Sheep associated malignant catarrhal fever: an emerging disease of bovids in India

Richa Sood · D. Hemadri · S. Bhatia

Received: 9 July 2013 / Accepted: 9 September 2013 / Published online: 21 September 2013
© Indian Virological Society 2013

Abstract Malignant catarrhal fever (MCF) is a fatal lymphoproliferative disease affecting bovids, cervids and other ruminant species caused by viruses belonging to subfamily *Gammaherpesvirinae*, genus *Macavirus*. Among the 10 MCF viruses known to cause the disease, alcelaphine herpesvirus 1 (AIHV-1) and ovine herpesvirus 2 (OvHV-2) are the two most widely prevalent causative organisms. The AIHV-1 naturally infects wildebeest and causes wildebeest associated MCF (WA-MCF) in cattle in regions of African sub-continent. The OvHV-2 is prevalent in all varieties of domestic sheep as a sub-clinical infection and causes sheep associated MCF (SA-MCF) in susceptible ruminants in most regions of the world. In India, the detection of cases of SA-MCF in cattle and OvHV-2 infection in sheep during the last decade has established the presence of the virus in native sheep of the country. The present review presents up to date information on various aspects of SA-MCF and its causative agent OvHV-2 with special reference to Indian scenario.

Keywords Sheep associated malignant catarrhal fever · Gamma herpesvirus · Transmission · Diagnosis · OvHV-2

Introduction

Malignant catarrhal fever (MCF) is a clinically conspicuous and generally lethal infection of many species of Bovidae and Cervidae [71]. MCF is defined by the recognition of characteristic lymphoid cell accumulations in non-lymphoid organs, vasculitis and T-lymphocyte hyperplasia in lymphoid organs, the main cause of which is either of two gammaherpesviruses viz., alcelaphine herpesvirus 1 (AIHV-1) and ovine herpesvirus 2 (OvHV-2). The AIHV-1 naturally infects wildebeest and causes wildebeest associated MCF (WA-MCF) in cattle in regions of African sub-continent while the OvHV-2 infects all varieties of domestic sheep as a sub-clinical infection and causes sheep associated MCF (SA-MCF) in susceptible ruminants in most regions of the world. In both forms of the disease, animals with clinical disease are not a source of infection as the virus is only excreted by the natural hosts—wildebeest and sheep, respectively. AIHV-1 has been isolated and can be grown in cell culture with demonstration of cytopathic effect consisting of multi-nucleate cellular foci [65]; however, attempts to isolate OvHV-2 have failed till date. Mixed farming of sheep/goat and cattle has brought this disease to the surface and it is being recognised as an emerging and economically important disease of cattle worldwide.

MCF is also reported in domestic pigs [7, 87] and has been shown to be experimentally produced in rabbits [69, 70]. A typical feature of MCF in cattle is that many outbreaks are sporadic, with single or only a few individuals in a herd being affected. It is because of this feature, MCF is usually not recognized as a major cause of economic losses to cattle husbandry; however, outbreaks of MCF have been recorded in domestic cattle, deer and bison herds causing huge economic losses [10, 14, 22, 31, 48, 49, 61]. In regions where mixed farming of the carrier species and the

R. Sood (✉) · S. Bhatia
High Security Animal Disease Laboratory, IVRI, Anand Nagar,
Bhopal 462021, Madhya Pradesh, India
e-mail: richa_bhatia@yahoo.com

D. Hemadri
Project Directorate on Animal Disease Monitoring and
Surveillance, Bengaluru, India

susceptible species is practised, MCF is a notable disease e.g. Bali cattle in Indonesia, bison in the USA and in pastoralist cattle herds in Eastern and Southern Africa [71]. Though SA-MCF in bovids has been well described in many textbooks, the latest developments in PCR-based diagnostics, epidemiology, transmission and viral etiology need to be reviewed to present up-to-date information about the disease and its causative agent, OvHV-2. Due to recent clinical cases of SA-MCF in cattle in India and subsequent detection of OvHV-2 in these animals, this virus has caught attention of field veterinarians and veterinary virologists in this region too. The present review focuses on various aspects of diagnosis, pathogenesis and epidemiology of SA-MCF as a disease of bovids and recent advances in understanding of molecular biology of OvHV-2 as the causative agent of SA-MCF.

Etiology

AIHV-1 and OvHV-2 are the two most widely prevalent causative organisms among the 10 MCF viruses (Table 1) known as the cause of the disease [51]. All the 10 MCF causing viruses belong to the genus *Macavirus* under the subfamily *Gammaherpesvirinae* in family *Herpesviridae*. Six of them clearly associated with clinical disease are, AIHV-1, OvHV-2, caprine herpesvirus-2 (CpHV-2), MCFV of unknown origin causing disease in white-tailed deer (MCFV-WTD), ibex MCFV (MCFV-ibex) carried by ibex, and an AIHV-2-like virus carried by Jackson hartebeest. The remaining four viruses carried by roan antelope [68], oryx, muskox [43], and aoudad [47] have not yet been associated with disease [51]. AIHV-1 is endemic in

wildebeest, in which it induces sub-clinical infection and causes WA-MCF in cattle and other susceptible ruminants [65]. OvHV-2 is enzootic worldwide in domestic sheep [5] and causes sheep-associated MCF when transmitted to a wide variety of domestic and wild ruminants, including pigs [54, 78, 84]. AIHV-2 causes MCF in hartebeest (*Alcelaphus buselaphus* P) and topi (*Damaliscus korrigum* O) [38, 58]. Hippotragine herpesvirus 1 (HiHV-1) has been detected in the roan antelope (*Hippotragus equinus* D) and oryx (*Oryx gazella*) L [43, 67]. Caprine herpesvirus 2 (CpHV-2) is enzootic in domestic goats [18, 41] and can cause MCF disease in several ruminants, including sika deer (*Cervus nippon* T.), white-tailed deer (*Odocoileus virginianus* Z.), moose (*Alces alces* L.) and roe deer (*Capreolus capreolus* L.) [19, 37, 44, 84].

OvHV-2 genome and latency

Viruses within the *Herpesviridae* family generally share a common structure with icosahedral capsid symmetry and a relatively large double stranded, linear DNA genome encoding 100–200 genes. The genomic DNA is packed into the capsid which is embedded in a complex amorphous layer composed of several proteins called the tegument. The entire structure is then enclosed by a glycoprotein containing lipid envelope [56]. In contrast to AIHV-1, which has been isolated and sequenced completely [26, 65], OvHV-2, has never been isolated due to the lack of a productive tissue-culture system. However, an OvHV-2-infected bovine T-lymphoblastoid cell line, BJ1035 that was derived from a clinically affected cow with SA-MCF [73], has been used for molecular characterization of the

Table 1 Causative agents and the host range in MCF

Causative agent	Carrier host	Susceptible host	References
<i>Alcelaphine herpesvirus 1</i>	African wildebeest	Bison	[65]
<i>Alcelaphine herpesvirus 2</i>	Hartebeest, topi	Barbery red deer	[38, 58]
<i>Ovine herpesvirus 2</i>	Sheep/goat	Cattle, water buffalo, bateng, antelopes, pigs,	[42, 54, 68, 78, 84]
<i>Caprine herpesvirus 2</i>	Goat	Several ruminants, sikka deer, white-tailed deer, moose, roe deer	[18, 19, 37, 41, 42, 84]
MCF of unknown origin-White tailed deer virus	Not known	White-tailed deer	[43]
MCF of unknown origin-MCFV- ibex	Ibex	Not known	[43]
<i>Hippotragine herpesvirus 1</i>	Roan antelope	Not associated with disease	[43, 68]
<i>Un-named virus resembling Caprine herpesvirus 2</i>	Oryx	Not associated with disease	[43]
	Muskox	Not associated with disease	[43]
	Aoudad	Not associated with disease	[47]

virus. The complete OvHV-2 genome, derived from BJ1035, has been cloned and sequenced [32]. Sequence analyses showed that the unique region of the OvHV-2 genome consists of 130 kbp with 73 open reading frames (ORFs). The arrangement of ORFs within the genome is highly similar to other gammaherpesviruses. The genome structure, ORF content and amino acid sequences are closest to those of AIHV-1. Three ORFs are entirely unique to OvHV-2, viz, Ov 2.5, Ov 3.5 and Ov 8.5; while there are 8 ORFs, which are only found in AIHV-1 and OvHV-2 viz., Ov2, Ov3, Ov4.5, Ov6, Ov7, Ov8, Ov9 and Ov10 [32]. Ov2.5 contains five exons and encodes a homologue of cellular IL-10. Ov3.5 encodes a 163 aa long protein, which contains a putative signal peptide. This protein, therefore, is likely to be secreted from the infected cell. Ov2 contains two exons and encodes a protein containing a basic leucine-zipper (bZIP) motif, and is likely to be a transcription factor. The product of Ov3 contains a putative signal peptide to residue 22 and is homologous to proteins of the semaphorin family. Ov3 is predicted to be involved in the modulation of the host response to OvHV-2. The Ov4.5 and Ov9 products promote survival of infected lymphocytes and the establishment of latency in the OvHV-2- infected cells. The Ov5 ORF is located downstream of ORF9/DNA polymerase and overlaps with ORF10. This ORF is predicted to encode a G protein-coupled receptor (GPCR). In between ORF50 and ORF52 lie three ORFs, Ov6, Ov7 and Ov8. The product of Ov6 contains a leucine-zipper motif in its C-terminal region and is believed to play a role in transcriptional transactivation. The product of Ov7 contains a predicted signal peptide and N-linked glycosylation motifs and is thus likely to be a viral glycoprotein, involved in receptor binding. The product of Ov8 was predicted to contain a transmembrane anchor near the C terminus and N-linked glycosylation sites and so, like Ov7, is likely to be a virus glycoprotein and may also be involved in binding to cellular receptors. Ov8.5 is predicted to encode a proline-rich (24 %) 42 kDa peptide of unknown function. The predicted Ov10 protein has a potential transmembrane anchor at the C terminus and four consensus nuclear-localization signals. Thus, the Ov10 protein may localize to the nucleus of infected cells [32].

Comparative studies of genome sequences of OvHV-2 isolated from nasal secretion of domestic sheep and cattle with MCF, showed an amino acid identity of 94–100 % between the predicted ORFs, barring for ORF 73, which was about 83 % identical [81]. It is not clear what functional difference this variability makes, nevertheless differences in ORF 73 sequence is thought to be useful for epidemiological studies.

Viruses in the *Gammaherpesvirinae* subfamily are usually specific for T or B lymphocytes, and latency is

frequently established in lymphoid tissue. Unlike alpha or beta herpesviruses, which undergo lytic replication, gammaherpesviruses seem to favour the initial establishment of latency, while only a subset support lytic replication [2]. Therefore, today it is a common belief that significant levels of subclinical or latent infections are present due to these viruses in a wide range of ruminant species, including cattle, bison, moose, reindeer, roe- and red deer (*Cervus elaphus* L.) [42, 84]. A detailed description of the gene expression of well studied gammaherpesviruses including the Epstein Barr virus (EBV), indicates that no common pattern of establishment, maintenance and reactivation of latency exists among gammaherpesviruses. Lethal MCF disease occurs when the immune response of the host is not adequate to control the number of infected cells. Animal species that are most vulnerable to develop lethal disease are those that did not participate in a co-evolution process with the virus. The outcome of infections with the gammaherpesviruses depends not only on the virus but also on the targeted animal. In vivo, viruses in this subfamily have evolved with their reservoir hosts and thus have been protected from being destroyed by the hosts' immune response and these reservoir hosts also have evolved to be infected without exhibiting any clinical signs and transmitting the viruses without showing any symptoms of the disease. Thus, these viruses exist in nature as inducers of inapparent infections in well adapted hosts. In animals not adapted to infection, such as in animals that did not co-evolve with the virus, lethal malignant catarrhal fever occurs [2]. Rabbits and Hamsters are excellent animal models of MCF; on being infected with OvHV-2 they develop a MCF-like syndrome which is very similar to the natural disease [4, 15].

Host range of OvHV-2

The natural host range of OvHV-2 is wide (Table 1). Sheep are the original host of OvHV-2, but the infection may naturally be transmitted to goats, cattle, bison, deer, and pigs [1, 3, 5, 12, 36, 43, 44, 52, 56, 72]. The sheep and goats remain healthy upon infection with OvHV-2, whereas the other susceptible hosts succumb to MCF. The diseased animals are dead-end hosts and do not transmit the virus. Interestingly, there is a wide spectrum of species-susceptibility to OvHV-2-induced disease, ranging from relatively resistant cattle species (*Bos taurus* and *B. indicus*), to the much more susceptible deer species, bison (*Bison bison*) and water buffalo (*Bubalus bubalis*), to the extremely susceptible Bali cattle (*Bos javanicus*) and Père David's deer (*Elaphurus davidianus*). The less susceptible species tend to experience a more prolonged infection and exhibit elaborate lesions, while more susceptible species

display less dramatic clinical signs and a shorter disease course.

Transmission and incidence

Sheep associated MCF occurs in all seasons. The causative agent, OvHV-2 is transmitted by contact or aerosol, mainly from less than a year old lambs [40]. A strong association between OvHV-2 outbreaks in different susceptible animals and exposure to sheep has been documented in the literature [5, 35, 54]. The respiratory tract is a common route for both entry and shedding of many herpesviruses in animals. The clinical disease occurs when the susceptible hosts are in contact with asymptomatic sheep either directly or indirectly [9]. OvHV-2 collected from ovine nasal secretions can infect naive sheep, cattle or bison [79, 80]. OvHV-2 viral DNA also has been detected in samples from the alimentary, respiratory and uro-genital tracts of sheep [35] indicating that some infection of offspring may occur during or shortly after lambing or calving. However, other studies have documented spread of OvHV-2 from sheep to bison over distances of up to 5 km [49]. The infection with OvHV-2 predominantly occurs during the perinatal period [6]. Incubation periods after experimental inoculation of cattle are 2–12 weeks [80]. Infectious OvHV-2 is present in ovine nasal secretions, but appears to be difficult to isolate from this source, since the period of virus shedding is short for any given animal [46]. Adult sheep can be infected by OvHV-2 via horizontal transmission through natural contact and it may intermittently shed large quantities of virus from nasal secretions. However, maximum nasal shedding of virus commonly occurs in adolescent sheep during 6–10 months of age [45]. While sheep and bison can be infected by intranasal nebulisation with 10^3 – 10^5 genome copies of OvHV-2, the infection of cattle is not reliable, even at 1,000-fold higher doses [80]. At very high doses, intranasal inoculation of OvHV-2 induced MCF-like clinical signs in naive sheep, confirming a previous report that this carrier species can develop a mild form of MCF [17, 46]. The MCF-susceptible species generally are thought to be dead-end hosts and do not transmit virus to other animals, which has the beneficial effect of limiting the spread of disease especially during outbreaks. The reason for lack of spread from MCF-susceptible animals is likely to be that the virus remains in a cell-associated manner in these species and cell-free virus is not produced. Although MCF is a sporadic disease in most ruminant species, it is an important cause of mortality in farmed American bison (*Bison bison*). In bison, mortalities have ranged from sporadic to as high as 100 % in small herds [76].

Clinical signs and pathology

The macroscopic and microscopic lesions caused by OvHV-2 in susceptible animals are similar to the ones caused by AIHV-1. These are generally widespread and involve many organs [25]. MCF in cattle may be present in distinct forms of clinical disease: peracute, head and eye, alimentary, neurological and cutaneous. Typical signs of the head and eye form are opacity of the cornea, with a narrow grey ring at the corneo–scleral junction. A study conducted to investigate a possible correlation between the severity and progression of certain typical clinical signs and disease outcome indicated that in the surviving cases there was improvement in uveitis and no deterioration of corneal oedema and which usually worsens in fatal cases as the disease progresses thus concluding that the progression of corneal edema correlates well with disease outcome, and that non improvement of uveitis is a bad prognostic sign [91]. The characteristics of the head and eye form include extreme dejection, agalactia, high fever (41 – 41 °C), rapid pulse rate (100–120 bpm), mucopurulent nasal discharge (Fig. 1), dyspnea due to nasal cavity obstruction, anorexia, lymphadenopathy, blepharospasm and congestion of scleral vessels. Superficial necrosis is evident in the anterior nasal mucosa and on the buccal mucosa.

Early stages of neurological form may involve nervous signs with weakness in one leg, in-coordination, muscle tremor and demented appearance, whereas in the final stages head pressing, nystagmus and paralysis are typical.

The per-acute form usually lasts for only 1–3 days and typical signs of head and eye form are absent, nevertheless, high fever, dyspnoea and gastroenteritis are evident. The alimentary form has several of the typical symptoms of head and eye form, except that there are only minor eye

Fig. 1 Mucopurulent nasal discharge in crossbred cattle. Courtesy @ Dr. G.V. Muralikrishna

changes and pronounced diarrhoea [66]. The mild form is rare and occurs most commonly in experimental animals. In these cases, mild erosions may be seen on the oral and nasal mucosa. In mild form of disease, there may be complete recovery or development of chronic MCF. A distinctive clinical feature in chronic MCF is persistent bilateral ocular leukomata [60].

As mentioned earlier, the clinical signs depend to some extent on the species infected, the virus and how long the animal survives after the onset of clinical signs. Some species appear to be particularly susceptible, for example, Père Davids deer [67], Bali cattle [88] and bison [10]. Deer mostly die within 48 h of the appearance of the first symptoms and bison within three days [61]. In contrast, cattle usually survive a week or more post infection [33] although chronic cases have been described in cattle and bison [59, 75]. Recovery from MCF is rare, nevertheless, there are reports of recovery in cattle that remained persistently infected [5, 60].

Gross findings at post-mortem examination include petechial haemorrhages on the tongue, buccal mucosa, in the gastrointestinal and respiratory tracts and urinary bladder. Commonly, there are raised pale foci on the surfaces of the kidneys and these may extend into the cortex. There is general enlargement of lymph nodes. Histologically, the distribution of lesions differs slightly depending upon the species affected, but the basic pathological features are consistent and are characterised by the accumulation of lymphocytes in a range of tissues [53]. The pathognomic lesions include necrotising vasculitis which shows infiltration of tunica media and adventitia by lymphoblast like cells and macrophages. Perivascular mononuclear cell cuffing is seen in most of the organs and an obliterative arteriopathy is a characteristic vascular lesion [66]. Once clinical MCF develops, the transmitting viral dose does not affect severity of the lesions [62]; however, it has significant effect on the incubation period and the timing of initial detection of viral genome by polymerase chain reaction (PCR) in peripheral blood leukocytes (PBL) [27]. Both clinical presentation and pathological features are of significant diagnostic value [61]. Detection of viral DNA by PCR in PBL and tissues can support the diagnosis of MCF [82]. Indications are there that an initial, transient period of OvHV-2 lytic replication occurs in the lungs, followed by systemic dissemination and viral latency with sporadic episodes of productive lytic replication in turbinate cells, which results in virus shedding [21, 50].

Pathogenesis

MCF is characterized by extensive pathological changes with little evidence of viral antigen in affected organs,

although viral DNA can be detected by in situ hybridisation or PCR [6, 13]. Experimental infection of OvHV-2 and AIHV-1 in rabbits has given insights into the pathogenesis of the disease. In rabbits, the disease has been seen as a progressive T cell hyperplasia involving local proliferation and infiltration of both lymphoid and non-lymphoid organs, associated with extensive vasculitis, which lead to tissue destruction caused by dysregulated cytotoxic lymphocytes [16, 73]. Detailed analysis of MCF in rabbits comparing MCF caused by OvHV-2 and AIHV-1 revealed OvHV-2-associated lesions were more apparent in visceral lymphoid tissue (e.g. mesenteric lymph nodes), whereas lesions associated with AIHV-1 were more frequent in peripheral lymph nodes. Also, OvHV-2 associated lesions contained more areas of necrosis than those of AIHV-1. However, with both viruses, lymphoid cell infiltrations consisted mainly of T cells, of which CD8⁺ T cells predominated, with very few CD4⁺ T cells, while a proportion of the infiltrating T cells were neither CD4⁺ nor CD8⁺ [4]. The study on rabbits with AIHV-1 induced MCF showed latency as the predominant phenomenon detected, indicated by the presence of ORF73 transcripts in tissues [24], which was in contrast to the OvHV-2 induced MCF which showed lytic gene expression profile, as indicated by detection of ORF25, ORF50 and ORF73 transcripts [51]. OvHV-2 has also been propagated in IL-2-dependent lymphoblastoid cell lines derived from diseased cattle and deer to study the growth regulation and cytokine transcription [74].

Study of MCF pathogenesis has been facilitated by the recent production of a bacterial artificial chromosome (BAC) clone carrying the entire pathogenic AIHV-1 genome [25]. This clone propagated infectious AIHV-1 virus in permissive cells and produced MCF in rabbits that was indistinguishable from the disease caused by wild-type virus. The AIHV-1 BAC is currently proving to be an useful tool for analysing the contribution of individual genes to the pathogenesis and host range of MCF and will help understand the intricacies of MCF pathogenesis.

Diagnosis of SA-MCF

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 pathogenesis. In the clinically affected animals, there is extreme variability in clinical signs and gross-pathological lesions which have been described in the previous section (clinical signs and pathology). Since the disease is sporadic and usually does not spread from one susceptible animal to another, a complete history of the affected animal along with clinical symptoms and its contact with carrier species gives an

insight into diagnosis. The age of the in-contact carrier species, its feeding milieu, grazing habits, calving, type of rearing, etc. are important to arrive to a tentative diagnosis. Before the introduction of molecular detection tools and tests, histopathology was considered as definitive diagnostic test for MCF and still has a distinct place along with genomic detection. However, confirmatory diagnosis is based on laboratory tests.

Genome detection assays

Detection of the viral nucleic acid is currently the accepted method of diagnosis for MCF. Recently developed molecular diagnostic assays have improved the detection and differentiation of MCF causative viruses. A number of different formats of PCR have been developed targeting various MCFVs since the first PCR for detection of AIHV-1 DNA was reported in 1990 [39]. The PCR test, developed by Baxter and co-workers [5], is the OIE approved diagnostic test for the detection of OvHV-2 infection. In this assay, the primers target a DNA fragment in the ORF75 of OvHV-2, a gene coding for a viral tegument protein. From the A8a subclone of a 5.2 kb *Hind*III insert from lymphocyte line MF629, two sets of primers were developed for a nested OvHV-2 PCR assay [5]. The primers are considered to be unique to OvHV-2. The outer set of primers (556 and 755) amplifies a 422 bp fragment of OvHV-2 DNA. The nested primer set (556 and 555) amplifies a 238 bp fragment. Both of the fragments could be cleaved with restriction endonuclease *Rsa*I into fragments that were consistent with their predicted size from the known base sequence of the A8a subclone. The primers did not amplify DNA from BoHV-1, BoHV-2, BoHV-4, or AIHV-1. This PCR assay has been used in many studies to detect latent OvHV-2 infections in sheep. The specificity for OvHV-2 arises from the primer (#556), which binds to a region of low homology between OvHV-2 and AIHV-1. This nested PCR has high sensitivity and is validated for detection of OvHV-2 DNA in infected sheep as well as in animals with clinical MCF. The assay has been widely used in veterinary diagnostic laboratories and is considered as gold standard [57], however, its use as a routine method to detect OvHV-2 DNA for confirmation of clinical SA-MCF in diagnostic laboratories may be problematic due to a high potential for carryover amplicon contamination leading to false positive results. In addition, it can only be used as a qualitative method for establishing infection in the in vivo model. As repeated attempts to isolate the virus had failed, a need to trace the agent in a quantitative manner along the time axis of a natural infection was felt and the second important step in MCF diagnosis was development of quantitative PCR or the real time PCR for OvHV-2 DNA by Hussy and co-workers [34]. The OvHV-2-specific

primer-probe set for the real-time PCR is based on the same sequence of genomic OvHV-2 DNA that had been used for the nested PCR (ORF75 gene) and showed adequate sensitivity for clinical samples. This qPCR was found equally suitable for detection of OvHV-2 DNA since its results corresponded to the previously established conventional nested PCR [82]. Moreover, it could trace the course of infection in real time in an experimental infection of sheep which was proved by a study in which OvHV-2-free animals were introduced into a conventional OvHV-2-positive flock. Two peaks of viral replication in the co-infected sheep were noticed, the first peak was observed after 8 weeks and the second, smaller peak was observed between weeks 20 and 22 indicating an active immune response reducing the viral replication. As expected for a herpesvirus, the virus was not entirely eliminated from the host and reactivation seemed to occur. Thus, in the absence of susceptible cell cultures, which can support replication of OvHV-2 in vitro, this molecular method was found to be appropriate for the tracing of OvHV-2 in vivo [34].

The development of a multiplex PCR for detection and differentiation of MCF causing viruses was the next significant advancement in MCF molecular diagnostics, especially for mixed-species operations, such as zoos, wildlife parks, and game farms. The multiplex real-time PCR used one pair of primer in conjunction with fluorescently labeled probes that target a polymorphic region in the viral DNA polymerase gene specific for OvHV-2, CpHV-2, MCFV-WTD, MCFV-ibex, and AIHV-1. The PCR was optimized and validated for the identification of these pathogenic MCFV in clinical samples using a single reaction. The assay was able to simultaneously detect and differentiate the viruses in clinical samples with high sensitivity (97.2 %) and specificity (100 %) [20].

Serological tests

There are various tests available for diagnosis of the disease. Antibody detection is useful in reservoir hosts and relies on use of AIHV-1 antigen which cross-reacts with antibodies against OvHV-2 [48]. All the serological tests developed so far have utilised antigen prepared from AIHV-1 since OvHV-2 has not been isolated. The fact that infected sheep develops no to low neutralising antibodies against AIHV-1 has made the use of neutralization tests impossible and therefore the diagnosis traditionally has relied on immunofluorescence, ELISA, cELISA and immunoperoxidase tests. These tests have good sensitivity but low specificity due to cross-reactivity with bovine herpesviruses [51]. A cELISA was developed using 15A epitope of AIHV 1 virus which is known to be conserved in all the MCFVs. This ELISA showed high sensitivity and specificity due to application of monoclonal antibody.

Uninfected lambs less than four months of age may give positive results due to presence of maternal antibodies. Low doses of virus also take longer than usual (almost 4 weeks) for antibody production. Therefore, paired sampling has to be carried out for conclusive identification and moreover ELISA, though specific, cannot differentiate between different MCFVs [42].

Global epidemiology

OvHV-2 is globally distributed and is spread by sheep and goat to the susceptible ruminant species such as cattle, bison and deer. The importance of the disease varies because of its dependence on the source of virus as well as the species affected by the virus. The two blue wildebeest and the black wildebeest species are the source of AIHV-1. This form of MCF is prevalent in the natural habitats of antelopes of Africa and zoological collections where mixed populations of members of Artiodactyla, including wildebeest are kept. In South Africa, although the prevalence of OvHV-2 was shown to be 77 % in 4 different sheep breeds from various regions, only about 10 % of bovine malignant catarrhal fever PCR-positive cases in South Africa were reported to be caused by OvHV-2 and the remaining 90 % by AIHV-1 [11]. Co-habitation of sheep and cattle in Turkey had also led to the development of clinical case of SA-MCF by OvHV-2 in cattle [90]. In another study conducted for serological identification of OvHV-2 in the captive ungulates in Turkish zoos; 39 Cameroon sheep (*Ovis ammon f aries*), 11 Barbary sheep (*Ammotragus lervia*), 57 pygmy goats (*Capra hircus*), 9 Angora goats (*Capra hircus*), 21 mountain goats (*Capra aegagrus-aegagrus*), 7 llamas (*Lama glama*), 8 Persian goitred gazelle (*Gazella subgutturosa subgutturosa*), 7 Caspian red deer (*Cervus elaphus maral*), 2 fallow deer (*Dama dama*), and 2 camels (*Camelus dromedarius*) were screened and ten sheep (37.0 %), 48 goats (84.2), and 1 Llama (14.2 %) were found positive for MCF antibodies [89]. CpHV-2, and not OvHV-2, was detected in a clinical case of MCF in buffalo in Switzerland [23]. Very recently presence of antibodies to OvHV-2 in 37.66 % out of 154 samples tested in a year has also been confirmed in sheep population in Japan [29]. Formalin fixed tissues were tested for determining the cause of death in a heifer, which was in contact with sheep population in Croatia and presence of OvHV-2 genome by fluorogenic real-time PCR was confirmed [83].

Recent reports have also added pigs in UK as one of the susceptible host of MCF [87]. An outbreak of MCF in a farrow-to-finish swine farm in the United States has been reported. The outbreak was confirmed by identification of OvHV-2 DNA in two clinically affected adult swine previously exposed to sheep [28]. In Germany, first report with clinical, pathological and histopathological findings in an

alpaca suffering from MCF caused by OvHV-2 was described recently [30]. Beef cattle housed next to sheep flock showing respiratory symptoms were identified to be suffering from SA-MCF in Jordan by collective clinical, genomic detection and histopathological testing. In a survey conducted in the North eastern region of Brazil, out of a total of 441 cattle tested which showed neurological signs, 6.3 % were found to be MCF positive [55].

Indian scenario

Although SA-MCF has been reported worldwide, the information on the epidemiology of the disease within India is very limited. Although the first confirmed description of the disease based on histopathology was reported from Punjab in 1975 [63], where 31 buffaloes and cattle reportedly died because of the disease, there was a silence on its incidence for almost next 30 years. One of the reasons could have been that the disease can symptomatically be very easily confused with other diseases like infectious bovine rhinotracheitis, vesicular stomatitis, bovine viral diarrhoea-mucosal disease complex, sporadic bovine encephalomyelitis etc. In addition, the confirmatory diagnosis requires sophisticated laboratory diagnostic tests and therefore the diagnosis of the disease has not been carried out effectively especially in field conditions. Another reason of lack of immediate concern for the diagnosis and the identification of the disease has been its sporadic nature and the fact that cattle/buffaloes are the dead end hosts and the disease does not spread from these animals might have led to non-pursuance of the disease diagnosis. It was almost three decades later the occurrence of SA-MCF was reported in cows [85] and then in sheep [86] from Kashmir. More recently, an investigation of 612 samples from sheep and goats in south Indian states revealed the presence of SA-MCF virus (OvHV-2 specific genome) in 17 samples [8] establishing the presence of MCF disease in India. The intermingling of domestic animals and wildlife has also increasingly become an issue of concern with regard to the spread and emergence of this disease. SA-MCF is an important animal disease that can cross-infect domestic animals and wildlife. Very recently, a SA-MCF in a captive bison was reported from Bannerghatta National Park, Bangalore. A clinical case in a young female bison was recorded which died within three days of development of clinical symptoms [77]. The first appearance of clinical signs included respiratory distress and straining, ocular discharge followed by corneal opacity (Fig. 2). The post mortem lesions included sloughing of mucosa, blood-tinged vaginal discharge haemorrhages on the base of trachea, congested lungs with yellow frothy material. The urinary bladder wall had haemorrhages

Fig. 2 Corneal opacity in bison. Courtesy@ Dr. Shilpa V.T.

Fig. 3 Haemorrhages on the wall of urinary bladder with dark brown urine of clinically infected bison. Courtesy@ Dr. Shilpa V.T.

(Fig. 3) and the kidneys were enlarged. MCF was never suspected in bison initially and was diagnosed only after the death of the animal. The diagnosis was carried out by OIE approved PCR test for diagnosis of MCF and confirmed by sequencing of the partial region in the tegument gene (GenBank accession number JQ801454) which matched 99 % with OvHV-2 reference sequence (GenBank accession number AY839756.1) [77]. There has been subsequent identification of more cases of SA-MCF in cattle and buffalo from the southern India (unpublished data). These cases were diagnosed by OIE approved PCR test for tegument region as well as by detection of glycoprotein region ORF 8 (2.8 kb) using primers specific to OvHV-2 and nucleotide sequencing (GenBank accession number KC577352).

It is to be emphasized that India has large population of susceptible species and mixed livestock farming system practised in most of the Indian states can lead to increased chances of close contact of carrier animals (sheep and goat) with clinically susceptible animals while co-housing or shared grazing. The main source of infection is the lambs, 9–12 months of age, which shed virus in high quantities without showing any clinical manifestation of harbouring the virus. Systematic disease monitoring with emphasis on

the epidemiology of the disease in sheep and goat for identification of positive flocks along with vigilance for clinical cases of MCF in other domestic and wild ruminants in captivity needs to be initiated to ascertain the status of the disease in the country.

Currently there is no reliable method to treat MCF in affected animals. Occasionally, supportive care with fluids and treatment with steroids and antibiotics has been effective in helping animals recover [64]. Whether treatment is actually effective in helping animals' recovery is still not proven, as treatment has not been shown to increase recovery in animals. Attempts to develop a vaccine to prevent infections with AIHV-1, have been made in the past; however, there has been no success. Currently, the only method of prevention available is proper management of susceptible species. The best method for controlling the spread of disease in susceptible hosts such as bison, deer, Bali cattle, water buffalo, is to prevent exposure to known carrier animals: wildebeest, sheep and goats.

Conclusions

In conclusion, SA-MCF is widely distributed globally and has shown its presence in India too. SA-MCF is an emerging and an important disease of cattle and is particularly significant in the Indian context where mixed farming is a common practice. With spread to a large part of the world, MCF has become one of the emerging diseases of ruminants with significant economic, trade and/or food security importance for a considerable number of countries. Due to its sporadic nature and overlapping clinical signs it produces, the disease has not received the due attention of veterinarians, researchers and policy makers in our country. With development of PCR based assays and their improved versions, it has now become easier to know the status of this disease in India. Countrywide survey of this disease coupled with molecular characterization of virus genome will provide new insights into epidemiology and economic losses associated with SA-MCF and will help in devising a control strategy for the whole country. Currently, there is no policy in place in India related to the control of MCF. In India, there is a mixed livestock farming system of cattle with sheep and goats, which leads to increased chances of close contact of carrier animals with clinically susceptible animal. The segregation of the susceptible host from the reservoir hosts is most important step for control of the disease. If cattle and sheep are reared under natural flock conditions, the grazing areas should be separate. It has been suggested that grazing sheep in the morning hours and cattle in the evening hours on the same land patch may also help as the virus may be destroyed in the day's sun as it is very heat labile.

A program to produce sheep free of infection should be initiated; although lambs can be infected at an early age, under natural flock conditions, lambs are not infected until after 2 months of age. If lambs are removed from contact with infected sheep prior to that age, they remain uninfected and can be raised virus free [42]. This knowledge should be used by sheep producers and zoos to produce sheep free of OvHV-2. If certain sheep flocks continue to be the source of infection for some years, disposal of these flocks for slaughter should be considered.

Zoological parks should introduce sero-negative animals only and follow strict quarantine restrictions for newly acquired animals. Systematic surveillance with emphasis on the epidemiology of the disease in sheep and goat for identification of transmitting flocks along with vigilance for clinical cases of MCF in other domestic and wild ruminants in captivity needs to be initiated to ascertain the current status of the disease in the country and to design effective control measures.

Acknowledgments The authors wish to thank Director IVRI for infrastructure provided to carry out MCF surveillance in the country. The assistance of Dr. Shipla V. T., Assistant Professor, Veterinary Pathology, Hassan Veterinary College, Karnataka and Dr. G. V. Muralikrishna, Deputy Director Animal Husbandry, Anekal, Bangalore for the photographs and the field work is also acknowledged.

References

- Ackermann M. Virus in sheeps skin. *Schweiz Arch Tierheilkd*. 2005;14:155–64.
- Ackermann M. Pathogenesis of gammaherpesvirus infections. *Vet Microbiol*. 2006;113:211–22.
- Albini S, Zimmermann W, Neff F, Ehlers B, Häni H, Li H, Hüssy D, Engels M, Ackermann M. Identification and quantification of ovine gammaherpesvirus 2 DNA in fresh and stored tissues of pigs with symptoms of porcine malignant catarrhal fever. *J Clin Microbiol*. 2003;41:900–4.
- Anderson IE, Buxton D, Campbell I, Russell G, Davis WC, Hamilton MJ, Haig DM. Immuno-histochemical study of experimental malignant catarrhal fever in rabbits. *J Comp Pathol*. 2007;136:156–66.
- Baxter SI, Pow I, Bridgen A, Reid HW. PCR detection of the sheep-associated agent of malignant catarrhal fever. *Arch Virol*. 1993;132:145–59.
- Baxter SI, Wiyono A, Pow I, Reid HW. Identification of ovine herpesvirus-2 infection in sheep. *Arch Virol*. 1997;142:823–31.
- Bedelian C, Nkedianye D, Herrero M. Maasai perception of the impact and incidence of malignant catarrhal fever MCF in southern Kenya. *Prev Vet Med*. 2007;78:296–316.
- Banumathi N, Sood R, Patil SS, Subramanian M, Pradhan HK. Genomic detection of Ovine herpes virus-2 in sheep and goat in states of southern India. *Ind J Anim Sci*. 2008;78:13–6.
- Benetka V, Krametter-Froetscher R, Baumgartner W, Moestl K. Investigation of the role of Austrian ruminant wildlife in the epidemiology of malignant catarrhal fever viruses. *J Wildl Dis*. 2009;45:508–11.
- Berezowski JA, Appleyard GD, Crawford TB, Haigh J, Li H, Middleton DM, O'Connor BP, West K, Woodbury M. An outbreak of sheep-associated malignant catarrhal fever in bison *Bison bison* after exposure to sheep at a public auction sale. *J Vet Diagn Invest*. 2005;7:55–8.
- Bremer CW. The prevalence of ovine herpesvirus-2 in 4 sheep breeds from different regions in South Africa. *J S Afr Vet Assoc*. 2010;81:93–6.
- Bridgen A, Reid HW. Derivation of a DNA clone corresponding to the viral agent of sheep-associated malignant catarrhal fever. *Res Vet Sci*. 1991;50:38–44.
- Bridgen A, Munro R, Reid HW. The detection of Alcelaphine herpesvirus-1 DNA by in situ hybridization of tissues from rabbits affected with malignant catarrhal fever. *J Comp Pathol*. 1992;106:351–9.
- Brown CC, Bloss LL. An epizootic of malignant catarrhal fever in a large captive herd of white-tailed deer *Odocoileus virginianus*. *J Wildl Dis*. 1992;28:301–5.
- Buxton D, Reid HW. Transmission of malignant catarrhal fever to rabbits. *Vet Rec*. 1980;106:243–5.
- Buxton D, Reid HW, Finlayson J, Pow I. Pathogenesis of sheep-associated malignant catarrhal fever in rabbits. *Res Vet Sci*. 1984;36:205–11.
- Buxton D, Reid HW, Finlayson J, Pow I, Berrie E. Transmission of a malignant catarrhal fever-like syndrome to sheep: preliminary experiments. *Res Vet Sci*. 1985;38:22–9.
- Chmielewicz B, Goltz M, Ehlers B. Detection and multigenic characterization of a novel gammaherpesvirus in goats. *Virus Res*. 2001;75:87–94.
- Crawford TB, Li H, Rosenberg SR, Norhausen RW, Garner MM. Mural folliculitis and alopecia caused by infection with goat-associated malignant catarrhal fever virus in two sika deer. *J Am Vet Med Assoc*. 2002;221:843–7.
- Cunha CW, Otto L, Taus NS, Knowles DP, Li H. Development of a multiplex real-time PCR for detection and differentiation of malignant catarrhal fever viruses in clinical samples. *J Clin Microbiol*. 2009;47:2586–9.
- Cunha CW, Traul DL, Taus NS, Oaks JL, O'Toole D, Davitt CM, Li H. Detection of ovine herpesvirus 2 major capsid gene transcripts as an indicator of virus replication in shedding sheep and clinically affected animals. *Virus Res*. 2008;132:69–75.
- Dabak M, Bulut H. Outbreak of malignant catarrhal fever in cattle in Turkey. *Vet Rec*. 2003;152:240–1.
- Dettwiler M, Stahel A, Krüger S, Gerspach C, Braun U, Engels M, Hilbe M. A possible case of caprine-associated malignant catarrhal fever in a domestic water buffalo *Bubalus bubalis* in Switzerland. *BMC Vet Res*. 2011;7:78.
- Dewals B, Boudry C, Gillet L, Markine-Goriaynoff N, de Leval L, Haig DM, Vanderplasschen A. Cloning of the genome of Alcelaphine herpesvirus 1 as an infectious and pathogenic bacterial artificial chromosome. *J Gen Virol*. 2006;87:509–17.
- Dewals B, Boudry C, Farnir F, Drion P-V, Vanderplasschen A. Malignant catarrhal fever induced by alcelaphine herpesvirus 1 is associated with proliferation of CD8 + T cells supporting a latent infection. *PLoS ONE*. 2008;32:e1627.
- Ensser A, Pflanz R, Fleckenstein B. Primary structure of the alcelaphine herpesvirus 1 genome. *J Virol*. 1997;71:6517–25.
- Gailbreath KL, O'Toole D, Taus NS, Knowles DP, Oaks JL, Li H. Experimental nebulization of American bison *Bison bison* with low doses of ovine herpesvirus 2 from sheep nasal secretions. *Vet Microbiol*. 2010;143:389–93.
- Gauger PC, Patterson AR, Kim WI, Stecker KA, Madson DM, Loynachan AT. An outbreak of porcine malignant catarrhal fever in a farrow-to-finish swine farm in the United States. *J Swine Health Prod*. 2010;18:244–8.
- Giangaspero M, Orusa R, Savini G, Gennaro AD, Osawa T. Serological survey to determine the occurrence of blue tongue virus, bovine leukemia virus and herpesvirus infections in the

- japanese small ruminant population from northern districts. Clin Microbiol. 2013;2:1–6.
30. Goerigk D, Merbach S. Clinical and pathological findings in an alpaca suffering from malignant catarrhal fever. Tierarztl Prax Ausg G Grosstiere Nutztiere. 2012;40:112–8.
 31. Hamilton AF. Account of three outbreaks of malignant catarrhal fever in cattle in the Republic of Ireland. Vet Rec. 1990;127:231–2.
 32. Hart J, Ackermann M, Jayawardane G, Russell G, Haig DM, Reid H, Stewart JP. Complete sequence and analysis of the ovine herpesvirus 2 genome. J Gen Virol. 2007;88:28–39.
 33. Holliman A, Daniel R, Twomey DF, Barnett J, Scholes S, Willoughby K, Russell G. Malignant catarrhal fever in cattle in the UK. Vet Rec. 2007;161:494–5.
 34. Hussy D, Stauber N, Leutenegger CM, Rieder S, Ackermann M. Quantitative fluorogenic PCR assay for measuring ovine herpesvirus 2 replication in sheep. Clin Diagn Lab Immunol. 2001;8:123–8.
 35. Hussy D, Janett F, Albin S, Stauber N, Thun R, Ackermann M. Analysis of the pathogenetic basis for shedding and transmission of ovine gamma herpesvirus 2. J Clin Microbiol. 2002;40:4700–4.
 36. Imai K, Nishimori T, Horino R, Kawashima K, Murata H, Tsunemitsu H, Saito T, Katsuragi K, Yaegashi G. Experimental transmission of sheep-associated malignant catarrhal fever from sheep to Japanese deer *Cervus nippon* and cattle. Vet Microbiol. 2001;79:83–90.
 37. Keel MK, Patterson JG, Noon TH, Bradley GA, Collins JK. Caprine herpesvirus-2 in association with naturally occurring malignant catarrhal fever in captive sika deer *Cervus nippon*. J Vet Diagn Invest. 2003;15:179–83.
 38. Klieforth R, Maalouf G, Stalis I, Terio K, Janssen D, Schrenzel M. Malignant catarrhal fever-like disease in Barbary red deer *Cervus elaphus barbarus* naturally infected with a virus resembling alcelaphine herpesvirus 2. J Clin Microbiol. 2002;40:3381–90.
 39. Lahijani RS, Sutton SM, Klieforth RB, Murphy MF, Heuschele WP. Application of polymerase chain reaction to detect animals latently infected with agents of malignant catarrhal fever. J Vet Diagn Invest. 1994;6:403–9.
 40. Li H, Snowden G, O'Toole DT, Crawford TB. Transmission of ovine herpesvirus 2 in lambs. J Clin Microbiol. 1998;36:223–6.
 41. Li H, Keller J, Knowles DP, Crawford TB. Recognition of another member of the malignant catarrhal fever virus group: an endemic gammaherpesvirus in domestic goats. J Gen Virol. 2001;82:227–32.
 42. Li H, Hua Y, Snowden G, Crawford TB. Levels of ovine herpesvirus 2 DNA in nasal secretions and blood of sheep: implications for transmission. Vet Microbiol. 2001;79:301–10.
 43. Li H, Gailbreath K, Bender LC, West K, Keller J, Crawford TB. Evidence of three new members of malignant catarrhal fever virus group in muskox *Ovibos moschatus* Nubian ibex *Capra nubiana* and gemsbok *Oryx gazella*. J Wildl Dis. 2003;39:875–80.
 44. Li H, Wunschmann A, Keller J, Hall DG, Crawford TB. Caprine herpesvirus-2-associated malignant catarrhal fever in white-tailed deer *Odocoileus virginianus*. J Vet Diagn Invest. 2003;15:46–9.
 45. Li H, Taus NS, Lewis GS, Kim O, Traul DL, Crawford TB. Shedding of ovine herpesvirus 2 in sheep nasal secretions: the predominant mode for transmission. J Clin Microbiol. 2004;42:5558–64.
 46. Li H, O'Toole D, Kim O, Oaks JL, Crawford TB. Malignant catarrhal fever-like disease in sheep after intranasal inoculation with ovine herpesvirus-2. J Vet Diagn Invest. 2005;17:171–5.
 47. Li H, Gailbreath K, Flach EJ, Taus NS, Cooley J, Keller J, Russell GC, Knowles DP, Haig DM, Oaks JL, Traul DL, Crawford TB. A novel subgroup of rhadinoviruses in ruminants. J Gen Virol. 2005;86:3021–6.
 48. Li H, Taus NS, Jones C, Murphy B, Evermann JF, Crawford TB. A devastating outbreak of malignant catarrhal fever in a bison feedlot. J Vet Diagn Invest. 2006;18:119–23.
 49. Li H, Karney G, O'Toole D, Crawford TB. Long distance spread of malignant catarrhal fever virus from feedlot lambs to ranch bison. Can Vet J. 2008;9:183–5.
 50. Li H, Cunha CW, Davies CJ, Gailbreath KL, Knowles DP, Oaks JL, Taus NS. Ovine herpesvirus 2 replicates initially in the lung of experimentally infected sheep. J Gen Virol. 2008;89:1699–708.
 51. Li H, Cunha CW, Taus NS. Malignant Catarrhal Fever: understanding molecular diagnostics in context of epidemiology. Int J Mol Sci. 2011;12:6881–93.
 52. Liggitt HD, McChesney AE, DeMartini JC. Experimental transmission of bovine malignant catarrhal fever to a bison (*Bison bison*). J Wildl Dis. 1980;16:299–304.
 53. Liggitt HD, DeMartini JC. The pathomorphology of malignant catarrhal fever. I. Generalized lymphoid vasculitis. Vet. Pathol. 1980;17:58–72.
 54. Løken T, Aleksandersen M, Reid H, Pow I. Malignant catarrhal fever caused by ovine herpesvirus-2 in pigs in Norway. Vet Rec. 1998;143:464–7.
 55. Luvizotto MCR, Ferrari HF, Cardoso TC. Malignant catarrhal fever-like lesions associated with ovine herpesvirus-2 infection in young calves (*Bos indicus*): a case report. J Venom Anim Toxins Trop Dis. 2010;16:178–85.
 56. McGeoch DJ, Rixon FJ, Davison AJ. Topics in herpesvirus genomics and evolution. Virus Res. 2006;117:90–104.
 57. Müller-Doblies UU, Li H, Hauser B, Adler H, Ackermann M. Field validation of laboratory tests for clinical diagnosis of sheep-associated malignant catarrhal fever. J Clin Microbiol. 1998;36:2970–2.
 58. Mushi EZ, Rossiter PB, Jessett D, Karstad L. Isolation and characterization of a herpesvirus from topi (*Damaliscus korrigum*, *Ogilby*). J Comp Pathol. 1981;91:63–8.
 59. O'Toole D, Li H, Roberts S, Rovnak J, DeMartini J, Cavender J, Williams B, Crawford T. Chronic generalized obliterative arteriopathy in cattle: a sequel to sheep-associated malignant catarrhal fever. J Vet Diagn Invest. 1995;7:108–21.
 60. O'Toole D, Li H, Miller D, Williams WR, Crawford TB. Chronic and recovered cases of sheep-associated malignant catarrhal fever in cattle. Vet Rec. 1997;140:519–24.
 61. O'Toole D, Li H, Sourk C, Montgomery DL, Crawford TB. Malignant catarrhal fever in a bison *Bison bison* feedlot 1993–2000. J Vet Diagn Invest. 2002;14:183–93.
 62. O'Toole D, Taus NS, Montgomery DL, Oaks JL, Crawford TB, Li H. Intra-nasal inoculation of American bison *Bison bison* with ovine herpesvirus-2 OvHV-2 reliably reproduces malignant catarrhal fever. Vet Pathol. 2007;44:655–62.
 63. Parihar NS, Rajya BS, Gill BS. Occurrence of malignant catarrhal fever in India. Ind Vet J. 1975;52:857–9.
 64. Penny C. Recovery of cattle from malignant catarrhal fever. Vet Rec. 1998;142:227.
 65. Plowright W, Ferris RD, Scott GR. Blue wildebeest and the aetiological agent of bovine malignant catarrhal fever. Nature. 1960;188:1167–9.
 66. Radostits OM, Gay CC, Hinchcliff KW, Constable PD. Veterinary medicine: a text book of diseases of cattle, sheep goat pigs and horses. 10th ed. Philadelphia: Saunders; 2010. p. 1245 [chap 21].
 67. Reid HW, Buxton D, McKelvey WA, Milne JA, Appleyard WT. Malignant catarrhal fever in Père Davids deer. Vet Rec. 1987;121:276–7.
 68. Reid HW, Bridgen A. Recovery of a herpesvirus from a roan antelope *Hippotragus equinus*. Vet Microbiol. 1991;28:269–78.
 69. Rossiter PB, Mushi EZ, Plowright W. Development of antibodies in rabbits and cattle infected experimentally with an African

- strain of malignant catarrhal fever virus. *Vet Microbiol.* 1977;2:57–66.
70. Rossiter PB, Mushi EZ, Plowright W. Antibody response in cattle and rabbits to early antigens of malignant catarrhal fever virus in cultured cells. *Res Vet Sci.* 1978;25:207–10.
 71. Russell GC, Stewart JP, Haig DM. Malignant catarrhal fever: a review. *Vet J.* 2009;179:324–35.
 72. Ruth GR, Reed DE, Daley CA, Vorhies MW, Wohlgemuth K, Shave H. Malignant catarrhal fever in bison. *J Am Vet Med Assoc.* 1977;171:913–7.
 73. Schock A, Reid HW. Characterisation of the lymphoproliferation in rabbits experimentally affected with malignant catarrhal fever. *Vet Microbiol.* 1996;53:111–9.
 74. Schock A, Collins RA, Reid HW. Phenotype growth regulation and cytokine transcription in Ovine Herpesvirus-2 OHV-2-infected bovine T-cell lines. *Vet Immunol Immunopathol.* 1998;66:67–81.
 75. Schultheiss PC, Collins JK, Austgen LE, DeMartini JC. Malignant catarrhal fever in bison acute and chronic cases. *J Vet Diagn Invest.* 1998;10:255–62.
 76. Schultheiss PC, Collins JK, Spraker TR, DeMartini JC. Epizootic malignant catarrhal fever in three bison herds: differences from cattle and association with ovine herpesvirus- 2. *J Vet Diagn Invest.* 2000;12:497–502.
 77. Sood R, Kumar M, Bhatia S, Pateriya AK, Khandia R, Siddiqui A, Kulkarni DD, Kumar S, Venkatesha MD. Transboundary Diseases: ovine herpesvirus type 2 infection in captive bison in India. *Vet Rec.* 2012;170:654.
 78. Syrjälä P, Saarinen H, Laine T, Kokkonen T, Veijalainen P. Malignant catarrhal fever in pigs and a genetic comparison of porcine and ruminant virus isolates in Finland. *Vet Rec.* 2006;159:406–9.
 79. Taus NS, Traul DL, Oaks JL, Crawford TB, Lewis GS, Li H. Experimental infection of sheep with ovine herpesvirus 2 via aerosolization of nasal secretions. *J Gen Virol.* 2005;86:575–9.
 80. Taus NS, Oaks JL, Gailbreath K, Traul DL, OToole D, Li H. Experimental aerosol infection of cattle *Bos taurus* with ovine herpesvirus 2 using nasal secretions from infected sheep. *Vet Microbiol.* 2006;116:29–36.
 81. Taus NS, Herndon DR, Traul DL, Stewart JP, Ackermann M, Li H, Knowles DP, Lewis GS, Brayton KA. Comparison of ovine herpesvirus 2 genomes isolated from sheep (*Ovis aries*) and a clinically affected cow (*Bos bovis*). *J Gen Virol.* 2007;88:40–5.
 82. Traul DL, Taus NS, Lindsay Oaks J, OToole D, Rurangirwa FR, Baszler TV, Li H. Validation of nonnested and real-time PCR for diagnosis of sheep-associated malignant catarrhal fever in clinical samples. *J Vet Diagn Invest.* 2007;19:405–8.
 83. Turk N, Habuš J, Friess M, Zobel R, Grabarevi Ž, Barbi L, Štritof Z, Milas Z, Starešina V. Demonstration and quantification of ovine herpesvirus 2 in Croatia—a case report. *Veterinarski Arhiv.* 2010;80:135–43.
 84. Vikøren T, Li H, Lillehaug A, Jonassen CM, Böckerman I, Handeland K. Malignant catarrhal fever in free-ranging cervids associated with OvHV-2 and CpHV-2 DNA. *J Wildl Dis.* 2006;42:797–807.
 85. Wani S. A. Bhat MA, Samanta I, Buchoo BA, Ishaq SM, Pandit F, Shah G-u-D, Buchh AS. Clinical serological and molecular evidence of sheep-associated malignant catarrhal fever in India. *Vet Rec.* 2004;155:242–4.
 86. Wani SA, Samanta I, Pandit F, Buchoo BA, Peer F, Bhat MA. Molecular epidemiology of ovine herpesvirus type 2 infection in Kashmir India. *Vet Rec.* 2006;159:587–90.
 87. Wessels M, Harwood D, Maley M, Willoughby K, Balfour C. Malignant catarrhal fever in *kune kune* pigs in the UK. *Vet Rec.* 2011;169:156.
 88. Wiyono A, Baxter SI, Saepullo M, Damayanti R, Daniels P, Reid HW. PCR detection of ovine herpesvirus-2 DNA in Indonesian ruminants—normal sheep and clinical cases of malignant catarrhal fever. *Vet Microbiol.* 1994;42:45–52.
 89. Yeşilbağ K, Alpaly G, Karakuzulu H. A serologic survey of viral infections in captive ungulates in Turkish zoos. *J Zoo Wildl Med.* 2011;42:44–8.
 90. Yildirim Y, Bilge Dağalp S, Yilmaz V, Faraji Majarashin AR. Molecular characterisation of ovine herpesvirus type 2 OvHV-2 in Turkey. *Acta Vet Hung.* 2012;60:521–7.
 91. Zemljić T, Pot SA, Haessig M, Spiess BM. Clinical ocular findings in cows with malignant catarrhal fever: ocular disease progression and outcome in 25 cases 2007–2010. *Vet Ophthalmol.* 2012;15:46–52.