An outbreak of malignant catarrhal fever in Murrah buffaloes in Minas Gerais, Brazil[1]

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ABSTRACT.- Costa E.A., Bastianetto E., Vasconcelos A.C., Bomfim M.R.Q., Fonseca F.G., Gomes A.D., Leite R.C. & Resende M. 2009. **An outbreak of malignant catarrhal fever in Murrah buffaloes in Minas Gerais, Brazil.** *Pesquisa Veterinária Brasileira 29(5):395-400.* Departamento de Microbiologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, Belo Horizonte, MG 31270-901, Brazil. E-mail: lvc@icb.ufmg.br

An outbreak of Malignant Catarrhal Fever (MCF) resulted in death of five female buffaloes and one domestic cow from the same farm. Four buffaloes died 10-15 days after the appearance of clinical signs, while the fifth was euthanized *in extremis,* after similar clinical signs. Histopathological lesions included multifocal histiolymphocytic epicarditis, myocarditis and lymphocytic interstitial pneumonia, which are commonly seen in cases of MCF in buffaloes. Furthermore, lymphocytic vasculitis centered in the adventitia, with occasional fibrinoid necrosis in the muscular layer, was found in the kidneys, liver, spleen, lymph nodes and brain. Nucleotide sequencing of DNA fragments from the central nervous system amplified by PCR revealed 98% similarity with known OHV-2 sequences from Genbank. Additionally, PCR analysis also revealed the presence of OHV-2 DNA in the peripheral mononuclear blood cells of two clinically healthy buffaloes. The diagnosis of MCF was based on epidemiological, clinical, gross and histopathological findings and on the results of a *semi-nested* PCR followed by nucleotide sequencing.

INDEX TERMS*:* Diseases of buffaloes, viral diseases, gammaherpesviruses.

RESUMO.- [**Surto de febre catarral maligna (FCM) em búfalos da raça Murrah no estado de Minas Gerais.**] É relatado um surto de febre catarral maligna (FCM) em Minas Gerais, que resultou na morte de 5 búfalas e uma vaca de uma mesma propriedade. Quatro búfalas morreram com 10-15 dias após o início dos sinais clínicos e uma búfala foi sacrificada *in extremis,* após manifestar sinais clínicos semelhantes. O exame histopatológico revelou lesões comumente observadas em búfalos com FCM como epicardite e miocardite histiolinfocítica multifocal e pneumonia linfocítica intersticial. Além disso, vasculite linfocítica, principalmente na camada adventícia, com necrose fibrinóide da camada muscular, foi observada no rim, fígado, baço, linfonodos e cérebro. A seqüência de nucleotídeos amplificada pela técnica de PCR revelou 98% de homologia entre o fragmento de DNA amplificado da amostra do sistema nervoso central (SNC) da búfala com seqüências de OHV-2 previamente depositadas no Genbank. Adicionalmente, a técnica de PCR revelou a presença do DNA viral no sangue total de 2 búfalas, clinicamente sadias. O diagnóstico de FCM foi baseado em dados epidemiológicos, clínicos, patológicos, histopatológicos e *semi-nested* PCR.

TERMOS DE INDEXAÇÃO: Doenças de búfalos, doenças virais, gammaherpesvirus.

INTRODUCTION

Malignant catarrhal fever (MCF) is a pansystemic, often fatal, viral disease that occurs as a complex of syndromes

affecting mainly ruminant species, such as domestic cattle, water buffalo, American bison and many cervids (Li et al. 2006, Anderson et al. 2007). MCF has long been a major problem among deer farmed in New Zealand and Norway, Indonesian swamp buffaloes in Australia and New Zealand, grazing cattle in Africa and American bison in North America (Li et al. 2006).

Several members of the MCF virus complex (MCFV) within a group of closely related ruminant rhadinoviruses (Gammaherpesvirinae) are currently linked to clinical MCF in ruminant species. The MCF-associated viruses occur endemically in latently infected carrier species that spread infection to MCFV-susceptible species. MCFV include the well-known alcelaphine herpesvirus 1 (AHV-1) and ovine herpesvirus 2 (OHV-2), and the recently identified caprine herpesvirus 2 (CpHV-2), which are respectively endemic in wildebeest, domestic sheep, and domestic goats. AHV-1 causes the classic African form of MCF, or wildebeest-associated MCF (WA-MCF), and OHV-2 is the causative agent of sheep-associated MCF (SA-MCF). Both occur worldwide in a range of ruminants while CpHV-2 has been found to cause serious disease only in deer (Li et al. 2003, Vikøren et al. 2006).

In Brazil, MCF has been reported in cattle (Barros et al. 1983, Garmatz et al. 2004, Rech et al. 2005) and deer (*Mazama gouazoubira)* (Driemeier et al. 2002), both linked to OHV-2 infection. MCF caused by AHV-1 or CpHV-2 has not yet been found in the country.

MCF is characterized by fever, depression, anorexia, lymphadenopathy, corneal opacity, ocular and nasal discharges, and central nervous system involvement. Histopathological lesions include generalized necrotizing vasculitis, multisystemic lymphoreticular proliferation, fibrinoid changes, and mononuclear inflammatory infiltrate in a variety of tissues (Hoffmann et al. 1984a,b, Powers et al. 2005).

The severity and distinctive features of an MCF outbreak is dependent on the susceptible species involved. In general, disease occurs sporadically among most ruminants, and is rarely seen among European breeds of cattle (*Bos taurus*) and swamp buffaloes. In contrast, indigenous Bali cattle, American bison and some cervid species are highly susceptible (Li et al. 2006).

Cattle and deer commonly develop the head and eye form of MCF characterized by bilateral keratoconjunctivitis, corneal opacity, fibrin clots in the anterior eye chamber, mucopurulent nasal discharge, encrusted muzzle and lymphadenopathy. In contrast, a fatal enteric clinical syndrome without vascular fibrinoid necrosis was observed in bisons (O'Toole et al. 2002). Additionally, the lymphoid infiltrates in the liver and kidney, and lymphoreticular proliferation of lymph nodes was less severe in bisons than is usually seen in cattle and deer (Schultheiss et al. 1998, Vikøren et al. 2006).

There are few reports of MCF in swamp buffaloes (Mohan 1968, Hill et al. 1993, Tham 1997). Common clinical signs in buffaloes include anorexia, high fever, depression and poor body condition (Hoffmann et al. 1984b). Necrosis and erosivo-ulcerative lesions of oral cavities are often not present. Neurological signs include ataxia and severe convulsions which lead to the death of the animal. Diarrhea and hematuria are also seen in buffalos (Hill et al. 1993, Tham 1997). MCF has not been documented in swamp buffaloes in Brazil. However, clinical diagnosis is difficult because this disease have several clinical forms. Therefore, the disease may be under-diagnosed in swamp buffaloes in Brazil.

The purpose of this paper is to describe an outbreak of MCF in Murrah buffaloes on a farm located in the state of Minas Gerais, Brazil. Diagnosis was based on clinical, macroscopic and histopathologic findings, and primer-specific semi-nested PCR assays for the detection of the partial tegument protein gene of OHV-2.

MATERIALS AND METHODS

We observed six spontaneous cases of MCF on one farm in Esmeraldas, Minas Gerais, Brazil. Necropsies were performed on five buffaloes and one domestic cow. Skin, lymph node, brain, eye, lung, heart, intestine and kidney samples were collected. Tissues were fixed in buffered 10% formalin, routinely processed for histology, and stained with haematoxylin and eosin.

For PCR assay, tissue, blood, and nasal mucous samples were collected from all sick animals and from healthy buffaloes at the time of the necropsy procedure, and were shipped on ice (chilled, not frozen) to the laboratory. Blood samples were taken from the jugular veins of ten sheep (of 50 total) sharing water and food with buffaloes and cattle via EDTA tubes. The procedures for DNA preparation were described previously (Boom et al. 1990). Peripheral blood leukocytes (PBL) were extracted from the sheep whole blood using the ammonium chloride lysis method (Toth et al. 1992). Purified DNA was quantified by a spectrophotometer at 260 nm (Gene Quant, Pharmacia). The PCR assay for detection of OHV-2 DNA was performed with the use of a nested protocol with primer sets targeting OHV-2 genetic sequences gene as described by Baxter (Baxter et al. 1993) using the primer sets: 556 (59-AGTCTGGGGTATATGAATCCAGAT GGCTCTC-39) and 775 (59-AAGATAAGCACCAGT TATGCATCTGATAAA-39) in the first step and primer sets 556 and 555 (59-TTCTGGGGTAGT GGCGAGCG AAGGCTTC-39) in the second step. All amplification reactions were performed in a 25 μl volume containing 10 mM Tris-HCl (pH 8.0); 2 mM MgCl2; 200 mM (each) dATP, dCTP, dGTP, and dTTP (Invitrogen-BRL.); 20 pM (each) primer; 2 U of *Taq* DNA polymerase (Invitrogen-BRL.) and 200 ng of extracted DNA as template. Thermal cycling conditions for both steps consisted of 5 min at 95ºC followed by 30 cycles of 94ºC for 1 min, 46ºC for 1 min, and 72ºC for 1 min; and a final 7 min at 72ºC for extension. Two microliters of the PCR mixture from the first step was used as target for the amplification in the second step. Amplification of the targeted OHV-2 polymerase gene results in a 238-bp product in the semi-nested reaction. To analyze 10 μl of the amplified PCR products, we added 1.5% agarose gel electrophoresis followed by ethidium bromide staining**.** An internal control of the amplification efficiency and oligonucleotide primers for the mammalian GADPH sequence was utilized (Shi & Roy-Burman 2000). The standard positive control was cloned in a previously study (unpublished), and

contained a 238 bp fragment of OHV-2 DNA obtained from PBL of a sheep.

The products of semi-nested PCR were sequenced three times in forward and reverse directions using ET Dynamic Terminator for MEGABACE (GE HealthCare, UK). Sequencing was carried out in a MEGABACE automated capillary DNA sequencer (GE HealthCare, UK). The nucleotide sequences were assembled using the CAP3 Sequence Assembling Program (http://asparagin.cenargen.embrapa.br/phph/). The sequence alignment between the detected fragment and OHV-2 previously described (accession number AY839756) revealed a similarity rate of up to 98%. The obtained partial sequence of the tegument protein coding gene was deposited in GenBank (Buffalo Strain, accession number EF199761).

RESULTS

Epidemiology

The farm possessed a total of 216 ruminants (145 buffaloes, 21 cattle, and 50 sheep) which grazed together at a common grass pasture. After clinical signs began, conditions worsened in five bufalloes, despite parenteral treatment with antibiotics (Tetracycline 50 mg/10 kg/BW), and four buffaloes died within 10 and 15 days later. One domestic cow showing clinical signs also died, and one sick buffalo was euthanized *in extremis*. For the historic period, the rate of morbidity and mortality caused by MCF in buffalo population were identical with 3.45% and the rate of mortality was 100%.

Clinical observations

Buffaloes sharing stables and meadows with sheep showed signals of anorexia and pyrexia (< 41ºC) after a clinical course of two to three days. Animals died between 10 and 15 days after the first sign of disease was noted. The first clinical signs were slight depression and fever (over 41ºC). Two or three days later, the buffaloes had bilateral ocular discharge, corneal edema and opacity, conjunctival and scleral hyperemia and photofobia. The animals showed severe mucopurulent nasal discharge (Fig.1), accompanied by drooling and red oral mucosa. Animals became anorexic and presented with severe weight loss. The cow showed the head and eye form with nasal and ocular discharges progressing from serous to mucopurulent and purulent. There were encrustation of the muzzle, intense hyperemia and multifocal or diffuse necrosis of the oral mucosa (usually on the lips, gums, and hard and soft palate). One week later, it was recumbent, with hyperventilation and tachycardia, and died on the next day.

Gross findings

Necropsy was performed 8 hours after the death of the buffaloes and in general the carcasses were in poor body condition. The mucosas of the respiratory tract, including nasal, pharyngeal and tracheal mucosa were hyperemic and covered by mucopurulent exudate. Ulcerative stomatitis, glossitis, pharyngitis, esophagitis, rumenitis-abomasitis were found. Petechiae were seen over the

Fig.1. Affected buffalo showing mucopurulent nasal discharge.

entire mucosa of the small intestine. In addition, rumen and abomasum were distended with gas, and their mucosa were congested and edematous.

All lymph nodes were enlarged and edematous on cut surface. Also, large amounts of fibrinous fluid were observed in the articular cavities. The lungs were diffusely reddened and had generalized emphysema and petechial hemorrhages scattered on the capsular surface and throughout the parenchyma. There were numerous small white foci on the capsular surface and in the parenchyma of liver and spleen. The kidneys were enlarged, friable and with numerous white foci on the cut surface. In the heart, multiple inflammatory infiltrate composed of macrophages and lymphocytes were observed in the epicardium and myocardium.

In the domestic cow, the main lesions observed were multifocal lymphadenopathy, cranial lobe consolidation of the lungs, abundant ocular and tracheal purulent exudates and bilateral opacity of cornea.

Histopathological findings

Lymphocytic vasculitis, mainly in the adventitia, with occasional fibrinoid necrosis of in the muscular layer of the arterioles, was found in the kidneys, heart, liver, lungs, spleen, lymph nodes and brain. The heart showed multifocal mononuclear myocarditis primarily near the epicardium (Fig.2). A severe multifocal mononuclear glomerulonephritis with predominant adventitial lymphocytic vasculitis and prominent fibrinoid necrosis (Fig.3), was found in the kidneys. The spleen showed lymphoid depletion (Fig.4), congestion and vasculitis. Acute bronchopneumonia with areas of hyperemia, exudation of polymorphonuclear neutrophils within the alveoli, thickened interalveolar septa, and hemorrhage was evident in the lungs. A nonsuppurative meningoencephalitis (Fig.5), characterized by lymphocytic perivascular cuffing, was detected in the cerebrum and cerebellum. In addition to these findings, one buffalo showed signs of amyloidosis in the liver, as evidenced by interstitial deposition of a flocculent amorphous eosinophilic Congo-red positive material (Fig.6).
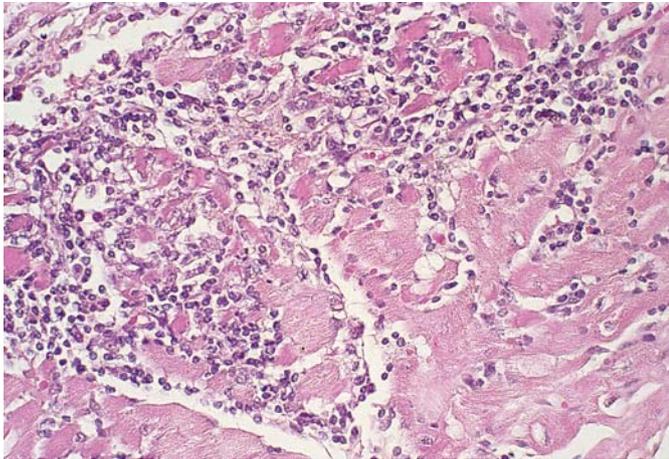
Fig.2. Multifocal mononuclear myocarditis, mainly near the epicardium, in a natural case of malignant catarrhal fever in a buffalo. HE, obj.60x.

In the cow, microscopic changes included diffuse vasculitis in the cranial lobe of the lungs. The alveolar septa were thickened with a large number of lymphocytes and fibrin strands. The corneal epithelium was thickened and showed focal intracytoplasmic vacuolation. The sclera was congested with multifocal infiltrates of mononuclear cells and vasculitis. In addition, the scleral stroma was proliferating underneath the cornea, increasing its thickness and opacity.

PCR results

DNA samples from buffaloes with clinical signs of MCF (n=5), buffaloes without clinical signs of MCF (n=11), and sheep (n=10) were tested by PCR for OHV-2. For all 5 sick buffaloes, the PCR generated a 238 bp amplicon identical to the positive control. Two out of 11 healthy buffaloes and 8 of the 10 sheep were also positive. These findings suggest that the virus causing the disease in this particular outbreak was indeed the OHV-2. A control PCR
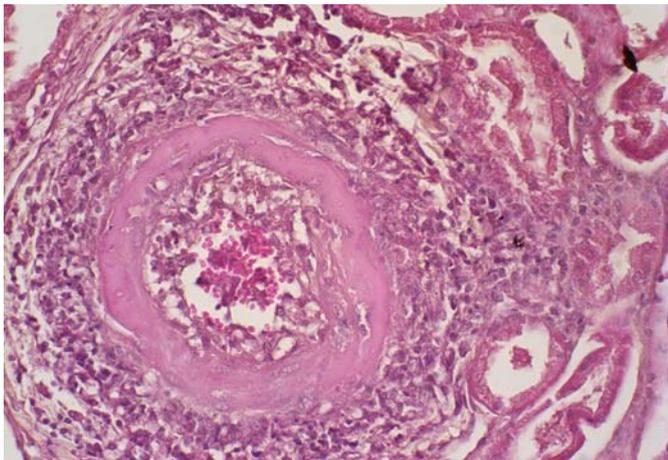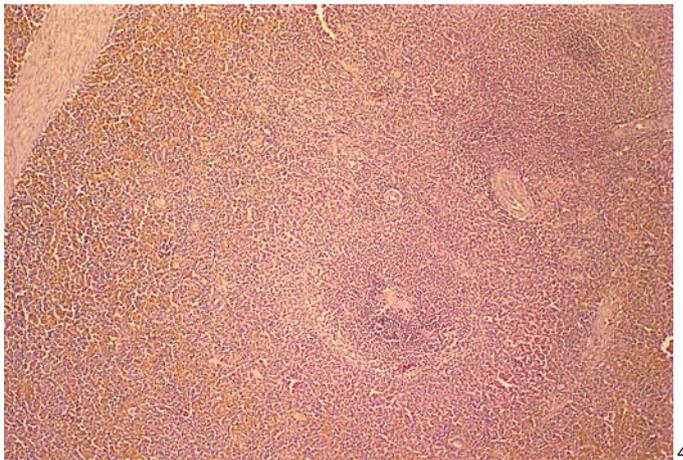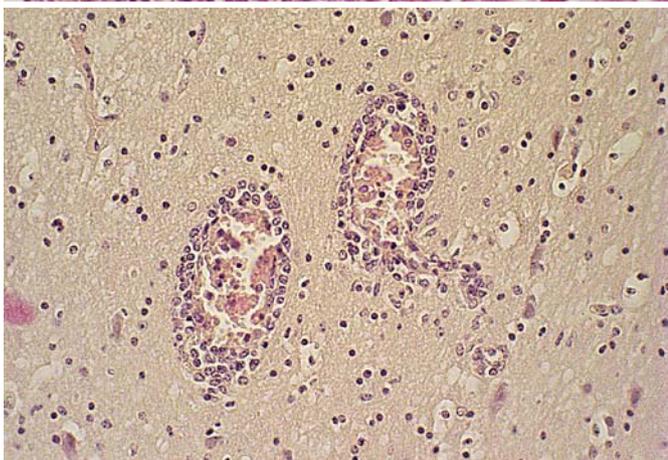
Fig.3. Lymphocytic vasculitis, mainly in the adventitia in the kidney of a buffalo naturally affected by malignant catarrhal fever. HE, obj.60x.
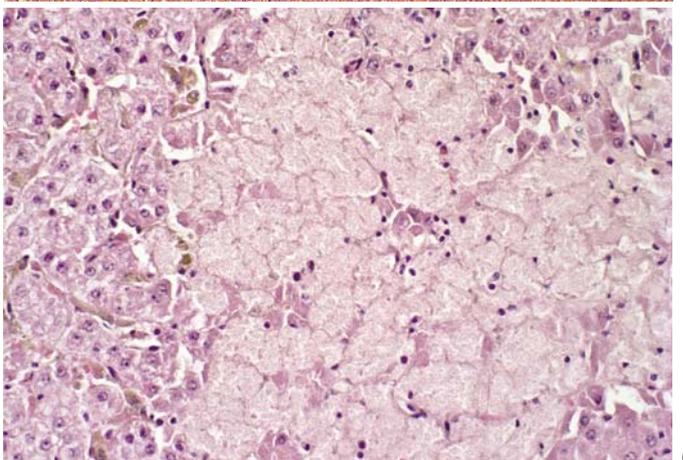
Fig.4. Lymphoid depletion and congestion in the spleen of a buffalo naturally affected by malignant catarrhal fever. HE, obj.20x.

Fig.5. Non-suppurative meningoencephalitis in the cerebellum of a natural case of malignant catarrhal fever in buffalo. HE, obj.60x.

Fig.6. Hepatic amyloidosis in by malignant catarrhal fever affected buffalo. HE, obj.60x.

detecting mammalian GADPH DNA was positive for all tested samples.

DISCUSSION

The clinical signs, macroscopic and histopathological findings, and the specific nested PCR, were consistent with malignant catarrhal fever caused by OHV-2 during the outbreak in Minas Gerais, Brazil. We believe that the sheep were the probable source of infection, because we detected OHV-2 DNA in samples from 8 out of 10 animals. These sheep were housed together and grazed in the same pasture with the sick buffaloes and cow. Co-housing ruminants (sheep, cattle and buffaloes) is still a common practice on farms in Minas Gerais. Obviously, this represents a risk for clinically susceptible cattle and buffaloes.

Unique features were observed in this outbreak in buffaloes such as the corneal opacity, serous to purulent nasal discharges, the complete lack of erosions in the oral cavity and the large quantities of fibrinous fluid in the articular cavities. The first three signs above have been associated with MCF in cattle, but the latter has not been previously noted as part of the MCF syndrome in cattle, although cases of nonsuppurative arthritis associated with MCF had been reported in buffaloes (Borghese 2005).

In the present study, vasculitis with characteristic adventitial mononuclear cell infiltration and fibrinoid necrosis of the tunica media was seen in the arterioles, and are consistent lesions for MCF in cattle. However, buffaloes rarely show vasculitis progressing beyond adventitial infiltration and involving the vascular wall (Hoffmann et al. 1984b). The multifocal myocarditis characterized by lymphocytic infiltrate dissociating myofibers close to the epicardium were also seen in buffaloes from many related outbreaks such as an Indonesian outbreak (Hoffmann et al. 1984b), in New Zealand (Hill et al. 1993) and in experimental infections of cattle (Mirangi & Kang'ee 1991). A marked lymphocytic bronchopneumonia has been reported in cases of MCF in buffaloes (Hoffmann et al. 1984a; Hill et al. 1993). In accordance with Hill et al. (1993), this lesion is probably due to vasculitis of the capillaries in the alveolar septa. Through experimental transmission of MCF from buffaloes to cattle it has been demonstrated that the lesions observed in the heart and lung of buffaloes do not occur in cattle. Those lesions might be considered specific features of MCF in buffaloes since the disease is multisystemic (Liggitt & DeMartini 1980).

In this work, we used primers derived from the tegument protein gene for the PCR assays. A fragment of 422 base pairs (bp) was amplified initially, followed by amplification of a truncated internal fragment of 238 bp. We obtained specific amplicons of OHV-2 in all samples from sick buffaloes, two healthy buffaloes and eight sheep. It has been proven that this test is capable of detecting as few as 35 viral genome equivalents, and that no product is nonspecifically amplified from AHV-1 or other bovid herpesviruses (Baxter et al. 1993). This PCR is thus both highly specific and sensitive for OHV-2, and has been employed worldwide in studies of the disease in clinically affected animals and the natural host. It is emerging as a robust test that can be employed to detect viral DNA in peripheral blood leukocytes of clinically affected animals as well as fresh tissues and paraffin-embedded samples collected at post-mortem (OIE 2004).

Detection of OHV-2 in PBL from two healthy buffaloes might indicate a prolonged period of incubation, or the establishment of an asymptomatic or even carrier state. Currently, cattle that have recovered from MCF remain chronically infected by OHV-2, suggesting that they can be a horizontal and vertical source of infection in the herd (Garmatz et al. 2004). On the other hand, although buffaloes are closely related to cattle, certain conditions are peculiar to buffaloes and the transmission of OHV-2 among them still needs to be elucidated (Hoffmann et al. 1984b).

Malignant catarrhal fever has emerged as a significant problem in the commercial buffalo industry. Although control of the disease represents a challenge to owners and veterinarians, the recognition of MCF in highly susceptible farmed species is an opportunity to characterize OHV-2 transmission pattern. The control strategy should be to separate sheep from susceptible animals such as buffaloes and cattle.

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