

CHAPTER 2.4.14.

MALIGNANT CATARRHAL FEVER

SUMMARY

Definition of the disease: *Malignant catarrhal fever (MCF) is an acute, generalised and usually fatal disease affecting many species of Artiodactyla. The disease has been most often described as affecting species of the subfamily Bovinae and family Cervidae, but is also recognised in domestic pigs as well as giraffe and species of antelope belonging to the subfamily Tragelaphinae. MCF is defined by characteristic lymphoid cell accumulations in nonlymphoid organs, vasculitis and T-lymphocyte hyperplasia in lymphoid organs. The disease occurs following infection with certain herpesviruses of the genus Macavirus. The best characterised of these are alcelaphine herpesvirus-1 (AIHV-1) and ovine herpesvirus-2 (OvHV-2). AIHV-1, which is maintained by inapparently infected wildebeest, causes the disease in cattle in regions of Africa and in a variety of ruminant species in zoological collections world-wide. OvHV-2, which is prevalent in domestic sheep as a subclinical infection, is the cause of MCF in most regions of the world. In both forms of the disease, animals with clinical disease are not a source of infection as virus is only excreted by the natural hosts, wildebeest and sheep, respectively.*

Description of the disease: *MCF usually appears sporadically and affects few animals, though both AIHV-1 and OvHV-2 can give rise to epizootics. There is a marked gradation in susceptibility to the OvHV-2 form of MCF ranging from the relatively resistant *Bos taurus* and *B. indicus*, through water buffalo, North American bison and many species of deer, to the extremely susceptible Père David's deer, and Bali cattle. The disease may present a wide spectrum of clinical manifestations ranging from the acute form, when minimal changes are observed prior to death, to the more florid cases characterised by high fever, bilateral corneal opacity, profuse catarrhal discharges from the eye and nares, necrosis of the muzzle and erosion of the buccal epithelium. Diagnosis is normally achieved by observing the characteristic histopathological changes, though detection of viral DNA in either form of the disease has become the preferred option.*

Identification of the agent: *AIHV-1 may be recovered from clinically affected animals using peripheral blood leukocytes or lymphoid cell suspensions, but cell viability must be preserved during processing, as infectivity cannot be recovered from dead cells. Virus can also be recovered from wildebeest, either from peripheral blood leukocytes or from cell suspensions of other organs. Most monolayer cultures of ruminant origin are probably susceptible and develop cytopathic effect (CPE). Primary isolates typically produce multinucleated CPE in which viral antigen can be identified by immunofluorescence or immunocytochemistry using suitable antisera or monoclonal antibodies. The OvHV-2 agent has never been isolated in culture, although lymphoblastoid cell lines propagated from affected animals contain OvHV-2-specific DNA. Both agents have been transmitted experimentally to rabbits and hamsters, which develop lesions characteristic of MCF.*

Viral DNA has been detected in clinical material from cases of MCF caused by both AIHV-1 and OvHV-2 using the polymerase chain reaction, and this is becoming the method of choice for diagnosing both forms of the disease.

Serological tests: *Infected wildebeest, the natural host, consistently develop antibody to AIHV-1, which can be detected in a variety of assays including virus neutralisation, immunoblotting, enzyme-linked immunosorbent assay (ELISA) and immunofluorescence. Antibody to OvHV-2 can be detected by using AIHV-1 as the source of antigen. Domestic sheep consistently have antibody that can be detected by immunofluorescence, ELISA or immunoblotting. However, the antibody response of clinically affected animals is limited, with no neutralising antibody developing, so that*

detection relies on the use of immunofluorescence, ELISA or immunoblotting although antibody is not always present in more acutely affected animals such as deer.

Requirements for vaccines: No vaccine is currently available for this disease.

A. INTRODUCTION

1. Description of the disease

Malignant catarrhal fever (MCF) is a generally fatal disease of cattle and many other species of *Artiodactyla* that occurs following infection with certain herpesviruses of the genus *Macavirus*. Six herpesviruses have been recognised as causing MCF, the best characterised being alcelaphine herpesvirus-1 (AIHV-1) and ovine herpesvirus-2 (OvHV-2). MCF is characterised by systemic lymphoproliferation and is usually fatal, with infected cells being detectable in blood and most tissues at necropsy by polymerase chain reaction (PCR) with virus-specific primers. Natural hosts of these viruses, including Wildebeest (*Connochaetes* spp. of the subfamily *Alcelaphinae*) for AIHV-1 and domestic sheep for OvHV-2, experience no clinical disease following infection, although experimentally, large doses of OvHV-2 produced clinical signs of MCF when inoculated into naive sheep (Li *et al.*, 2005b).

The clinical signs of MCF are highly variable and range from peracute to chronic with, in general, the most obvious manifestations developing in the more protracted cases. In the peracute form, either no clinical signs are detected, or depression followed by diarrhoea and dysentery may develop for 12–24 hours prior to death. In general, the onset of signs is associated with the development of a high fever, increased serous lachrymation and nasal exudate, which progresses to profuse mucopurulent discharges. Animals may be inappetent and milk yields may drop. Characteristically, progressive bilateral corneal opacity develops, starting at the periphery. In some cases skin lesions appear (characterised by ulceration and exudation), which may form hardened scabs associated with necrosis of the epidermis, and are often restricted to the perineum, udder and teats. Salivation associated with hyperaemia may be an early sign, progressing to erosions of the tongue, hard palate, gums and, characteristically, the tips of the buccal papillae. Superficial lymph nodes may be enlarged and limb joints may be swollen.

Nervous signs such as hyperaesthesia, incoordination, nystagmus and head pressing may be present in the absence of other clinical signs or as part of a broader more characteristic syndrome. In addition, a number of cases of MCF with dermatological presentation have recently been described in Sika deer infected with caprine herpesvirus 2 (CpHV-2; Foyle *et al.*, 2009 and references cited therein). These cases exhibited cutaneous lesions combined with lymphocytic vasculitis characteristic of MCF, with CpHV-2 being detected by PCR and DNA sequencing.

Wildebeest-associated MCF occurs in the cattle-raising regions of eastern Africa where pastoralists use areas grazed by wildebeest, and in southern Africa in areas where wildebeest and cattle are grazed together. The disease, however, can also affect a variety of other ruminant species in zoological collections world-wide and so, apart from antelope of the subfamilies *Alcelaphinae* and *Hippotraginae*, it is advisable to regard all ruminants as susceptible.

Sheep-associated MCF occurs world-wide in cattle and other species, normally appearing sporadically and affecting only one or a few animals. However, on occasion, incidents occur in which several animals become affected, and this appears to be associated with certain sheep flocks that may continue to transmit disease for a number of years. The disease can also infect and cause substantial losses in North American Bison (*Bison bison*), red deer (*Cervus elaphus*), other deer species and water buffalo (*Bubalus bubalis*) and even more readily in Père David's deer (*Elaphurus davidianus*) and Bali cattle (*Bos javanicus*). OvHV-2 is also responsible for causing MCF in zoological collections, where disease has been reported in a variety of species including giraffe.

Reports from several countries, and in particular from Norway, that the disease affects domestic pigs have recently been confirmed by the detection of virus DNA in affected animals (Loken *et al.*, 1998; Syrjala *et al.*, 2006). Experimental infection of pigs with OvHV-2 has also been documented (Li *et al.*, 2012). Signs are very similar to those seen in acutely affected cattle.

The more resistant species tend to experience a more protracted infection and florid lesions, while in the more susceptible species the disease course tends to be shorter and the clinical signs less dramatic. A mild form of the disease described in 1930 was regarded with some scepticism because the disease could be confirmed only by histological changes observed at post-mortem. However, recent investigations using molecular and serological methods would appear to confirm that a few infected animals may recover following mild or even quite severe

clinical reactions (Michel *et al.*, 1994). Some studies indicate that substantial numbers of animals may become infected without developing clinical disease.

Gross pathological changes reflect the severity of clinical signs, but are generally widespread and may involve most organ systems. Erosions and haemorrhages may be present throughout the gastrointestinal tract, and in the more acute cases can be associated with haemorrhagic intestinal contents. In general, lymph nodes are enlarged, although the extent of lymph node involvement varies within an animal. Catarrhal accumulations, erosions and the formation of a diphtheritic membrane are often observed in the respiratory tract. Within the urinary tract characteristic echymotic haemorrhages of the epithelial lining of the bladder are often present, especially in bison.

Histological changes have been the basis for confirming cases of MCF and are characterised by epithelial degeneration, vasculitis, hyperplasia and necrosis of lymphoid organs, and widespread interstitial accumulations of lymphoid cells in nonlymphoid organs. Vasculitis is generally present and may be pronounced in the brain, affecting veins, arteries, arterioles and venules. It is characterised by lymphoid cell infiltration of the tunica adventitia and media, often associated with fibrinoid degeneration. The brain may also show a nonsuppurative meningoencephalitis with lymphocytic perivascular cuffing and a marked increase in the cellularity of the cerebrospinal fluid.

Lymph-node hyperplasia is characterised by an expansion of lymphoblastoid cells in the paracortex, while degenerative lesions are generally associated with the follicles.

The interstitial accumulation of lymphoid cells in nonlymphoid organs, in particular the renal cortex and periportal areas of the liver, is typical, and in the case of the kidney may be very extensive with development of multiple raised white foci, each 1–5 mm in diameter.

The pathological features of MCF, irrespective of the agent involved, are essentially similar. However, apart from histological examination, the methods available for diagnosing AIHV-1- and OvHV-2-induced disease tend to be virus-specific and are indicated below for each virus.

2. Causal pathogen

Disease caused by AIHV-1 is restricted to those areas of Africa where wildebeest are present and to zoological collections elsewhere, and has been referred to as wildebeest-associated (WA-) MCF. The OvHV-2 form of the disease occurs world-wide wherever sheep husbandry is practised and has been described as sheep-associated (SA-) MCF. Both forms of the disease may present a wide spectrum of clinical entities, though the characteristic histopathological changes are very similar in all cases. These two viruses belong to a group of closely related ruminant macaviruses that infect three subfamilies of *Bovidae* (*Alcelaphinae*, *Hippotraginae* and *Caprinae*); all probably have a potential to cause typical MCF. On rare occasions members of this group of viruses other than AIHV-1 and OvHV-2 have been identified as a cause of MCF.

3. Disease control

Control at present relies on segregating natural hosts from susceptible species, the extent to which this is enforced depending on the species involved. MCF-affected animals never or rarely transmit infection; hence it is only the natural hosts that can act as a source of infection. Wildebeest are relatively efficient transmitters of infection, and hence their segregation in mixed collections is important. Pastoralists should segregate cattle from wildebeest and pastures recently grazed by them, particularly around the time of wildebeest calving.

With OvHV-2, the requirement to segregate sheep depends on the susceptibility of the species involved. Thus with Père David's deer and Bali cattle, strict separation and avoidance of contact through fomites must be ensured. Equally, every reasonable effort must be taken to segregate bison and farmed deer from sheep, although fallow deer (*Dama dama*) appear to be more resistant to MCF. Cattle are generally managed with sheep without taking precautions to guard against disease transmission.

4. Zoonotic risk and biosafety requirements

Disease has only been observed in MCF-susceptible species as described above, and only following contact with reservoir host species. The inability of MCF-susceptible species to propagate cell-free virus makes them terminal hosts of the virus. Biosafety requirements should therefore focus on the separation of susceptible hosts from reservoir species, particularly for those considered especially susceptible, such as bison, deer or Bali cattle.

5. Differential diagnosis

The clinical signs of the 'head and eye' form of MCF resemble those of other diseases that cause oral lesions (Holliman, 2005). Thus BVD/mucosal disease, rinderpest, foot and mouth disease, bluetongue and vesicular stomatitis may be considered as potential differential diagnoses where MCF is suspected. A clear diagnosis of MCF may be supported by additional evidence such as detection of MCF virus DNA, virus-specific antibody response and/or histopathology consistent with MCF.

B. DIAGNOSTIC TECHNIQUES

Table 1. Test methods available for the diagnosis of malignant catarrhal fever and their purpose

Method	Purpose			
	Natural host species		Clinically affected animals	All animals
	Population freedom from infection	Individual animal freedom from infection	Confirmation of clinical cases	Prevalence of infection – surveillance
Agent identification¹				
PCR	+	+	+++	++
Virus isolation	+(AIHV-1)	+(AIHV-1)	+(AIHV-1)	+(AIHV-1)
Detection of immune response				
C-ELISA	+++	+++	++	+++
Virus neutralisation	+(AIHV-1)	+(AIHV-1)	–	–
IFAT	+	+	+	–
Immunoperoxidase test	+	+	+	–

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors limit its application; – = not appropriate for this purpose.

Although not all of the tests listed as category +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

PCR = polymerase chain reaction; C-ELISA = competitive inhibition enzyme-linked immunosorbent assay;

IFAT = indirect fluorescent antibody test.

Note that virus isolation and virus neutralisation have only been documented for AIHV-1.

It must be emphasised that the viral cause of SA-MCF cannot be reliably isolated and evidence for OvHV-2 relies on: (a) the presence of antibody in sera of all domestic sheep that cross-reacts with AIHV-1 antigens in the immunofluorescent antibody (IFA) test, enzyme-linked immunosorbent assay (ELISA) and/or immunoblots (Hart *et al.*, 2007, Li *et al.*, 2001), but not in neutralisation assays; (b) the development of antibody that cross-reacts with AIHV-1 in the IFA test and competitive enzyme-linked immunosorbent assay (C-ELISA) in most cattle with SA-MCF and in experimentally infected animal models; (c) the detection and cloning of DNA from lymphoblastoid cell lines derived from natural cases of SA-MCF that cross-hybridises with, but is distinct from, AIHV-1 DNA; (d) the detection by PCR of amplicons unique to OvHV-2 in peripheral blood and affected tissues of animals with SA-MCF. More recently, OvHV-2 collected in sheep nasal secretions has been used to infect rabbits, cattle and bison, inducing MCF with characteristic clinical signs and histopathology (Li *et al.*, 2011; Taus *et al.*, 2006).

Diagnosis of MCF based on clinical signs and gross pathological examination cannot be relied on as these can be extremely variable. Histological examination of a variety of tissues including, by preference, kidney, liver, urinary bladder, buccal epithelium, cornea/conjunctiva and brain, are necessary for reaching a more certain diagnosis. However, detection of antibody to the virus and/or viral DNA can now also be attempted and these are rapidly becoming the methods of choice.

¹ A combination of agent identification methods applied on the same clinical sample is recommended.

Most laboratory-based tests to detect virus-specific antibody have relied on one attenuated isolate (WC11) that has been subjected to many laboratory passages as a source of viral antigen and DNA (Plowright *et al.*, 1960). The full nucleotide sequence of the virulent low passage virus (C500) is now available and will form the basis of further studies of this virus (Ensser *et al.*, 1997). Laboratory passage of the AIHV-1 C500 strain leads to attenuation of virulence and the ability to propagate in a cell-free manner, accompanied by genomic changes (Wright *et al.*, 2003). This high passage derivative of AIHV-1 C500 has been used as a candidate vaccine for wildebeest-associated MCF and as a source of antigen for serological analysis (Haig *et al.*, 2008; Russell *et al.*, 2012).

1. Identification of the agent

1.1. Clinically affected animals

1.1.1. Isolation

A striking feature of MCF is the lack of detectable viral antigen or herpesvirus-specific cytology within lesions. Confirmation of infection by virus recovery can only be performed for AIHV-1 to date, while attempts to recover the disease-causing virus from clinical cases of SA-MCF have failed consistently. However, lymphoblastoid cell lines have been generated from affected cattle and deer, some of which transmit MCF following inoculation into experimental animals (Reid *et al.*, 1989).

Generally, AIHV-1 infectivity is strictly cell associated and thus isolation can be achieved only from cell suspensions either of peripheral blood leukocytes, lymph nodes or other affected tissues. Cell suspensions are prepared in tissue culture fluid, approximately 5×10^6 cells/ml, and inoculated into preformed monolayer cell cultures. Bovine thyroid cells have been used extensively, but most primary and low passage monolayer cell cultures of ruminant origin will probably provide a suitable cell substrate for isolating the virus. Following 36–48 hours' incubation, culture medium should be changed and monolayers should be examined microscopically ($\times 40$) for evidence of cytopathic effects (CPE). These appear characteristically as multinucleate foci within the monolayers, which then progressively retract forming dense bodies with cytoplasmic processes that may detach. This is followed by regrowth of normal monolayers. A CPE may take up to 21 days to become visible and is seldom present before day 7. Infectivity at this stage tends to be largely cell associated and thus any further passage or storage must employ methods that ensure that cell viability is retained. Specificity of the isolate should be determined using specific antisera or monoclonal antibodies (MAbs) in fluorescence or immunocytochemical tests.

1.1.2. Viral DNA

Characteristically, very little viral DNA can be detected within affected tissues, hence it is necessary to amplify the viral genome either by conventional culture or the polymerase chain reaction (PCR).

The full sequence of the C500 isolate of AIHV-1 and of two isolates of OvHV-2 have been published, permitting the design of primers for PCR reactions from conserved regions of the genome (Ensser *et al.* 1997; Hart *et al.*, 2007; Taus *et al.*, 2007). Nested and real-time PCR assays have been developed for AIHV-1 and OvHV-2 (Baxter *et al.*, 1993; Flach *et al.*, 2002; Hussy *et al.*, 2001; Traul *et al.*, 2005) while a pan-herpesvirus PCR (VanDevanter *et al.*, 1996) has been used to identify CphV-2 in Sika deer with MCF (Foyle *et al.*, 2009) and a virus associated with MCF in White-tailed deer (Li *et al.*, 2000). This assay targets the viral DNA polymerase gene sequence and has been employed for phylogenetic comparison of AIHV-1 and related viruses (Li *et al.*, 2005a).

1.1.2.1. PCR protocols

These protocols are based on published nested PCR assays designed to detect OvHV-2 (Baxter *et al.*, 1993) or to distinguish AIHV-1 and OvHV-2 (Flach *et al.*, 2002) in DNA samples from natural hosts or MCF-affected species. DNA purification methods should avoid organic extraction as this can leave residues that inhibit PCR. Silica-based genomic DNA extraction methods have been extensively used and appear reliable. Methods for extraction of DNA from fixed tissue samples should be validated before use in these assays. An example protocol is given below, however optimal reaction conditions should be validated for each system of enzymes and buffers. Protocols for real-time PCR to detect MCF virus DNA (Traul *et al.*, 2005; Hussy *et al.*, 2001) are not given as these should be optimised for each reagent set and analysis system used.

1.1.2.2. Protocol 1: Hemi-nested PCR to detect OvHV-2 DNA (Baxter *et al.*, 1993)**i) Primers**

Name	Length	Sequence
556	30 mer	5'-AGT CTG GGT ATA TGA ATC CAG ATG GCT CTC-3'
555	28 mer	5'-TTC TGG GGT AGT GGC GAG CGA AGG CTTC-3'
755	30 mer	5'-AAG ATA AGC ACC AGT TAT GCA TCT GAT AAA-3'

ii) Primary amplification (product size 422 bp)

Prior to PCR, a master mix is made up, comprising all components except template DNA. This is then dispensed into PCR tubes containing test or control DNA. This approach minimises pipetting errors when assaying large numbers of samples. The master mix comprises (per reaction): 10× PCR buffer, 5 µl; MgCl₂ (25 mM), 1 µl; dNTP mix (1 mM), 5 µl; primer 556 (10 µM), 1 µl; primer 755 (10 µM), 1 µl; Taq DNA polymerase (5 u/µl), 0.125 µl; and nuclease-free water, 31.875 µl; making a total of 45 µl per reaction. Samples of 5 µl, containing up to 1 µg of test or control DNA, are placed in PCR tubes and 45 µl of master mix are added to each tube. The tubes are then used for PCR according to the following protocol, using a PCR machine with heated lid. To use a PCR machine without a heated lid, mineral oil should be overlaid on each PCR reaction to prevent evaporation.

Suggested cycling conditions are: Hot-start activation at 95°C for 15 minutes; followed by 15 cycles of 94°C for 60 seconds, 60°C for 60 seconds and 72°C for 60 seconds; with a final extension at 72°C for 10 minutes. The conditions should be adjusted according to the Taq polymerase and the thermocycler used.

iii) Secondary amplification (product size 238 bp)

The master mix for the secondary amplification comprises (per reaction): 10× PCR buffer, 5 µl; MgCl₂ (25 mM), 1 µl; dNTP mix (1 mM), 5 µl; primer 556 (10 µM), 1 µl; primer 555 (10 µM), 1 µl; Taq DNA polymerase (5 u/µl), 0.125 µl; and nuclease-free water, 33.875 µl; making a total of 48 µl. Samples of 2 µl of each primary amplification product are placed in PCR tubes and 48 µl of master mix are added to each tube. Cycling conditions for the secondary PCR are the same as for the primary PCR, except that 30 cycles of amplification are used. After amplification approximately 10 µl of each secondary PCR reaction should be run on a 1.8 % agarose gel to visualise the PCR products.

1.1.2.3. Protocol 2: Hemi-nested PCR to distinguish AIHV-1 and OvHV-2 DNA (Flach *et al.*, 2002)**i) Primers**

Name	Length	Sequence*
Primer POL1	24-mer	5'-GGC (CT)CA (CT)AA (CT)CT ATG CTA CTC CAC-3'
Primer POL2	21-mer	5'-ATT (AG)TC CAC AAA CTG TTT TGT-3'
Primer OHVPol	20-mer	5'-AAA AAC TCA GGG CCA TTC TG-3'
Primer AHVPol	20-mer	5'-CCA AAA TGA AGA CCA TCT TA-3'

*base positions in parentheses are degenerate – the oligonucleotide will contain either of the two indicated bases at these positions.

The primers POL1 and POL2 target a segment of the DNA polymerase gene which is conserved in both OvHV-2 and AIHV-1, amplifying a fragment of 386bp. OHVPol and AHVPol are specific primers for OvHV-2 and AIHV-1 respectively, which amplify 172bp products.

ii) Primary amplification

Master mix, per reaction: 10× buffer, 2.5 µl; MgCl₂ (25 mM), 0.5 µl; dNTP mix (1 mM), 2.5 µl; primer POL1 (10 µM), 1 µl; primer POL2 (10 µM), 1 µl; Taq DNA polymerase (5 u/µl), 0.125 µl; nuclease-free water, 12.375 µl (to 25 µl). Samples of 5 µl, containing up to 1 µg of test or control DNA, are placed in PCR tubes and 20 µl of master mix are added to each tube. The tubes are then used for PCR according to the following protocol: Hot-start activation at 95°C for 15 minutes; followed by 25 cycles of 94°C for 60 seconds, 60°C for 60 seconds and 72°C for 60 seconds; with a final extension at 72°C for 10 minutes. The conditions should be adjusted according to the Taq polymerase and the thermocycler used.

iii) Secondary amplification

Master mix, per reaction: 10× buffer, 2.5 µl; MgCl₂ (25 mM), 0.5 µl; dNTP mix (1 mM), 2.5 µl; primer AHVpol or OHVpol (10 µM), 1 µl; primer POL2 (10 µM), 1 µl; Taq DNA polymerase (5 u/µl), 0.125 µl; nuclease-free water, 12.375 µl (to 25 µl). Samples of 2 µl of each primary amplification product are placed in PCR tubes and 23 µl of master mix are added to each tube. Cycling conditions for the secondary PCR are the same as for the primary PCR, except that 30 cycles of amplification are used. After amplification approximately 10 µl of each secondary PCR reaction should be run on a 1.8% agarose gel.

1.2. Natural hosts

It is almost certain that all free-living wildebeest are infected with AIHV-1 by 6 months of age, virus having been spread intensively during the perinatal period. The species *Connochaetes taurinus taurinus*, *C.t. albojubatus* and *C. gnu* are all assumed to be infected with the same virus. Infection also appears to persist in most groups of wildebeest held in zoological collections. However, it is possible that infection may be absent in animals that have been isolated during calf-hood or that live in small groups. Natural infection has been successfully demonstrated by *in-situ* hybridisation on lung sections from *C.t. taurinus* calves in South Africa (Michel *et al.*, 1997).

Following infection there is a brief period when virus is excreted in a cell-free form and can be isolated from nasal swabs. Virus can also be isolated from blood leukocytes at this time, but in older animals this is less likely to be successful unless the animal is immunosuppressed either through stress or pharmacological intervention. In addition, virus may be isolated by establishing cultures of tissues from apparently normal animals, and this has been achieved in monolayer cultures of both kidney and thyroid cells from adult animals.

The domestic sheep is the natural host of OvHV-2 and probably all sheep populations are infected with the virus in the absence of any clinical response. Studies of the dynamics of infection within sheep flocks have however, generated conflicting results with some suggesting productive infection occurs in the first weeks of a lamb's life while others suggest infection of most lambs does not occur until 3 months of age with excretion of infectious virus occurring between 5 and 6 months (Li *et al.*, 2004). There is also evidence that some lambs may become infected *in utero* while other studies suggest that removal of lambs from their dams during the first week permits the establishment of virus-free animals. There may therefore be considerable variation in the dynamics of infection in different flocks. However, circumstantial evidence of the occurrence of MCF in susceptible species does suggest that the perinatal sheep flock is the principal source of infection, but that periodic recrudescence of infection may occur in sheep of all ages.

In addition to domestic sheep, domestic goats and other members of the subfamily *Caprinae* have antibody that reacts with AIHV-1 in a similar pattern to sheep serum. This implies that these species are infected with viruses similar to OvHV-2. Some goats have been found to be OvHV-2 positive by PCR, while a few cases of MCF caused by CpHV-2 have been reported (Foyle *et al.*, 2009). Other large antelope of the subfamilies *Alcelaphinae* and *Hippotraginae* are also infected with antigenically closely related gammaherpesviruses (Li *et al.*, 2005a), but there is no evidence that they can spread to other species and cause MCF, except rarely in captive populations.

2. Serological tests

2.1. Clinically affected animals

The antibody response of clinically affected animals is limited, with no neutralising antibody developing. Antibody to OvHV-2 has only been detected using AIHV-1 as the source of antigen. Antibody to AIHV-1 can be detected in 70–80% of clinically affected cattle by IFA or immunoperoxidase test (IPT) procedures, but may not be present in affected deer or animals that develop acute or peracute disease. A C-ELISA was first developed for detecting antibody to OvHV-2 (Li *et al.*, 1994) using an MAb (15-A) that targets an epitope that appears to be conserved among all MCF viruses and is probably also applicable to AIHV-1 infected animals (Li *et al.*, 2005a). The test has been employed to detect antibody in serum of wild and domestic ruminants in North America and appears to have some merit (Section B.2.4) (Li *et al.*, 2001). A comparative study of MCF diagnosis by histopathology, C-ELISA and OvHV-2-specific PCR showed that most cattle classified as MCF-positive by histopathology also had detectable MCF virus-specific antibodies and OvHV-2 DNA in the blood (Muller-Doblies *et al.*, 1998). A direct ELISA based on WC11 antigens has also been developed and assay results correlated well with parallel tests using the C-ELISA (Fraser *et al.* 2006).

2.2. Natural hosts

Antibody appears to develop consistently in wildebeest following infection and can be identified by neutralisation assays using the cell-free isolate WC11, or by immunofluorescence, again using the WC11 isolate and anti-bovine IgG, which has been shown to react with wildebeest IgG. The Minnesota MCF virus strain, which is indistinguishable from the WC11 strain of AIHV-1, is used for C-ELISA antigen production.

Antibody to OvHV-2 has only been detected using AIHV-1 as the source of antigen. In a study on the reaction of sheep serum to the structural proteins of AIHV-1 in immunoblots, the reactivity of different sera varied strikingly, indicating that individual sheep responded differently with regard to antibody recognition of cross-reacting epitopes of AIHV-1.

There has been no attempt so far to standardise the IFA test and the IPT, but the two methods below are given as examples. The C-ELISA is available as a commercial kit.

2.2.1. Indirect fluorescent antibody test

The IFA is less specific than virus neutralisation (VN); it can be used to demonstrate several varieties of 'early' and 'late' antigens in AIHV-1-infected cell monolayers. Antibodies reacting in the IFA test or the IPT develop in cattle and experimentally infected rabbits during the incubation period, and later in the clinical course of the disease, though cross-reactions with some other bovine herpesviruses, as well as OvHV-2, reduce the differential diagnostic value. Detection of such cross-reacting antibodies can sometimes be useful in supporting a diagnosis of SA-MCF.

2.2.1.1. Preparation of fixed slides

Inoculate nearly or newly confluent cell cultures with AIHV-1 (strain WC11). Uninoculated control cultures should be processed in parallel. At about 4 days – when the first signs of CPE are expected to appear but before overt CPE is visible – treat the cultures as follows: discard the medium, wash with PBS, remove the cells with trypsin–versene solution, spin down cells at approximate 800 *g* for 5 minutes, discard the supernatant fluid, and resuspend the cells in 10 ml of phosphate buffered saline (PBS) for each 800 ml plastic bottle of cell culture.

Make test spots of the cell suspension on two wells of a polytetrafluoroethylene-coated multiwell slide; air-dry and fix in acetone. Stain the spots with positive standard serum and conjugated anti-IgG to the appropriate species. Examine the incidence of positive and negative cells under a fluorescence microscope. Adjust the cell suspension by adding noninfected cells and/or PBS to give a suitable concentration that will form a single layer of cells when spotted on to the slide, with clearly defined positive cells among a background of negative cells.

Spot the adjusted positive cell suspension and the control negative suspensions on to multiwell slides in the desired pattern, and air-dry. Fix in acetone for 10 minutes. Rinse, dry and store over silica gel in a sealed container at –70°C.

An alternative procedure, which is easier to evaluate, is to prepare monolayers of infected and noninfected cells in Leighton tubes or chamber slides. The cell monolayers are infected with from 150 to 200 TCID₅₀ (50% tissue culture infective dose) of virus that has been diluted in cell culture medium. The infected and noninfected slides are fixed in acetone and stored, as above, at –70°C.

2.2.1.2. Test procedure

- i) Rehydrate the slides for 5 minutes with PBS, rinse in distilled water and air-dry.
- ii) Dilute sera 1/20 in PBS. Samples that give high background staining may be retested at higher dilutions. Apply diluted fluids to one MCF virus-positive cell spot and one negative control spot for each sample. Include positive and negative serum controls. Ideally, the test should be validated by titrating the control positive to determine its end-point.
- iii) Incubate at 37°C for 30 minutes in a humid chamber.
- iv) Drain the fluids from the spots. Wash the slides in two changes of PBS, for 5 minutes each.
- v) Wash in PBS for 1 hour with stirring, and then air-dry the slides.
- vi) Apply rabbit anti-bovine IgG fluorescein isothiocyanate (FITC) conjugate at a predetermined working dilution.
- vii) Incubate at 37°C for 20 minutes, drain the slides, and wash twice in PBS for 10 minutes each.
- viii) Counterstain in Evans blue 1/100,000 for 30 seconds, and wash with PBS for 2 minutes. Dip in distilled water, dry and mount in PBS/glycerol (50/50).
- ix) Examine by fluorescence microscopy for specific binding of antibody to the infected cells.

2.2.2. Immunoperoxidase test

A dilution of bovine turbinate (BT) cell-cultured AIHV-1 containing approximately 10³ TCID₅₀ is made in a freshly trypsinised suspension of BT cells and seeded into Leighton tubes containing glass cover-slips, 1.6 ml per tube, or four-chambered slides, 1.0 ml per chamber.

Observe the cell cultures at 4–6 days for CPE and fix the cultures with acetone when signs of CPE begin. Remove the plastic chambers, but not the gaskets, from the slide chambers before fixation, and use acetone (e.g. UltimAR grade) that will not degrade the gasket. Store the fixed cells at –70°C.

2.2.2.1. Test procedure

- i) Prepare IPT diluent (21.0 g NaCl and 0.5 ml Tween 20 added to 1 litre of 0.01 M PBS, pH 7.2) and washing fluid (0.5 ml Tween 20 added to 1 litre of 0.01 M PBS, pH 7.2).
- ii) Dilute the serum to be tested 1/20 in IPT diluent and overlay 150–200 µl on to a fixed virus-infected cover-slip or slide chamber.
- iii) Incubate the cover-slip in a humid chamber at 37°C for 30 minutes.
- iv) Dip the cover-slip three times in washing fluid.
- v) Overlay 150–200 µl of diluted (1/5000 in IPT diluent) peroxidase-labelled anti-bovine IgG on to the cover-slip or slide chamber.
- vi) Incubate the cover-slip or slide chamber in a humid chamber at 37°C for 30 minutes.
- vii) Dip the cover-slip three times in washing fluid.
- viii) Dilute the AEC substrate (3-amino-9-ethylcarbazole, 20 mg/ml in dimethyl formamide) in distilled water (5 ml of distilled water, 2 drops 50 mM sodium acetate buffer pH 5.0, 2 drops hydrogen peroxide (30%), and 3 drops AEC) and apply to the cover-slip or slide chamber.
- ix) Incubate in a humid chamber at 37°C for 8–10 minutes.
- x) Dip the cover-slip in distilled water, air-dry, and mount on a glass slide. Slide chambers are read dry.
- xi) The slide is read on a light microscope. The presence of a reddish-brown colour in the nuclei of the infected cells indicates a positive reaction.

2.2.3. Virus neutralisation

Tests have been developed for detecting antibodies to AIHV-1 in both naturally infected reservoir and indicator hosts. The first of these is a VN test using cell-free virus of the WC11 strain, and another uses a hartebeest isolate (AIHV-2). The attenuated strain of AIHV-1 C500 may also be used. These viruses have cross-reactive antigens and therefore either strain can be used in the test. The test is laborious, but can be performed in microtitre plates using low passage cells or cell lines. The main applications have been in studying the range and extent of natural gammaherpes virus infection in wildlife, captive species in zoos and, to a lesser extent, sheep populations. It has also been useful in attempts to develop vaccines, including the recent AIHV-1 vaccine that induced neutralising antibodies in cattle blood plasma and nasal secretions (Haig *et al.*, 2008). The VN test is of no value as a diagnostic test in clinically affected animals as no VN antibody develops in clinically susceptible species.

AIHV-1 stock (e.g. strain WC11) is grown in primary or secondary cell cultures of bovine kidney, bovine thyroid, low passage bovine testis, or another permissive cell type. The virus is stored in aliquots at -70°C . The stock is titrated to determine the dilution that will give 100 TCID₅₀ in 25 μl under the conditions of the test.

2.2.3.1. Test procedure

- i) Inactivate the sera for 30 minutes in a water bath at 56°C .
- ii) Make doubling dilutions of test sera in cell culture medium from 1/2 to 1/16 using a 96-well flat-bottomed cell-culture grade microtitre plate, four wells per dilution and 25 μl volumes per well. Positive and negative control sera are also included in the test. No standard sera are available, but internal positive standards should be prepared and titrated in an appropriate range.
- iii) Add 25 μl per well of AIHV-1 virus stock at a dilution in culture medium calculated to provide 100 TCID₅₀ per well.
- iv) Incubate for 1 hour at 37°C . The residual virus stock is also incubated.
- v) Back titrate the residual virus in four tenfold dilution steps, using 25 μl per well and at least four wells per dilution.
- vi) Add 50 μl per well of bovine kidney cell suspension at 3×10^5 cells/ml.
- vii) Incubate the plates in a humidified CO₂ atmosphere at 37°C for 7–10 days.
- viii) Read the plates microscopically for CPE. Validate the test by checking the back titration of virus (which should give a value of 100 TCID₅₀ with a permissible range 30–300) and the control sera. The standard positive serum should give a titre within 0.3 log₁₀ units of its predetermined mean.
- ix) The test serum results are determined by the Spearman–Kärber method as the dilution of serum that neutralised the virus in 50% of the wells.
- x) A negative serum should give no neutralisation at the lowest dilution tested (1/2 equivalent to a dilution of 1/4 at the neutralisation stage).

2.2.4. Competitive inhibition enzyme-linked immunosorbent assay (C-ELISA)

A C-ELISA was developed for detecting antibody to OvHV-2 (Li *et al.*, 1994; 2001) using an MAb (15-A) that targets an epitope on a complex of glycoproteins that appears to be conserved among all MCF viruses. The MAb was raised against the Minnesota isolate of virus, which is indistinguishable from the WC11 strain of AIHV-1. The test has been employed to detect antibody in serum of wild and domestic ruminants in North America and antibody to the following pathogenic viruses has been detected: AIHV-1, AIHV-2, OvHV-2, CpHV-2 and the herpesvirus of unknown origin observed to cause classic MCF in white-tailed deer, as well as the MCF-group viruses not yet reported to be pathogenic, such as those carried by the oryx, muskox, and others (Li *et al.* 2005a). The C-ELISA has the advantage of being faster and more efficient than the IFA or IPT. Additional validation data will become available as its use is expanded to more laboratories in other parts of the world. One comparison between C-ELISA, PCR and histopathological diagnosis of SA-MCF (Muller-Doblies *et al.*, 1998) suggested that the three approaches had good concordance.

The complete reagent set for the C-ELISA, including pre-coated plates, labelled MAb and control sera, is commercially available. For laboratories wishing to prepare their own antigen-

coated plates, the following protocol is provided. ELISA plates are coated at 4°C (39°F) for 18–20 hours with 50 µl of a solution containing 0.2 µg per well of semi-purified MCF viral antigens (Minnesota or WC11 isolates of AIHV-1) in 50 mM carbonate/bicarbonate buffer (pH 9.0). The coated plates are blocked at room temperature (21–25°C, 70–77°F) for 2 hours with 0.05 M PBS containing 2% sucrose, 0.1 M glycine, 0.5% bovine serum albumin and 0.44% NaCl (pH 7.2). After blocking, wells are emptied and the plates are then dried in a low humidity environment at 37°C for 18 hours, sealed in plastic bags with desiccant, and stored at 4°C (39°F) (Li *et al.*, 2001). MAb 15-A is conjugated with horseradish peroxidase using a standard periodate method.

2.2.4.1. Test procedure

- i) Dilute positive and negative controls and test samples (either serum or plasma) 1/5 with dilution buffer (PBS containing 0.1% Tween 20, pH 7.2).
- ii) Add 50 µl of diluted test or control samples to the antigen-coated plate (four wells for negative control and two wells for positive control). Leave well A1 empty and for use as a blank for the plate reader.
- iii) Cover the plate with parafilm and incubate for 60 minutes at room temperature, (21–25°C, 70–77°F).
- iv) Using a wash bottle, wash the plate three times with wash buffer (same as dilution buffer: PBS containing 0.1% Tween 20, pH 7.2).
- v) Prepare fresh 1 × antibody-peroxidase conjugate in dilution buffer according to previous titration/optimisation for each conjugate preparation, or to the manufacturer's instructions.
- vi) Add 50 µl of diluted antibody-peroxidase conjugate to each sample well. Cover the plate with parafilm and incubate for 60 minutes at room temperature (21–25°C, 70–77°F).
- vii) Wash the plate with wash buffer three times.
- viii) Add 100 µl of tetramethylbenzidine substrate solution to each sample well. Incubate for 60 minutes at room temperature (21–25°C; 70–77°F). Do not remove the solution from the wells.
- ix) Add 100 µl of stop solution (0.18 M sulphuric acid) to each well. Do not remove the solution from the wells.
- x) Read the optical densities (OD) on an ELISA plate reader at 450 nm.
- xi) Calculating % inhibition:

$$100 - \frac{\text{Sample OD (Average)} \times 100}{\text{Mean negative control OD}} = \% \text{inhibition}$$
- xii) *Interpreting the results:* If a test sample yields equal to or greater than 25% inhibition, it is considered positive. If a test sample yields less than 25% inhibition, it is considered negative.
- xiii) *Test validation:* The mean OD of the negative control must fall between 0.40 and 2.10. The mean of the positive control must yield greater than 25% inhibition.

C. REQUIREMENTS FOR VACCINES

At present no vaccine has been licensed for this disease.

Vaccination against MCF could be considered for use in those farmed species that have higher exposure or susceptibility to MCF, such as cattle in regions of East and South Africa where breeding wildebeest are prevalent, Bali cattle, bison in North America, farmed deer worldwide and susceptible species in zoological collections. Vaccination of reservoir hosts, such as wildebeest or sheep, is unlikely to be commercially viable and this is also the case for the majority of cattle herds that are at risk of sporadic SA-MCF. Numerous attempts to produce a protective vaccine against the AIHV-1 form of the disease have met with disappointing results. However, recent trials that focussed on stimulating high titres of neutralising antibody in nasal secretions of cattle have produced encouraging results (Haig *et al.*, 2008). This live attenuated vaccine induced protection against intranasal experimental challenge with pathogenic AIHV-1. Protection was also found to persist for at least 6 months (Russell *et al.*, 2012). This approach is likely to be the target for further research, including field trials.

As OvHV-2 cannot be successfully propagated in the laboratory no attempts at developing a vaccine have been attempted. However, recent work to develop a cattle challenge system for OvHV-2 using virus from sheep nasal secretions (Taus *et al* 2006) makes the testing of OvHV-2 vaccine candidates a possibility.

REFERENCES

- BAXTER S.I.F., POW I., BRIDGEN A. & REID H.W. (1993). PCR detection of the sheep-associated agent of malignant catarrhal fever. *Arch. Virol.*, **132**, 145–159.
- ENSSER A., PFLANZ R. & FLECKSTEIN B. (1997). Primary structure of the alcelaphine herpes virus 1 genome. *J. Virol.*, **71**, 6517–6525.
- FLACH E.J., REID H., POW I. & KLEMT A. (2001). Gamma-herpesvirus carrier status of captive artiodactyls. *Res. Vet. Sci.*, **73**, 93–99.
- FOYLE K.L., FULLER H.E., HIGGINS R.J., RUSSELL G.C., WILLOUGHBY K., ROSIE W.G., STIDWORTHY M.F. & FOSTER A.P. (2009). Malignant catarrhal fever in sika deer (*Cervus nippon*) the UK. *Vet. Rec.*, **165**, 445–447.
- FRASER S.J., NETTLETON P.F., DUTIA B.M., HAIG D.M. & RUSSELL G.C. (2006). Development of an enzyme-linked immunosorbent assay for the detection of antibodies against malignant catarrhal fever viruses in cattle serum. *Vet. Microbiol.*, **116**, 21–28.
- HAIG D.M., GRANT D., DEANE D., CAMPBELL I., THOMSON J., JEPSON C., BUXTON D. & RUSSELL G.C. (2008). An immunisation strategy for the protection of cattle against alcelaphine herpesvirus-1-induced malignant catarrhal fever. *Vaccine*, **35**, 4461–4468.
- HART J., ACKERMAN M., JAYAWARDANE G., RUSSELL G., HAIG D.M., REID H. & STEWART J.P. (2007). Complete sequence and analysis of the ovine herpesvirus 2 genome. *J. Gen. Virol.*, **88**, 28–39.
- HOLLIMAN A. (2005) Differential diagnosis of diseases causing oral lesions in cattle. *In Practice* **27**, 2–13.
- HUSSY D., STAUBER N., LEUTENEGGER C.M., RIDER S. & ACKERMAN M. (2001). Quantitative fluorogenic PCR assay for measuring ovine herpesvirus 2 replication in sheep. *Clin. Diagn. Lab. Immunol.*, **8**, 123–128.
- LI H., BROOKING A., CUNHA C.W., HIGHLAND M.A., O'TOOLE D., KNOWLES D.P. & TAUS N.S. (2012). Experimental induction of malignant catarrhal fever in pigs with ovine herpesvirus 2 by intranasal nebulization. *Vet. Microbiol.*, **159**, 485–489.
- LI H., CUNHA C.W., GAILBREATH K.L., O' TOOLE D., WHITE S.N., VANDERPLASSCHEN A., DEWALS B., KNOWLES D.P. & TAUS N.S. (2011). Characterization of ovine herpesvirus 2-induced malignant catarrhal fever in rabbits. *Vet. Microbiol.*, **150**, 270–277.
- LI H., GAILBREATH K., FLACH E.J., TAUS N.S., COOLEY J., KELLER J., RUSSELL G.C., KNOWLES D.P., HAIG D.M., OAKS J.L., TRAUL D.L. & CRAWFORD T.B. (2005a). A novel subgroup of rhadinoviruses in ruminants. *J. Gen. Virol.*, **86**, 3021–3026.
- LI H., KARNEY G., O'TOOLE D. & CRAWFORD T.B. (2008). Long distance spread of malignant catarrhal fever virus from feedlot lambs to ranch bison. *Can. Vet. J.*, **49**, 183–185.
- LI H., MCGUIRE T.C., MULLER-DOBLIES U.U. & CRAWFORD T.B. (2001). A simpler, more sensitive competitive inhibition enzyme-linked immunosorbent assay for detection of antibody to malignant catarrhal fever virus. *J. Vet. Diagn. Invest.*, **13**, 361–364.
- LI H., O'TOOLE D., KIM O., OAKS L. & CRAWFORD T.B. (2005b). Malignant catarrhal fever-like disease in sheep after intranasal inoculation with ovine herpesvirus-2. *J. Vet. Diagn. Invest.*, **17**, 171–175.
- LI H., SHEN D.T., KNOWLES D.P., GORHAM J. & CRAWFORD T. (1994). Competitive inhibition enzyme-linked immunosorbent assay for antibody in sheep and other ruminants to a conserved epitope of malignant catarrhal fever virus. *Clin. Microbiol.*, **32**, 1674–1679.
- LI H., TAUS N.S., LEWIS G.S., KIM O., TRAUL D.L. & CRAWFORD T.B. (2004). Shedding of ovine herpesvirus 2 in sheep nasal secretions: the predominant mode for transmission. *J. Clin. Microbiol.*, **42** (12), 5558–5564.
- LI H., DYER N., KELLER J. & CRAWFORD T.B. (2000). Newly recognized herpesvirus causing malignant catarrhal fever in white-tailed deer (*Odocoileus virginianus*). *J. Clin. Microbiol.*, **38**, 1313–1318.

- LOKEN T., ALEKSANDERSEN M., REID H. & POW I. (1998). Malignant catarrhal fever caused by ovine herpesvirus-2 in pigs in Norway. *Vet. Rec.*, **143**, 464–467.
- MICHEL A.L. & ASPELING I.A. (1994). Evidence of persistent malignant catarrhal fever infection in a cow, obtained by nucleic acid hybridisation. *J. S. Afr. Vet. Assoc.*, **65**, 26–27.
- MICHEL A.L., VAN DER LUGT J.J., BENGIS R.G. & DE VOS V. (1997). Detection of AHV-1 DNA in lung sections from blue wildebeest (*Connochaetes taurinus*) calves by *in situ* hybridisation. *Onderstepoort J. Vet. Res.*, **64**, 169–172.
- MULLER-DOBLIES U.U., LI H., HAUSER B., ADLER H. & ACKERMANN M. (1998). Field validation of laboratory tests for clinical diagnosis of sheep-associated malignant catarrhal fever. *J. Clin. Microbiol.*, **36**, 2970–2972.
- PLOWRIGHT W., FERRIS R.D. & SCOTT G.R. (1960). Blue wildebeest and the aetiological agent of bovine catarrhal fever. *Nature*, **188**, 1167–1169.
- REID H.W., BUXTON D., POW I. & FINLAYSON J. (1989). Isolation and characterisation of lymphoblastoid cells from cattle and deer affected with 'sheep-associated' malignant catarrhal fever. *Res. Vet. Sci.*, **47**, 90–96.
- RUSSELL G.C., BENAVIDES J., GRANT D., TODD H., DEANE D., PERCIVAL A., THOMSON J., CONNELLY M. & HAIG D.M. (2012). Duration of protective immunity and antibody responses in cattle immunised against alcelaphine herpesvirus-1-induced malignant catarrhal fever. *Vet. Res.* **43**, 51.
- TAUS N.S., HERNDON D.R., TRAU D.L., STEWART J.P., ACKERMANN M., LI H., KNOWLES D.P., LEWIS G.S. & BRAYTON K.A. (2007). Comparison of ovine herpesvirus 2 genomes isolated from domestic sheep (*Ovis aries*) and a clinically affected cow (*Bos bovis*). *J. Gen. Virol.*, **88**, 40–45.
- TAUS N.S., OAKS J.L., GAILBREATH K., TRAU D.L., O'TOOLE D. & LI H. (2006) Experimental aerosol infection of cattle (*Bos taurus*) with ovine herpesvirus 2 using nasal secretions from infected sheep. *Vet. Microbiol.*, **116**, 29–36.
- TRAU D.L., ELIAS S., TAUS N.S., HERRMANN L.M., OAKS J.L. & LI H. (2005). A real-time PCR assay for measuring alcelaphine herpesvirus-1 DNA. *J. Virol. Methods*, **129**, 186–190.
- TRAU D.L., LI H., DASGUPTA N., O'TOOLE D., ELDRIDGE J.A., BESSER T.E. & DAVIES C.J. (2007). Resistance to malignant catarrhal fever in American bison (*Bison bison*) is associated with MHC class IIa polymorphisms. *Anim. Genet.*, **38** (2), 141–146.
- VANDEVANter D.R., WARRENER P., BENNET L., SCHULTZ E.R., COULTER S., GARBER R.L. & ROAS, T.M. (1996). Detection and analysis of diverse herpesviral species by consensus primer PCR. *J. Clin. Microbiol.*, **34**, 1666–1671.
- WRIGHT H., STEWART J.P., IRERI R.G., CAMPBELL I., POW I., REID H.W. & HAIG D.M. (2003). Genome re-arrangements associated with loss of pathogenicity of the γ -herpesvirus alcelaphine herpesvirus-1. *Res. Vet. Sci.*, **75**, 163–168.

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