 min or till the samples were completely lysed. Then the samples were vortexed for 15 seconds before further proceeding for DNA extraction.

Processing of blood sample.

Fifty microlitre of whole blood was transferred to 2 ml microcentrifuge tubes, 20 µl of proteinase K and 150 µl 1X PBS was added to all the tubes to make total volume to 220 µl for each sample. To this mixture, 200 µl of buffer ATL was added, the samples were vortexed and incubated at 56 °C for 10 min or till the samples were completely lysed. Then samples were vortexed for 15 seconds before proceeding for DNA extraction.

Processing of nasal swab samples

Swab samples were mixed with one ml of 1X PBS, 200 µl of samples were transferred to 2 ml microcentrifuge tubes and 20 µl of proteinase K was added then the samples were vortexed for 15 seconds before proceeding for DNA extraction. DNA extraction was done using DNeasy blood and tissue kit (QIAGEN, Germany). The purity of extracted DNA was checked by using spectrophotometer (Nano Drop-1000).

Polymerase Chain Reaction

The PCR was carried out using the primers P32-F 5' ACACAGGGGGATATGATTTTACC 3' and P32 R-5' ATACCGTTTTTCATTTCGTTAGC 3' (Reddy *et al.*, 2015) [8] and RPO30 gene-based species-specific primers (Sumana *et al.*, 2020a) [9]. A PCR reaction was set to a volume of 25 µl using Taq PCR master mix (QIAGEN, Germany) with initial denaturation at 94 °C for 5 min, then 94 °C for 30 sec, 52 °C for 30 sec and 72 °C for 45 seconds for 35 cycles. And final extension of 72 °C for 10 min in thermal cycler (PeQ Star2x, Eppendorf). The PCR product was subjected to agarose gel electrophoresis and photographs were documented (GelDock: Box, Syngene, India).

Results and Discussion

The sheeppox outbreak in May 2024 in a migratory sheep flock of 35 nos, Mouli breed at the border of Kalaburagi and Vijayapura districts had a morbidity of 42 percent and case fatality rate of 100 percent. Clinical symptoms such as high fever, nasal discharge, swollen eyelid and head with generalized pox lesions on the entire body. These clinical signs were variable according to the form of the disease. In the malignant form that generally seen in young ones, wherein the body temperature may shoot up to 40-42 °C with dyspnoea and oculo-nasal discharge and pox lesions on un-wooled skin, whereas in benign form only skin lesions occur particularly under the tail (Hurisa *et al.*, 2018; Manjunatha Reddy *et al.*, 2017) [2, 6]. Post-mortem examination of the dead animals revealed pock lesions in lungs and liver (Fig.1). Similar observations were also reported by (Bowden *et al.*, 2008; Rather *et al.*, 2020; Manjunatha Reddy *et al.*, 2017; 2024b) [1, 7, 6, 5].

(A) (B)

Fig 1: Sheeppox affected animal showing the nodular pox lesions all over the body (A). and the lungs with gunshot wounds on the lung surface of sheep died of sheeppox (B).

The animals during the course of their migration might have picked p the infection by ingesting Pasteur/water contaminated with SPV. We learnt that the flock was an unvaccinated one. During the investigation, we also learnt that these shepherds throw away the sheep that died due to sheeppox which could be a potential source of infection to any other flocks following the same path.

Although sheeppox can be diagnosed by pathognomonic clinical signs and lesions, further confirmation of the disease was done by PCR targeting the partial P32 gene of SPV from skin scab, nasal swab and PM samples. All the samples were found positive with an expected amplicon size of 237 bp on agarose gel electrophoresis (Fig.2). Thus confirming the virus belonging to the genus Capripox (Reddy *et al.*, 2015; Sumana *et al.*, 2020b) [8, 10]. Capripox viruses are considered to be species specific (Tulman *et al.*, 2002) [11] and in the present investigation too the affected host was sheep and the disease was confirmed as sheeppox.

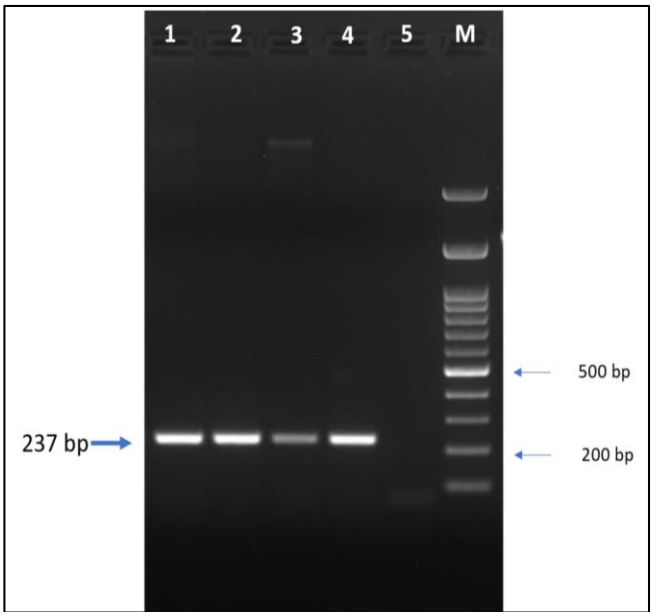

Fig 2: Agarose gel electrophoresis of the partial amplification of P32 gene by PCR, yielding 237 bps. M: 100 bp marker, 1: Positive Control, 2-4: Outbreak samples, 5: Negative control.

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Conclusion

The sheeppox outbreak reported in May 2024 in a migratory flock of Mouli sheep in northern Karnataka highlights the significant impact of the disease, particularly in unvaccinated populations. With a morbidity rate of 42% and a case fatality rate of 100%, the outbreak emphasized the need for preventative measures such as vaccination. The clinical symptoms, post-mortem findings, and PCR analysis confirmed the presence of the Capripoxvirus, specifically sheeppox. The unvaccinated flock's exposure to contaminated environments and improper disposal of infected carcasses likely facilitated the spread of the virus. This outbreak underscores the importance of vaccination and proper biosecurity measures to control the spread of sheeppox.

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