CASE REPORT
JIVA 21 (1) April 2023
https://doi.org/10.55296/JIVA/21.1.2023.86-89

Received: 16/12/2022
Accepted: 13/03/2023

DIAGNOSIS OF SHEEP ASSOCIATED MALIGNANT CATARRHAL FEVER IN BUFFALO BY HEMINESTED POLYMERASE CHAIN REACTION

Neeraja, D.[1]*, Vimala Devi, B.[2], Sireesha, G.[3], Ratna Kumari, L.[4] and Amarendra Kumar, R.[5]

[1, 2] Veterinary Assistant Surgeons, [3] Assistant Director, [4] Joint Director, [5] Director
Veterinary Biological and Research Institute, Labbipet, Vijayawada-520010
Animal Husbandry Department, Andhra Pradesh.
*Corresponding author: dneerajareddy46@gmail.com

ABSTRACT

A case of sheep associated malignant catarrhal fever (MCF) in buffalo was observed in Badudandla village, Panyam mandal, Kurnool district of Andhra Pradesh. The buffalo was reported to have suffered from high temperature, severe conjunctivitis and hyper salivation before death. Nasal swabs, spleen and lung tissue were received from Animal Disease Diagnostic Lab, Kurnool district. The samples were subjected to MCF heminested PCR using 556- 755 and 556 -555 primer pairs. All the three samples were found positive for MCF with specific amplicon size of 422 bp and 238 bp in outer and inner PCR, respectively confirming the presence of sheep associated Malignant Catarrhal Fever. The samples were also tested for infectious bovine rhinotracheitis (IBR) by Real Time PCR kit and found negative.

Keywords: Heminested PCR, Malignant Catarrhal Fever, Ovine Herpes Virus -2 (OvHV-2)

INTRODUCTION

Malignant catarrhal fever (MCF) is an acute, fatal and sporadic disease caused by Gammaherpes virus affecting all artiodactyl ruminants and is commonly reported in cattle and buffaloes where mixed farming with sheep is practised. The morbidity rate in cattle is estimated at 15% to 100% and herd mortality rate at 60% to 100%. (Zakharova *et al*., 2020). In India, though first case of MCF was reported in 1975 (Parihar *et al.,* 1975), MCF remained undiagnosed for the most parts, since it occurs sporadically. In recent times, due to availability of molecular tools like PCR, more cases are being reported (Sood *et al*., 2014 and Vinod *et al*., 2014).

The present paper describes a case of sheep associated MCF in buffalo caused by Ovine herpes virus type 2 detected by heminested Polymerase Chain Reaction.

CASE HISTORY

A buffalo died in Badudandla

village, Panyam mandal, Kurnool district of Andhra Pradesh after showing high temperature for one week and not responding to antibiotics. Symptoms like severe conjunctivitis and hyper salivation were observed. On post mortem examination, multifocal haemorrhages were observed in all vital organs and lungs was oedematous and enlarged**.** No symptoms were observed in other animals in the herd. A sheep farm was located adjacent to the cattle shed.

MATERIALS AND METHODS

Nasal swabs, spleen and lung tissue samples were received from Animal Disease Diagnostic Laboratory, Kurnool. Initially, genomic DNA was extracted from all samples by using Nucleospin Tissue kit (Macherey-Nagel, Bioanalysis). Ovine herpes virus type 2 MCF heminested PCR was done by using three primers as described by Baxter *et al.* (1993). In first step PCR, 556 and 755 primers were used

556 primer (5'-AGTCTGGGTATATGAA TCCAGATGGCTCTC-3')

755 primer (5'-AAGATAAGCACCAGTT ATGCATCTGATAAA-3')

In second step PCR 556 and 555 primer pair was used.

556 primer (5'-AGTCTGGGTATATGAA TCCAGATGGCTCTC-3')

555 primer (5'-TTCTGGGGTAGTGGCG AGCGAAGGCTTC-3')

Both PCRs were standardised for 25µl reaction volume with 12.5 µl of master mix. (Emerald Amp GT PCR - Takara bio), 1 µl each of Primer pair (20pm/µl), 5 µl of template and 5.5µl of Nuclease free water. Thermal profile for both was set as 94°C for 5 min as initial denaturation and 94°C for 30 sec as denaturation, 60°C for 30 sec as annealing, 72ºC for 30 sec as extension for 35 cycles with a final extension at 72°C for 7 min. Finally, the PCR products were loaded in 1.5 % agarose gel for determining the product size.

Then DNA was also tested for IBR by using Real time PCR kit (Bovine Herpes virus 1, Ingenetix).

RESULTS AND DISCUSSION

Based on the case history submitted, the samples were tested for MCF and IBR. All the three samples tested were positive for MCF by Ovine herpes virus type 2 MCF heminested PCR and negative for IBR by RT PCR. All samples yielded specific amplicon of 422 bp with 556 and 755 primers and 238 bp with 556 and 555 primers as shown in Image 1. The PCR products matched to the reported size by Baxter *et al.* (1993).

Detection of viral DNA is declared

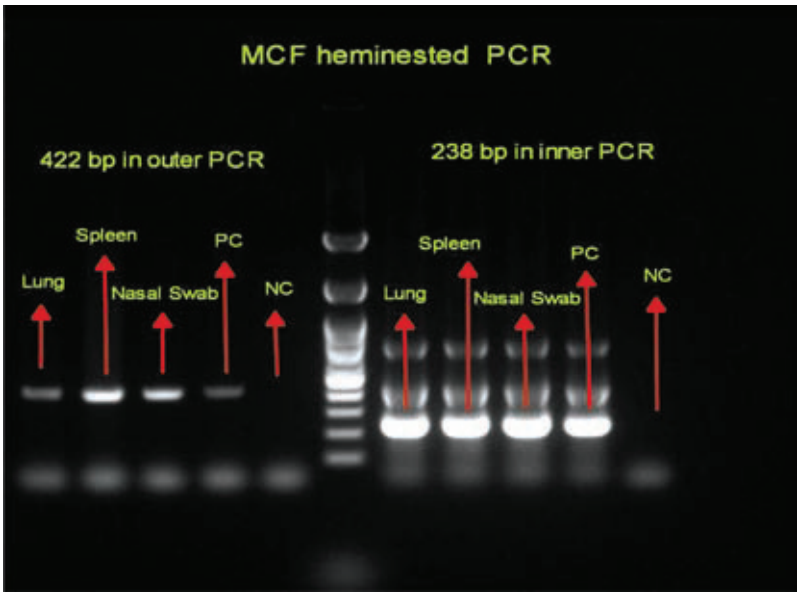

Image1. Heminested PCR for MCF (OvHV-2)

as one of the confirmatory test for MCF by OIE (OIE 2018). As heminested PCR is more specific than normal PCR, the results obtained are specific to MCF caused by Ovine herpes virus type 2. As a sheep flock was present in the same premises with the buffaloes, the disease might have spread from sheep. The same was reported by Vinod *et al*., 2014 that in Andhra Pradesh mixed livestock farming practices are very common and this may be the reason for sheep associated MCF outbreaks.

In this incident only one animal was affected with conjunctivitis and temperature for more than a week suggesting head and eye form of MCF, which is the most common form as per Pardon *et al*. (2009) and Russel *et al*. (2009). No symptoms were observed in other animals. The single case of MCF observed might be due to the fact that cattle act as dead end host and spread among cattle is generally not observed as reported by Sood *et al*. (2014) and Vinod *et al*., 2014.

CONCLUSION

The heminested PCR confirmed that the infection was from OvHV-2 MCF, probably spread from the sheep around. Further studies need to be done on the role of sheep as reservoir hosts. Though MCF occurs as single cases sporadically, economic losses are high due to acute and lethal nature of disease. Hence attention needs to be focussed on diagnosis and control measures as there are no vaccines and specific treatment available.

ACKNOWLEDGEMENT

The authors express their gratitude to the Animal Disease Diagnostic Laboratory, Kurnool for sending clinical material to VBRI, Vijayawada.

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