Bovine malignant catarrhal fever: case reporting in Central Italy

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Summary
A case of malignant catarrhal fever (MCF) occurred in a 4-month-old calf housed in a semi-intensive herd in central Italy is described. The herd was in strict cohabitation with a group of domestic sheep. The calf displayed clinical signs that resembled the acute form of MCF and, after a few days of antibiotic and anti inflammatory therapy, died in September 2016. The diagnosis was confirmed *in vivo* in blood by detection of ovine herpesvirus type 2 DNA through real-time PCR. At necropsy, the gross post-mortem findings were typical of MCF and the histological and molecular assays confirmed the presence of the virus. The sheep flock was suspected to be the source of the infection. In Italy, as well as in Europe, there is little data regarding the epidemiology and the recurrence of the disease in herds of cattle, due to the lack of an active surveillance plan and to a major consideration of MCF between differential diagnosis.

Febbre catarrale maligna in un bovino dell'Italia centrale

Parole chiave
Ovine herpesvirus 2,
Bovini,
Pecora,
Italia,
Febbre catarrale maligna.

Riassunto
Viene descritto un caso di febbre catarrale maligna (FCM) in un vitello di razza Chianina di 4 mesi presso un allevamento semi-intensivo in Italia centrale. Nella stessa stalla era stabulato un gregge ovino. Al momento dell'insorgenza della sintomatologia all'esame clinico il vitello mostrava depressione del sensorio, diarrea, dimagrimento, febbre, scolo nasale, epifora, pallore delle mucose apparenti, rafforzamento del murmure vescicolare della porzione apicale dei polmoni. Nel settembre 2016, dopo 10 giorni dall'insorgenza della malattia, l'animale veniva a morte. La diagnosi è stata effettuata *in vivo* nel sangue rilevando il DNA di *ovine herpesvirus* di tipo 2 attraverso un saggio di *real-time PCR*. Alla necroscopia, i reperti post mortem erano tipici di FCM e i test istologici e molecolari hanno confermato, infatti, la presenza del virus. Si presume che la fonte dell'infezione sia da rintracciare nel gregge di pecore con cui i bovini condividevano la stalla. In Italia, così come in Europa, sono disponibili pochi dati sull'epidemiologia e sulla ri-emergenza della malattia negli allevamenti bovini. L'intento della seguente indagine è quello di arricchire la bibliografia già presente di segnalazioni riferibili a questa patologia e di sensibilizzare i colleghi veterinari ad includere la FCM in diagnosi differenziale con altre patologie.

Malignant catarrhal fever (MCF) is a systemic viral disease of domestic and wild ruminants such as deer, bison and buffalo, and occasionally pigs. The disease is spread worldwide and it is caused by six viruses belonging to the subfamily *Gammaherpesvirinae*, genus *Macavirus* including alcelaphine herpesvirus 1 (AlHV-1), ovine herpesvirus 2 (OvHV-2), caprine herpesvirus 1 (CpHV-2), ibex malignant catarrhal fever virus, alcelaphine herpesvirus 2 like virus and a virus responsible for MCF in deer (Modesto *et al.* 2015). The most prevalent virus in Europe is OvHV-2 which is associated with outbreaks of the disease in domestic cattle. Sporadic cases are usually observed in European cattle breeds (*Bos taurus*), because they

are a relatively resistant species. Bali cattle, bison, and some but not all cervid species (eg., white-tailed deer, Pere David's deer) instead are highly susceptible to the virus (Wiyono *et al.* 1994, Berezowski *et al.* 2005). MCF outbreaks in herds typically have low morbidity and high mortality rates, and generally affect younger individuals (8-24 months). A feature of MCF, with regards to cattle, is that many outbreaks are sporadic and affect a single or only a few individuals in the herd. However, occasionally there are more serious outbreaks that can affect up to 40% of the herd.

The disease has been reported worldwide, including North and South America (Rech *et al.* 2005), Europe (Yus *et al.* 1999), the Middle East (Abu Elzein *et al.* 2003), Asia (Dabak and Bulut 2003), Africa (Rossiter 1981) and New Zealand (Wilson 2002). In Italy, few cases of MCF have been described over the past 20 years; in 2003 Decaro and colleagues (Decaro *et al.* 2003) reported the first two cases of MCF in the Country that were confirmed by PCR assays; in 2015 a case of MCF was also described in a captive pudu (*Pudu puda*) by Modesto and colleagues (Modesto *et al. 2015)* and, more recently, in 2017 a MCF outbreak was reported in two water buffalo farms in Southern Italy (Amoroso *et al.* 2017). Moreover, other cases have been described in cattle and in bison increasing the attention to MCF (Campolo *et al.* 2008, Grattarola *et al.* 2011). However, even if sporadic, the prevalence of the disease seems to be underestimated in cattle. This is probably due to the lack of reports and, in some cases, of accurate diagnosis. To the best of the authors' knowledge, there are no reports of MCF in Central Italy.

The study describes a case of MCF in a 4-month-old Chianina calf. The case occurred in August 2016. The calf belonged to a semi intensive herd of 15 Chianina and mixed-breed individuals, living in close proximity with a group of domestic sheep. Clinical symptoms started in August 2016 when the calf showed hyperthermia (41 °C), anorexia, reluctance to stand, depression, ocular and nasal discharge, lacrimation, generalized lymph nodes enlargement, diarrhoea, bilateral keratoconjunctivitis. Auscultation of the lungs assessed an increased pulmonary murmur of the apical lobes of the lungs. The animal died ten days after the onset of the symptoms. Serum sample and nasal swab were collected intra vitam for laboratory testing. The nasal swab was analyzed by end point PCR and real-time PCR for *Histophilus somni, Mycoplasma* spp., bovine respiratory syncytial virus, bovine coronavirus, bovine viral diarrhea virus, bovine parainfluenza type 3 virus (Moustacas *et al.* 2013, Lierz *et al.* 2007, Vilcek *et al.* 1994, Decaro *et al.* 2008, OIE 2018, Thonur *et al.* 2012). The serum sample was analyzed by ELISA for bovine parainfluenza type 3 virus, bovine coronavirus, bovine respiratory syncytial virus, bovine herpesvirus type 1and bovine viral diarrhea virus antibodies (Prima Check® PI-3 Ab, Agrolabo Spa Scarmagno - Italy; Svanovir® BCV Ab, Boeringer Ingelheim Svanova, Uppsala, SE; Prima Check® BRSV, Agrolabo Spa, Scarmagno - Italy; Ab IDEXX® BVDV p80 Ab, IDEXX Laboratories, Westbrook, ME), whereas nucleic acids purified from the blood sample were tested by real-time PCR for the detection of OvHV-2 (Traul *et al.* 2004). PCRs performed on the nasal swab resulted constantly negative. The blood sample was also analyzed also for the determination of total protein concentration and biochemical values. Analysis of the blood sample revealed no significant alteration regarding total protein concentration and biochemical values but revealed the presence of OvHV-2 DNA by real-time PCR (Ct 27.84).

Samples from different tissues were collected for molecular analysis. In details, spleen was analyzed by end point RT-PCR and real-time PCR for detection of bovine viral diarrhoea virus and OvHV-2, respectively. real-time PCR on nucleic acids purified from spleen resulted positive for OvHV-2. Our genetic study was based on the ORF50 characterization (Russell *et al.* 2014). Viral DNA was extracted from blood sample previously identified as OvHV2-positive. The purified amplicons were sequenced on both strands. Nucleotide sequences were aligned using Clustal W algorithm. Manual editing was performed using BioEdit software, version 7.0. The phylogeny was estimated using the neighbour-joining algorithm (NJ).

The strain described in this study (36409/UM/2016; GenBank accession number LR697102) shared a 99.80 % identity to strain KU499856.1/iTcal allele, previously identified in two water buffalo farms in Southern Italy (Amoroso *et al.* 2017). The products of the polymerase chain reaction of the expected size (444 nt) was obtained after amplification with the primer pair designed for the ORF50 region. The topology of the tree indicated that the isolate clustered into known genotypes (Figure 1).

The post mortem examination showed severe bilateral keratoconjunctivitis. Grossly, enlarged lymph nodes and mucosal erosive and ulcerative lesions on the gastrointestinal tract were observed (Figure 2).

Renal congestion and necrohemorrhagic cystitis were also detected. Lastly, lung showed mild oedema and congestion of the anteroventral lobes. Bacteriological examination was carried out on visceral tissue samples by culturing on blood agar, MacConkey agar and Mannitol salt agar but the samples did not showed any significant pathogenic bacteria.

Tissue samples from the spleen, omasum, abomasum, larinynx, liver, kidney, urinary bladder, brain, eye globes, mesenteric lymph nodes, prescapolar lymph

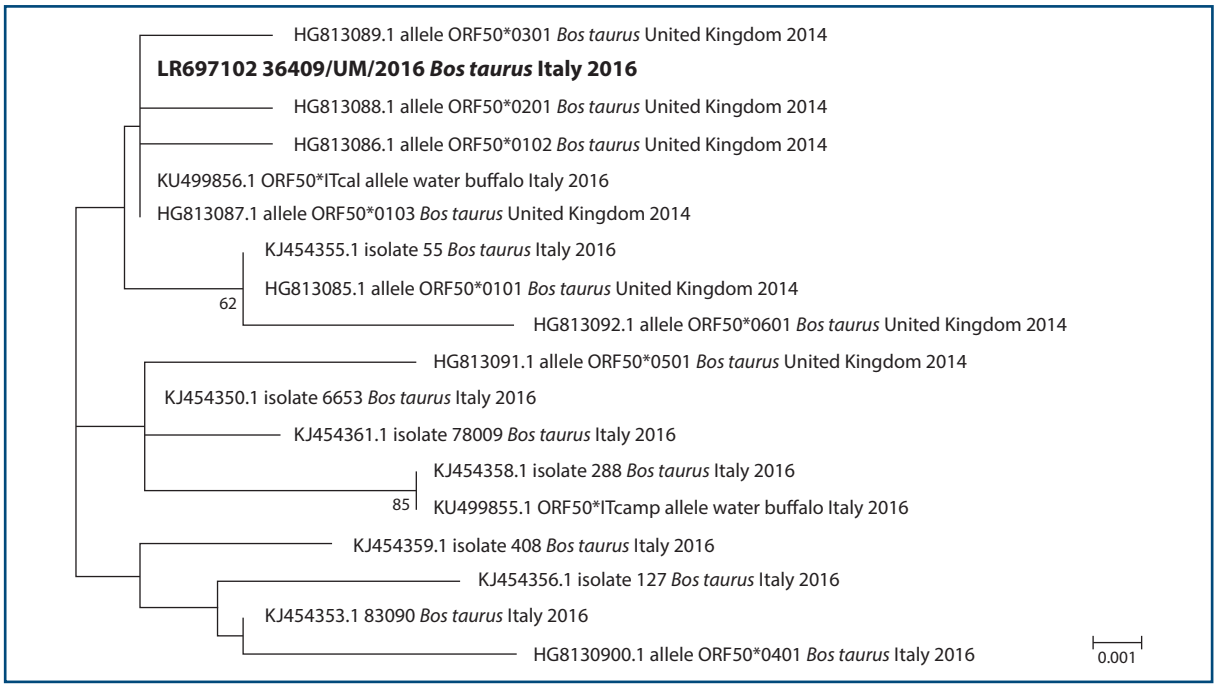

Figure 1. *Phylogenetic tree based on ORF50 sequences.* Phylogenetic analysis was performed on an alignment of 420 nucleotides with MEGA7 using the NJ method. Distances were computed using the Kimura two-parameter model. Bootstrap values higher than 60% are shown for 10,000 replicate data sets. The tree describes the relationship between selected ORF50 sequences retrieved from the GenBank database and the Italian novel nucleotide sequence (36409/UM/2016; GenBank accession number LR697102) labelled in bold. Bar number of substitutions per site.

nodes and mediastinal lymph nodes were fixed in 10% neutral buffered formalin and embedded in paraffin. Paraffin-wax embedded tissue samples were submitted for histological examination via hematoxylin-eosin (HE). Microscopical examination revealed systemic mild to marked necrotizing lymphocytic vasculitis. The gastrointestinal tract, brain, and eye globes were most affected. In particular, severe necrotic and hemorrhagic transmural omasitis with lymphocytic endarteritis and necrotizing vasculitis was observed. In the central nervous system, a marked nonsuppurative meningoencephalitis, with disseminated perivascular cuffing and small areas of malacia were detected especially in the brainstem. Histological eye lesions consisted in perivascular and intramural lymphocytic infiltrates (perivasculitis and vasculitis) in the periocular skeletal muscle, sclera, and in the connective tissues of the cornea associated with haemorrhages, oedema, neovascularization and keratinocytes necrosis.

Overall, the histological findings suggested a viral infection that was afterconfirmed by molecular tests.

The clinical manifestations and the post mortem findings of the affected calf were strongly suggestive of MCF. The diagnosis was then confirmed by the detection of OvHV-2 DNA in the blood and in the spleen by real-time PCR and by histological examination. Although OvHV-2 has been found worldwide, data regarding the epidemiology of the disease in Italy is limited; the first two cases were described in a Holstein calf and in a cow in Southern Italy by Decaro and colleagues (Decaro *et al.* 2003) and diagnosed by PCR test and immunofluorescence. An interesting case was reported in 2006 in a Mediterranean water buffalo farm in which calves presented the "head and eye"

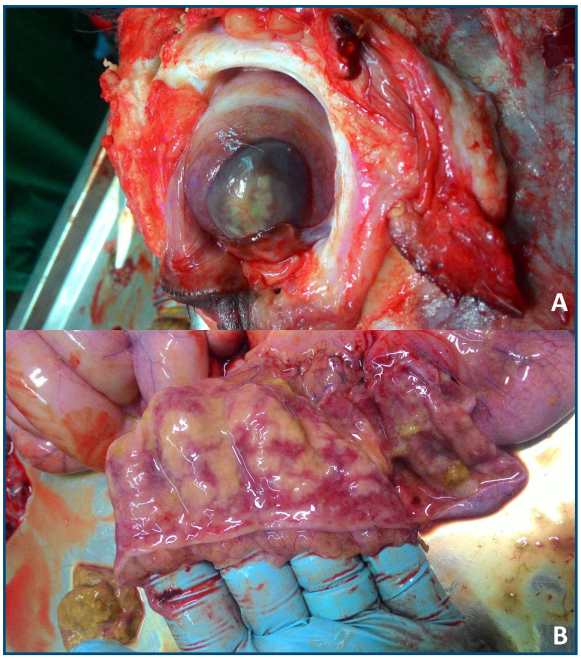

Figure 2. *Chianina calf showing MCF lesions: (**A**) corneal opacity, (**B**) mucosa of the small intestinal tract with congestion and ulcerative lesions.*

form and died within 8 days (Martucciello *et al.* 2006). Later, in 2008, an adult American bison, housed in a zoo in Southern Italy, displayed clinical signs of MCF that was confirmed by molecular and serological assays (Campolo *et al.* 2008). Other two cases, one in a cattle farm in Northern Italy and the other in a captive pudu (*Pudu puda*), have been registered until 2015 (Grattarola *et al.* 2011, Modesto *et al.* 2015). The latest outbreak of MCF was reported in Southern Italy in two water buffalo farms (Amoroso *et al.* 2017). In the majority of the cases described above, a contact between the affected animals and carriers like sheep was documented.

Diagnosis of MCF is first based on clinical signs and gross pathological examination even if these can be extremely variable, thus causing difficulties during diagnosis. Histological examination can be considered a useful diagnostic tool and it can be performed on a variety of tissues. However, detection of viral DNA is rapidly becoming the method of choice for confirmation of clinical cases. As presented in this case report, the inclusion of MCF in differential diagnosis can represent the first step toward the final diagnosis (OIE 2018). Primary differential diseases include bovine viral diarrhoea/mucosal disease, infectious bovine rhinotracheitis, bluetongue, epizootic haemorrhagic disease, foot and mouth disease, vesicular stomatitis, or ingestion of caustic materials or some toxic plants. Clinical signs can be elusive if not related to risk factors such as evidence of contact with a carrier species (sheep, goats, or wildebeest) as suspected in this case and as described in literature (Decaro *et al.* 2003, Campolo *et al.* 2008, Martucciello *et al.* 2006). In this occasion, unfortunately, the sheep flock could not be adequately investigated to identify the potential reservoirs of the virus. Anyway, the present study provides new information regarding the circulation of OvHV-2 in Central Italy. Malignant catarrhal fever is an important and underestimated disease with many unclear questions concerning transmission, prevalence and pathogenesis. The authors believe that the inclusion of this pathology in routine differential diagnosis protocols could help to fill these gaps. Importantly, some susceptible species, including cattle and bison, may be latently infected. Recrudescence of latent infections is possible and must be considered for cases with unknown history of contact with carriers. In this regard, it could be important to consider the establishment of an active surveillance plan for MCF in European herds. In addition due to the lack of a valid immunization strategy and the rapid course of the disease, is of fundamental importance to rapidly report field outbreaks in order to create a baseline data for more extensive epidemiological investigations.

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