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Incidence of sheep associated-malignant catarrhal fever in Karnataka, India

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Abstract
Malignant Catarrhal Fever (MCF) is a viral disease primarily affecting cloven-footed animals including cattle, bison, sheep, deer, and rarely giraffes. Even though the disease is reported scarcely, it has a significant economic impact on the livestock industry. The present study was aimed at the identification of Ovine gammaherpes virus 2 in one incidence. Samples were collected from 36 animals on the farm. Heminested PCR targeting the partial tegument gene of the virus was used. Overall, 6 animals were positive for PCR. Among them, 2 positive samples were sent for sequencing. The Phylogenetic tree revealed a close association with Indian and Egyptian isolates. Sheep were harbouring the virus and were the most probable source of infection.

Keywords: Malignant catarrhal fever, ovine gammaherpes virus, PCR

Introduction
India is a nation with a multifarious livestock population. The population of sheep in India is 11.05 million and that of goats is 6.16 million, according to the book 'Basic Animal Husbandry Statistics', 2020, published by the Indian government. Mixed farming is most commonly practised in which sheep, goats, cows, and buffalos are maintained together. So, there is a high possibility of disease transmission between animals. Malignant Catarrhal Fever (MCF) is one of those viral diseases that might be fatal to cattle, buffaloes, bison, deer, pigs, and rarely giraffes (Loken *et al.*, 1998; Russel *et al*., 2009; Zakharova *et al*., 2020; De Jonge *et al*., 2021; Tomana *et al*., 2021; Ivan *et al*. 2022) [10, 18, 26, 3, 22]. There are 10 known MCF-causing viruses and they belong to the *Macavirus* genus of the *Gammaherpesvirinae* subfamily, a part of the *Herpesviridae* family (O'Toole and Li, 2014). Among these, Ovine gammaherpes virus 2 (OvHV-2) is the causative agent of sheep-associated-MCF (SA-MCF) in India. Sheep act as reservoirs for the disease and transmit it to susceptible domestic and wild animals (OIE, 2018) [11]. The disease is transmitted through aerosols, nasal secretions, and ocular secretions (WOAH,2022) [22]. Clinical signs of the disease include high fever, congestion of mucous membranes, enlargement of lymph nodes, depression, unilateral or bilateral corneal opacity, profuse nasal and ocular discharge, and rarely nervous signs (Wallman and Thompson, 1982; Headley *et al*., 2020) [23, 5]. Sheep-associated-MCF was reported in 1975 (Parihar *et al*., 1975) [13] for the first time in India and many have reported the same ever since (Wani *et al*., 2004; Wani *et al*., 2006; Banumathi *et al*., 2008; Sood *et al*., 2014; Kumar *et al*., 2021) [24, 26, 1, 19, 8]. This research paper will discuss a disease incidence of OvHV-2 in Karnataka state, India.

Materials and Methods
Samples and History
A recent death of an animal with respiratory symptoms was reported in an unorganized farm located in Jigani Hobli, Anekal taluk, Bengaluru urban district. Blood samples were collected in EDTA from 12 bovines (4 Holstein Friesian crossbreds, two buffaloes, and six Gir crossbreds), and all 24 sheep (Bannur crossbreds), two nasal swabs were collected from two Murrah buffaloe crossbreds. All the staff on the farm provided excellent support in collecting samples. All the samples were transported for processing.

Viral DNA extraction and PCR

Genomic DNA was extracted from all samples of sheep and cattle using the QIAamp DNA Mini Kit (Qiagen: Catalogue No. 51306). The initial step was running a heminested PCR on each sample (Baxter *et al*., 1993; Sood *et al*., 2014) [2, 19]. In a nutshell, primer pairs 556 (5'-AGTCTGGGGTATATGAATCCAGATGGCTCTC-3') and 775 (5'-AAGATAAGCACCAGTTATGCATCTGATAAA-3') were used in the first stage of the hemi-nested PCR, and primer pairs 556 and 555 (5'-TTCTGGGGTAGTGGCGAGCGAAGGCTTC-3') in the second stage (Baxter *et al*., 1993) [2]. A 25 µl volume containing 10 mM Tris-HCl (pH 8.0), 2 mM MgCl2, 200 µM each of dATP, dCTP, dGTP, and dTTP (Fermentas), 20 pM of primers, and 2 U of Taq DNA polymerase (Fermentas) was used for all amplification operations. Thermal cycling conditions for both stages were initial denaturation at 95 °C for 5 min and then 34 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 2min; this was followed by a final extension step at 72°C for 7 min. Three microliters of the first-stage PCR product were used as the template for the second stage's amplification. 10µl of the amplified PCR products were electrophoresed on a 1.2% agarose gel and stained with ethidium bromide for product visualization.

The PCR mix was prepared in a dedicated hood, away from the room where DNA extraction and gel electrophoresis of PCR products were performed. All PCR runs included NTC (No template controls - water), which were consistently negative. A gel extraction kit (QIAquick kit-Catalogue No. 28706) was used to elute DNA and later the samples were sent for Sanger's method of sequencing.

Phylogenetic analysis was performed on obtained sequences using Gene Tool 1.0 and MEGA 11 (Molecular Evolutionary Genetics Analysis) software.

Results

Detection of Ovine gammaherpes virus 2

The tegument coding region of OvHV-2 was amplified in heminested PCR with amplicons of 422 bp (Fig 1) and 238 bp (Fig 2) as reported by Baxter *et al*. (1993) [2].

In the Bengaluru urban district farm, two cows and four sheep were positive by PCR. One cow and one sheep sample were sent for sequencing. Purified PCR products were sequenced and accession numbers were obtained (Bengaluru-OR604335 and OR604336). The sequences obtained were edited in Gene Tool 1.0 software. 2 sequences obtained in this study were in alignment with partial OvHV-2 tegument gene sequences when BLAST (Basic Local Alignment Search Tool) was done in NCBI (National Center for Biotechnology Information).

Phylogenetic analysis was done (Fig.3) with sequences from Pakistan (MK840962.1), Brazil (KJ658293.1, KC123170.1), India (KF303530.1, KF303529.1, MF977714.1, KR092145.1, JQ801454.1, KR092147.1), Egypt (KP015737.1), sequences obtained in study (Bengaluru-OR604335 and OR604336) whole genome sequences of OvHV-2 (AY839756.1) and Alcelaphine gammaherpes virus 1 (AF005370.1). The evolutionary history was inferred by using the Maximum Likelihood method and Kimura 2-parameter model (Kimura, 1980). The tree with the highest log likelihood (-11649.38) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 14 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 4123 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura *et al.*, 2021) [21].

Discussion

Malignant catarrhal fever is an extremely complex illness that has been the subject of numerous investigations but is still only dimly understood. Sheep that are natural hosts of Ovine gammaherpes virus 2 do not experience clinical disease following infection and act as carriers (OIE, 2018) [11]. The disease's typically sporadic character has made it difficult to create effective control measures (Li *et al*., 1994) [9].

In the present study, efforts have been made for the detection of OvHV-2 in sheep and bovines and characterized by nucleotide sequencing. Samples from sheep and bovines from Bengaluru urban of Karnataka were collected

In the farm, one month before the study, an HF crossbred cow failed to respond to treatment and died after showing severe respiratory symptoms. So, the owner requested to test the animals on the farm for MCF. The farm had sheep in the close adjoining area. Two cows and four sheep were found positive for OvHV-2. The owner was advised to house all sheep distantly. Two cows that tested positive for MCF by PCR have been kept under observation and have not come up with clinical disease for six months.

It was observed that the transmission pattern of OvHV-2 from sheep to bovines was puzzling as the cow that was found positive for OvHV-2 did not exhibit clinical disease so far

The two possibilities are either the animals with subclinical infections were able to eliminate the pathogen or the infected animals developed lifelong persistent infections, similar to those caused by other gammaherpesvirus infections (Roizman, 1996; Powers *et al*., 2005) [17, 15]. The owner was advised to observe for clinical signs in future if any and report back

In this study nucleotide sequences for the tegument gene coded by ORF 75 were used to construct the Maximum likelihood tree. Sequences from Pakistan, Brazil, India, and Egypt were compared with sequences obtained in this study. When the tree was constructed using MEGA11 software, the sequences obtained in this study clustered with the available sequences from Karnataka and Andhra Pradesh states of India indicating their similarity. Unrestricted movement of sheep between states may have been the reason for transmission.

Overall, it was observed that the mixed farming practice has contributed to the disease incidence.

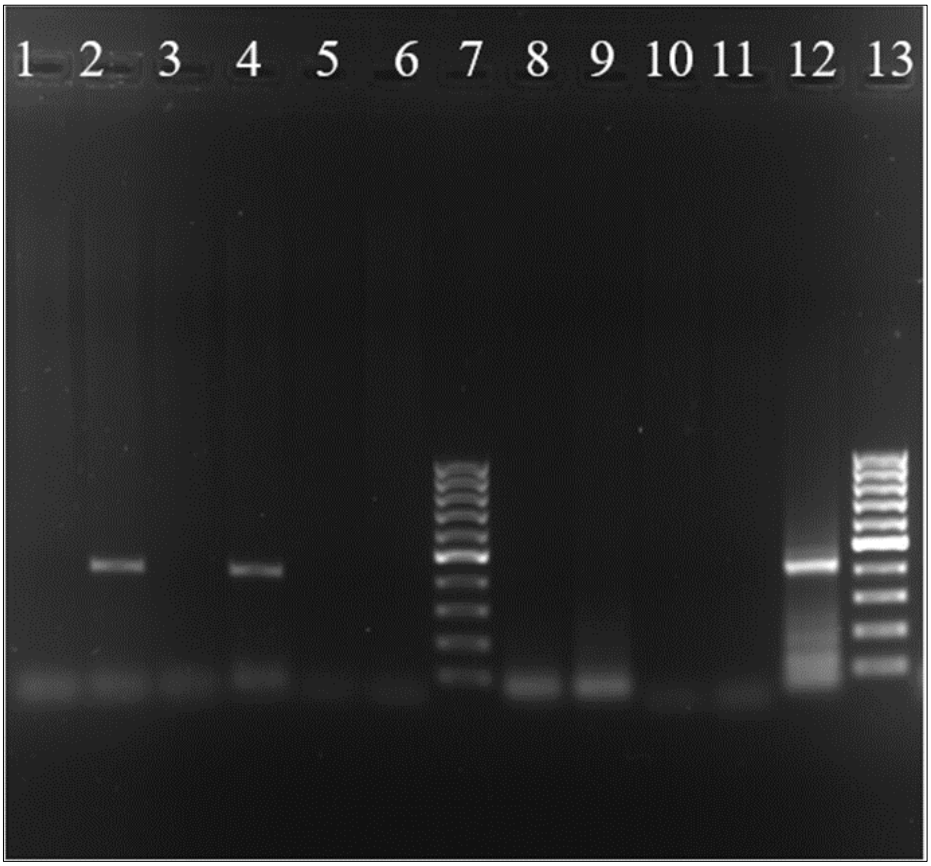

Fig 1: First stage of heminested PCR

2,4-Positive for 422bp (MCF tegument gene)
11-Non template control, 12-positive control, 7,13- 100bp ladder

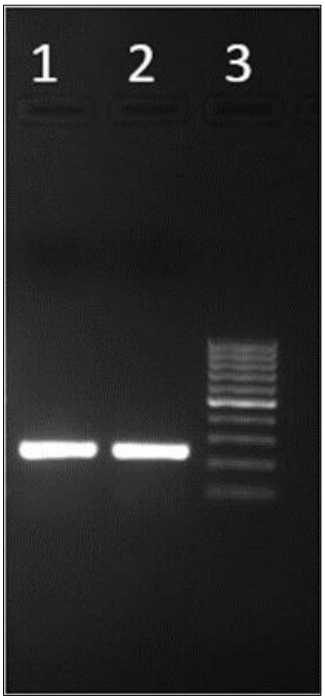

Fig 2: Second stage of heminested PCR

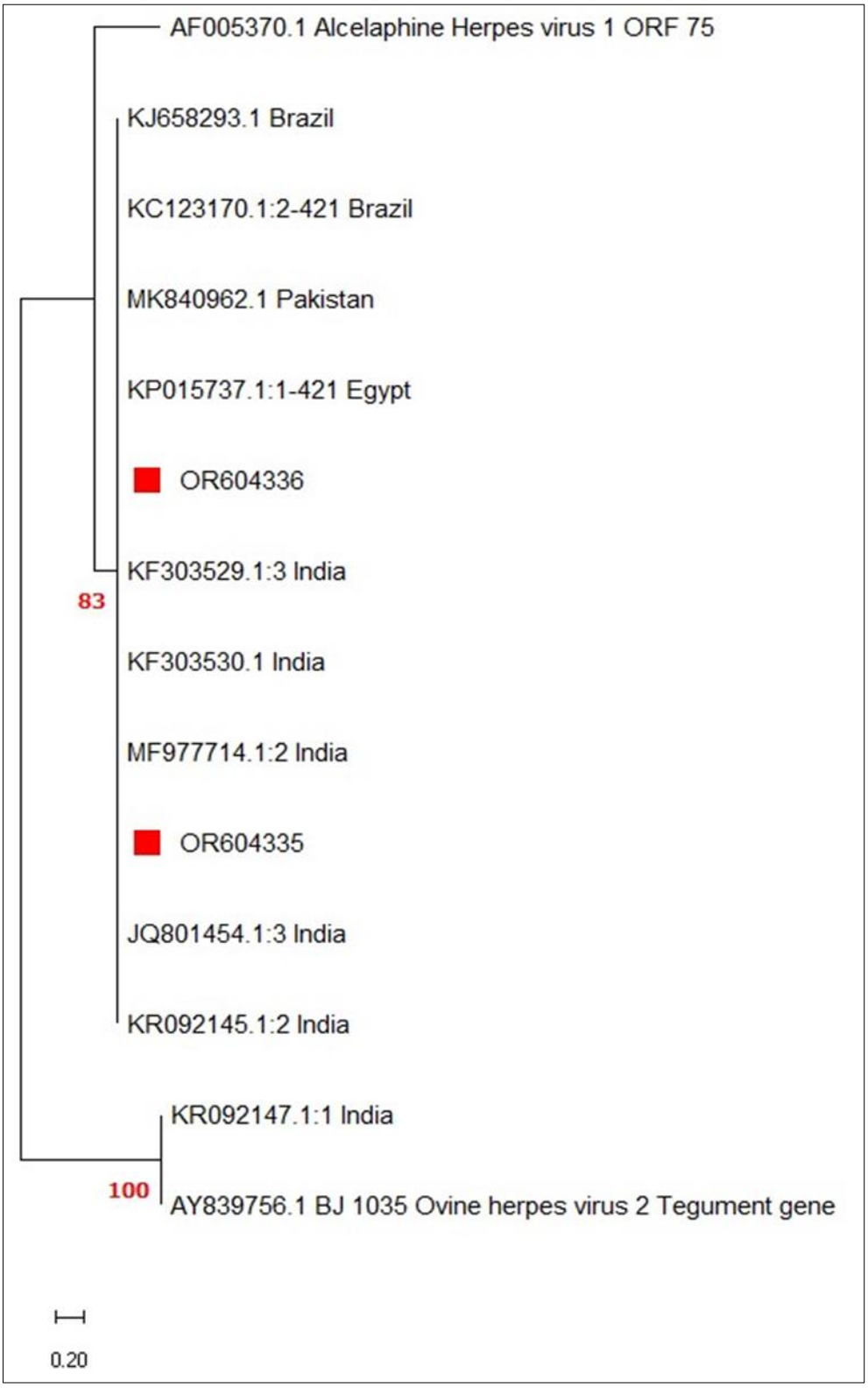

Fig 3: Phylogenetic tree

Conclusion

The severity and enigmatic nature of MCF make it an area of concern in Indian conditions where unorganized mixed farming is most commonly practised and the livelihood of farmers is dependent on it. Unrestricted movement of domestic animals for trading purposes poses a major challenge in the control of MCF transmission. Educating farmers regarding the severity of disease and efficient utilization of available systems could help in understanding the true prevalence and transmission of disease in the country.

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