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Evidence of sheep associated malignant catarrhal fever in young calves of cross bred cattle from Andhra Pradesh

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Abstract
Malignant catarrhal fever is a fatal herpes viral infection of domestic and wild ruminants. Potential survival of the virus in the reservoir hosts including sheep, goat and wild beasts and association of various farm animals in a single area is responsible for occurrence in cross bred dairy cows and calves. During this study period fatal cases of sheep associated Malignant Catarrhal fever in calves were recorded from the state of Andhra Pradesh. Total of 19 calves of different age groups were shown fatal clinical signs of pyrexia, conjunctivitis, purulent nasal and lachrymal discharges, redness of muzzle with anorexia. Peripheral blood samples were collected from all the calves and further processed for separation of buffy coat layer. The viral DNA was extracted from peripheral blood leukocytes and subjected to PCR targeting the OIE approved tegument gene of OVH-2. Out of 19 blood samples screened, 12 samples were found positive by PCR amplifying 422bp of the tegument gene and confirmed the presence of OVH2 infection in young calves.

Keywords: MCF, bovine, blood samples, tegument gene, hemi nested PCR-confirmation

Introduction
Malignant catarrhal fever (MCF) is a fatal lympho proliferative disease of cattle and other ungulates caused by the ruminant gamma-herpes viruses, alcelaphine herpes virus-1 (AIHV-1) and ovine herpes virus-2, OVH-2 (Kirbas *et al*., 2013) [6]. Alcelephine herpes virus-1 (AIHV-1) naturally infects wild beast and persists as subclinical infection in wild beast and causes wild beast associated MCF (WA-MCF) in cattle in the regions of African subcontinent (Plowright, 1990) [10]. The ovine herpes virus -2 (OVH2) infects all varieties of domestic sheep as a subclinical infection and causes sheep associated MCF in susceptible ruminants in most regions of the world. These viruses cause unapparent infection in their reservoir host (wild beast for AIHV-1 & sheep and goat for OVH-2); however it becomes fatal lympho proliferative disease when they infect MCF-susceptible hosts (Bisons for AIHV-1 and cattle, water buffalo, bantengs, antelopes and pigs for OVH2).
Sheep associated malignant catarrhal fever is an emerging and an important disease of cattle and it is particularly significant in the Indian continent where mixed farming is a common practice. In India, there is mixed livestock farming system of cattle with sheep and goats which leads to increased chances of close contact of carrier animals with clinically susceptible animals. Transmission occurs among animals when the virus is excreted mainly through nasal and ocular secretions (Gelay *et al*., 2013) [4] and also reported in feaces and semen Hussy *et al*., 2002) [5].
Diagnosis of SA-MCF poses significant challenges to veterinarians due to multi systemic involvement of the disease and symptomatic resemblance to many other diseases in the field and its complex diagnosis (Priya *et al*., 2019) [19]. Hence, laboratory confirmation of clinical diagnosis of MCF is important. The diagnostic approaches may vary in different clinically affected animals and reservoir hosts due to biology of the MCF viruses and host responses to the viruses. Clinical signs, serology, Polymerase Chain Reaction, histopathology, isolation and identification may be used for detection of MCF virus or diagnosis of disease caused by the viruses. Viral DNA can be detected in clinical material from cases of MCF caused by OVH-2 using PCR. Hence, PCR is the method of choice for rapid diagnosis of the disease. Any animals that are suffering from MCF will have significant levels of viral DNA in their leukocytes and tissues to be readily detected by PCR. Present study reported the occurrence of MCF in young calves of cross bred cattle from Andhra Pradesh.

Materials and Methods

During April, 2019 to December, 2020, 19 young calves of cross bred cattle of different age groups were shown clinical signs of Temperature-104^{0}F, conjunctivitis, purulent nasal and lacrimal discharges, redness of muzzle with anorexia suspected for MCF. Out of 19 animals, 6 animals were died with similar clinical symptoms.

Sample collection

Whole blood was collected in EDTA vials from ailing animals and brought to the lab for processing. The diagnostic work has been carried out at State Level Diagnostic Laboratory, SVVU, Tirupati, Andhra Pradesh.

Separation of Buffy coat and DNA extraction

Fresh blood sample (1 ml) collected in EDTA vials was taken and mixed with equal volume of PBS, pH 7.2. To this mixture equal volume of lympho prep (Hi-media) was added slowly and centrifuged at 2,000 rpm for 30 minutes. Buffy coat layer was separated carefully from the mixture and stored at 4 ^{0}C for extraction of Viral Genomic DNA. Genomic DNA was extracted from the peripheral blood leucocytes (PBL)/buffy coat using Qiamp blood mini DNA extraction kit (Qiagen, USA) as per manufacturer' s protocol and the DNA eluted was stored at -20 ^{0}C for PCR template. For detection of infection, PCR was performed with primers (Table.1) coding the tegument gene of OVH-2 as per the method described by Baxter *et al*, 1993 [2].

Table 1: Details of Primers used for partial amplification of tegument protein of OVH2

Primer name	Primer sequence	Reference
556-forward	**AGTCTGGGTATATGAATCCAGATGGCTCTC**	Baxter *et al*. (1993) [2]
755-Reverse	AAGATAAGCACCAGTTATGCATCTGATAAA	

PCR protocol and cyclic conditions

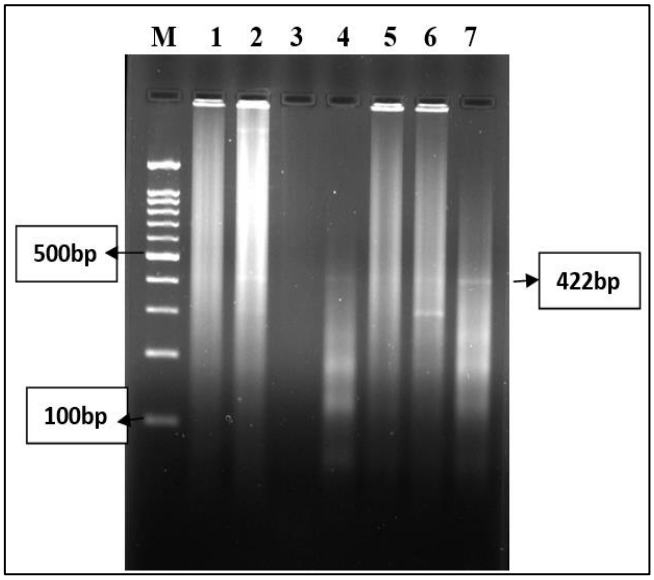
PCR for detection of OvHV-2 sequences was performed according to the amplification reaction cycles described by Muller-Doblies *et al*, 1998 [8] with little modifications. The amplification reaction used 25pico moles each of the primer set (556 and 755) designed by Baxter *et al*, 1993 [2]. The total reaction mixture was prepared for 25µl consisting of 2.5µl of 10x PCR buffer; 0.5µl of dNTP's (10mM); 4µl of Ammonium sulphate (16mM); 2µl of DMSO (10%),1.5µl $MgCl_2$(25mM); 5U of Taq DNA polymerase (Bangalore genei);10µl of Template DNA and remaining volume were made with nuclease free water. A buffy coat sample from a healthy calf was used as Negative template control (NTC). The reaction conditions include denaturation of DNA at 94 ºC for 2 min followed by 39 cycles of denaturation at 94 ºC for 20sec; annealing at 60 ºC for 30seconds and 72 ºC for 30sec extension step and a final terminal extension step at 72 ºC for 5 min.

Amplified PCR products were resolved by 2% agarose gel electrophoresis with added ethidium bromide at a concentration of 0.5µg/ml. The DNA fragments were documented with a gel documentation system (Bio-Rad).

Results

PCR amplification of tegument gene of MCF virus

Blood samples were collected in EDTA vials from bovine calves with symptoms of high fever (104^0 F), conjunctivitis, purulent nasal and ocular discharges, redness of muzzle and anorexia suspected for MCF. The blood samples were processed and buffy coat layer was separated. Viral genomic DNA was extracted and subjected to PCR targeting the tegument gene of MCF from the blood of infected animals. Out of 19 blood samples, twelve samples were found positive for MCF amplifying OVH-2 tegument encoding gene yielding 422bp (fig.1) and confirmed the presence of MCF in bovine calves.



Lane-M-100bp DNA ladder
Lane-1-Negative control
Lane-2-Positive control
Lane-3-PCR control
Lane-4 to7 – Samples showing 422bp amplification of partial tegument gene of OVH-2

Fig 1: PCR amplification of tegument gene of MCF

Discussion

The occurrence of MCF in India was confirmed and reported by Parihar *et al*, 1975 [9]. Later after long gap few incidences of SA-MCF in cattle and bison (Wani *et al.*, 2004; Sood *et al*., 2012) [14] and OVH-2 infection in sheep were reported (Wani *et al.*, 2006; Banumathi *et al*., 2008) [15, 1]. Due to sporadic occurrence of the disease many cases were under diagnosed in cattle during past years. But recently many cases of MCF in cattle were reported due to availability of sensitive and specific molecular tools. Both conventional and quantitative real time PCR assays have been developed for the detection of OVH-2 and AIHV-1 viral DNA (Baxter *et al*., 1993; Hussy *et al*., 2001; Traul *et al*., 2005; Cunha *et al*., 2009) [2, 13, 3].

In the present study, PCR was used to detect MCF from blood (Buffy coat) of infected calves. Earlier, Baxter *et al*, 1993 [2] used hemi nested PCR targeting the OVH-2 tegument gene and detected MCF from the blood of infected bovines. The OIE approved nested PCR was found to be 10 fold more sensitive than quantitative PCR. However, real time PCR assays have the potential to define viral loads in a range of tissues from both natural and MCF susceptible hosts. Conventional PCR is very useful and easy test for routine diagnosis of clinical cases of MCF. PCR also found to be more sensitive than CI-ELISA either in reservoir or susceptible host (Li *et al*., 1995a; Muller Dobles *et al*., 1998) [7, 8].

The use of PCR allows sensitive confirmation of the presence of MCF viruses in infected animals. Further, PCR is used not only for diagnosing the clinical cases from organized and unorganized farm but also useful for phylogenetic and epidemiological studies during the disease outbreaks.

Conflict of Interest: None.

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