MALIGNANT CATARRHAL FEVER

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Introduction

Malignant Catarrhal Fever (MCF), a fatal disease of a large number of different ruminants, is caused by infection with either a blue or black wildebeest-associated alcephaline herpesvirus 1 (AlHV-1) or the sheep-associated ovine herpes virus - 2 (OvHV-2). Wildebeest and sheep are the natural hosts of these viruses respectively. Both these forms of MCF are of great economic importance, particularly in cattle, but may also cause losses of captive and farmed species of antelope, deer and bison (Bison bison), water buffalo (Bubalus bubalis) and occasionally domestic pigs. Neither of these viruses cause clinical disease in their respective natural hosts (wildebeest and sheep). The clinical course of MCF may vary considerably in individual affected animals – from peracute to acute, chronic or mild. MCF tends to appear sporadically with mostly low morbidity and high mortality rates. However, in some outbreaks, relatively large numbers of animals may be affected.

No effective vaccine is available. Transmission to MCF-susceptible species tends to be erratic and eradication of carrier species (wildebeest and sheep) is usually impractical. Control is therefore extremely difficult and is most effectively achieved by separating carrier species from susceptible animals. It has generally been accepted that infectious virus is only excreted by the natural hosts (wildebeest and sheep). Transmission between MCF-susceptible species has only been reported following experimental inoculation.

There are also other viruses in this group although not all have been associated with clinical MCF. There is evidence of the existence of a group of related gammaherpesviruses in four subfamilies of bovidae which may cause MCF in certain animal species following experimental transmission without these natural hosts developing any clinical signs. Caprine herpesvirus 2 has been described in clinically healthy goats and has been associated with disease in deer and recently water buffalo. MCF virus of white tailed deer is also of importance.

EPIDEMIOLOGY
Malignant Catarrhal Fever due to both forms of the disease occurs worldwide. Wildebeest-associated MCF occurs commonly in areas of Africa where these antelope are naturally distributed. It is also frequently diagnosed in zoological collections where wildebeest are kept. Sheep-associated MCF occurs wherever domestic sheep are kept which includes all African countries. The susceptibility of animal species to infection varies considerably and a number of different species may be affected by both forms. Clinical MCF has been described in most of the Bovinae (including cattle, waterbuffalo, American and European bison, Yak) and all species of Cervidae, except fallow deer. In African wildlife species clinical confirmed MCF has now been described in kudu, sitatunga, eland, roan, black impala, giraffe and African buffalo.

Of the two viruses, the most commonly reported worldwide is OvHV-2. The aetiological agent of the sheep-associated virus has never been isolated either from clinical cases of MCF or from sheep. However, it has been possible to generate it in lymphoblastoid cell lines originating from clinically affected cattle and deer. Sequence data from one of these clones has formed the basis of a polymerase chain reaction (PCR) specific for OvHV-2 which allows detection of the virus. Cattle (B. taurus and B. indicus) are relatively resistant to infection with OvHV-2 whilst Bali cattle of Indonesia (B. javanicus) and Pere David’s deer (Elaphurus davidianus), are extremely susceptible to infection. The latter must be kept well-separated from sheep to avoid high mortality rates. Other species of deer and water buffalo have an intermediate susceptibility to the virus.

The sheep-associated disease in domestic cattle occurs sporadically, usually affecting only one or a few animals. This may occur following intimate contact with sheep or personnel and fomites that have had contact with sheep, as well as where no obvious contact with sheep can be established. Disease generally occurs following the lambing period but this is not necessarily always the case as lambs between the ages of 6 and 9 months shed more virus than sheep of other ages. Nasal secretions are the predominant means by which the virus is spread.

In the South African context blue wildebeest (Connochaetes taurinus) are considered as the major reservoir host of AIHV-1 but, it is also accepted that black wildebeest (Connochaetes gnou) are a reservoir host. Personal experience in the diagnosis of MCF over the years is that most cases diagnosed were confirmed to be wildebeest-associated (mostly blue wildebeest) with occasional cases being due to the sheep-associated form.

South Africa is the only natural habitat of the black wildebeest. All black wildebeest herds tested thus far in South Africa were serologically positive for antibodies against AIHV-1. In a more recent study by Pretorius et al (2008), specimens were collected from several game farms and conservation areas in central South Africa, in geographical areas historically recognized as the natural habitat of black wildebeest. Samples from 304 black wildebeest of different ages and sexes and 51 black wildebeest foetuses at different stages of gestation were collected. Virus isolated from a black wildebeest calf was morphologically and antigenically classified as a gammaherpesvirus closely related to AIHV-1. By means of a competitive inhibition enzyme-linked immunosorbent assay (CI-ELISA), the serum samples of all the animals tested positive for group-specific MCF virus antibody. Following screening of these samples with a real-time PCR assay, it was found that only 15.8% of the animals tested positive for the virus whereas 90% of the foetuses tested positive.
The authors concluded that these findings suggested that in black wildebeest the virus is mainly transmitted in utero or soon after birth. This is in contrast to OvHV-2 infection in lambs in which the infection takes place after weaning. These findings also seem to suggest that black wildebeest are latent carriers of the virus and furthermore it would seem that all black wildebeest are likely to be persistently infected.

Transmission of AlHV-1 in free-living populations of blue wildebeest is considered to be very efficient and all calves seem to become infected within the first few months after birth. It was established many years ago that all adults had neutralizing antibody to the virus. Virus could also be recovered from a proportion of their foetuses. Serum samples tested from wildebeest calves were positive for antibodies in the majority of calves aged less than 4 weeks to 12 months.

It is therefore accepted that most calves do receive colostral antibody. Calves are infected either in utero or during the first few months of life. Maternal antibody is still present in the calves at this time. Nasal or ocular secretions of wildebeest calves aged six to eight weeks yielded virus, making the respiratory tract the most likely route of contagious spread. It is therefore believed that in free-living wildebeest herds, a proportion of foetuses are infected in utero and intense transmission takes place within the population during which all calves become infected in the first few months of life.

Natural spread from the host to cattle is generally accepted to be exclusively by means of aerosols. Infection does not seem to take place horizontally between MCF-affected cattle and other in-contact susceptible animals or even from the very few clinically affected animals that survived. However, vertical (transplacental) transmission in cattle has been described. Such calves were either born infected but normal or died sooner or later after birth.

In East Africa, a classical seasonal pattern of occurrence of MCF has been recognized in cattle. MCF is predominantly seen in March and April in northern Tanzania and from April to July in southern Kenya. These periods have always been associated with the blue wildebeest calving seasons and clinical MCF is more common when the wildebeest calves are two to three months old. Calves older than three months of age are unlikely to be a source of infection. In contrast, evidence from South Africa suggests that older calves may also be a source of infection.

In South Africa, blue wildebeest-associated MCF occurs wherever free-living or semi-captive wildebeest are found. The majority of cases occur in the Limpopo and North West provinces in two distinct peaks. This has been observed from January to May, with the highest numbers in early April following the wildebeest calving season in December, January and February. A second peak, when the prevalence is higher, is seen from September to November with the highest number of cases being in mid-September, when the wildebeest calves are 9 to 11 months old. This period coincides with the weaning period of the wildebeest calves.

There are still many unanswered questions about transmission of the virus. There are reports of MCF occurring in cattle or other susceptible species in zoological gardens separated from wildebeest by up to 800 meters. This has led to speculation that spread by arthropod vectors may occur but this has not been proven yet. Clinical sheep-associated MCF has been reported in bison (Bison bison) with suspected virus transmission over distances as far as 4.8 kilometres, against prevailing winds, and in the absence of common water sources. Latent infection could explain clinical disease in bison or cattle, where direct close contact with sheep or other reservoir species is absent or cannot be confirmed. It has therefore been
proposed that a significant level of subclinical or latent MCF infections may be present in both cattle and bison and recrudescent disease may occur during periods of stress.

The existence of latent infection in clinically susceptible hosts is poorly understood, but is documented in some ruminant species. Consistent with previous observations in deer and other species, results from a study by Palmer et al (2013) suggested the existence of latent infection of white-tailed deer with OvHV-2. Serological or PCR-based testing of apparently healthy cattle and free-living bison and caribou (Rangifer tarandus) has demonstrated infection in the absence of clinical signs. This all suggests that inapparent infection with OvHV-2 or recovery from MCF may be more frequent than was previously thought.

The relatively common occurrence of sheep-associated MCF in domestic pigs in Norway and also to a lesser extent in Finland, Sweden, Germany and Switzerland also poses a number of epidemiological questions which have, so far, gone unanswered.

PATHOGENESIS

Despite years of research into the pathogenesis of MCF, it is still not fully understood. It has generally been accepted that lesions do not arise through direct virus-induced cytopathology as these viruses do not establish a productive infection. Virus instead replicates in a cell-associated fashion in T-lymphocytes, leading to systemic immune dysregulation and, in general, a fatal outcome. These cells have been characterized as large granular lymphocytes (LGLs) that exhibit non-antigen specific cytotoxicity similar to that of natural killer (NK) cells.

Some studies have demonstrated that CD8+ cells and CD4+ cells predominate within vascular infiltrates in cattle and bison. Polychromatic immune-phenotyping analyses in a study by Nelson et al (2010) showed that CD8(+)perforin(+) WC1(-) gammadelta T-cells, CD4(+)/perforin(−) alphabeta T-cells, and B-cells made up the infiltrate in the vascular lesions seen in the urinary bladder, kidney and liver of bison following experimentally-induced sheep-associated MCF. CD8(+) alphabeta T-cells and WC1(+) gammadelta T-cells were only infrequently and inconsistently identified. These findings support the authors’ hypothesis that the predominant CD8+ lymphocytes infiltrating the vascular lesions of bison with sheep-associated MCF are cytotoxic lymphocytes of the innate immune system, not CD8+ alphabeta T cells and, the notion that MCF is fundamentally a disease of immune dysregulation.

It has been recognized that the pathology of MCF includes the three major phenomena of:

1- hyperplasia of T-lymphocytes in lymphoid organs and accumulation of these cell in non-lymphoid tissue
2- degeneration and necrosis of epithelial cells and hyperkeratosis
3- the development of lesions of vasculitis.

In a review article by Russell (2009), it has been quoted that, despite the severe pathological lesions developing, little evidence of the presence of viral antigen in affected organs can be demonstrated although viral DNA can be detected by in situ hybridisation or PCR.

Progressive T-cell hyperplasia in both lymphoid and non-lymphoid organs, associated with extensive vasculitis and followed by tissue destruction caused by cytotoxic lymphocytes, has been experimentally reported in rabbits. Further studies in rabbits have suggested specific differences between MCF caused by OvHV-2 and AlHV-1. OvHV-2-associated lesions were
more apparent in visceral lymphoid tissue (e.g. mesenteric lymph nodes), in contrast to AIHV-1 where lesions were more frequently seen in the peripheral lymph nodes. It was also observed that more areas of necrosis were present in OvHV-2 associated lesions than those of AIHV-1. Very recent studies by Russell et al (2012) into AIHV-1 induced MCF in cattle confirms the results of these previous studies in rabbits and OvHV-2-infected cattle. Hence e supporting the possible role of increased cytotoxicity and IFN-stimulated immune- and inflammatory responses.

Levy et al (2012) describes 8 miRNAs encoded by OvHV-2 expressed in an OvHV-2 bovine lymphocyte cell line. Herpesvirus-encoded miRNAs have been shown to be effective regulators of both cellular and viral gene expression and are able to influence cell processes. The authors suggest that OvHV-2 also encodes miRNAs which may play a role in the cellular deregulation seen in infected bovine T-cells.

The different species of animals susceptible to MCF generally seem to be dead-end hosts and they do not transmit virus to other animals, although some transmission between infected deer has been reported as an unusual event. The failure of spread between MCF-susceptible animals is attributed to the fact that the virus replicates in a cell-associated manner and therefore cell-free virus is not produced.

**CLINICAL SIGNS**

The clinical signs seen in MCF are similar and cannot be reliably differentiated from each other, regardless of whether the affected animals are infected with AIHV-1 or OvHV-2.

Natural incubation periods are often difficult to determine and can be very variable, but are reported to probably range from two weeks to nine months. The clinical course of the disease is also highly variable in individual animals and may be peracute, acute or chronic. Peracute disease is characterized by sudden death with very few clinical signs observed beforehand. Clinical signs may include: rapid onset of depression, high fever and diarrhoea, which may become haemorrhagic, usually followed by death within 12 to 24 hours.

Generally, the onset of clinical signs in all forms of the disease are characterized by fever (rectal temperature of >42°C), inappetence, photophobia, lachrymation and a serous nasal exudate. The ocular and nasal discharges may become profuse and mucopurulent. Such exudates may cause matting of the facial hair and may block off the nares with difficulties in breathing as a consequence. Ocular lesions are typically bilateral with corneal opacity developing from the periphery of the eye and progressively affecting the entire cornea. The cornea may become oedematous and blindness, corneal ulceration and hypopyon may all be consequences. In the early stages of clinical disease, hyperaemia of the oral epithelium may be present, progressing to erosion of the mucosa. The latter is mostly seen on the ventral tongue surfaces, hard palate, gums and the tips of the buccal papillae. Skin lesions may develop in some affected animals and affected areas may show exanthema, exudation and encrustation. Such lesions are often restricted to the perineum, udder, teats, interdigital spaces and skin at the base of the horns and hooves. Hyperaemia may be seen in affected, unpigmented skin. The muzzle epithelium may become severely encrusted, necrotic and may slough. Skin lesions are very often overlooked during clinical examination. Superficial lymph nodes may enlarge and a drop in milk production may be seen in lactating animals.

In some cases, clinical signs of meningoencephalitis may be observed. Nervous signs including hyperaesthesia, inco-ordination, nystagmus, behavioural changes and head pressing
have been described. These may be observed without any other clinical signs present or, may be part of a more typical clinical picture. Aggression may be seen in some clinically affected animals. In a recent report by Mitchell et al (2009), the authors described nervous signs in calves which they considered unusual as only young calves were involved; with an unusually high morbidity (five out of a group of 20 affected); and neurological signs predominating. Ocular and other signs were not prominent in these calves, occurring only in one calf.

More severe clinical signs may develop in the more protracted cases and death may be seen 9 to 10 days after the first clinical signs are noticed. In general, disease in the more susceptible species follows an acute or peracute course.

The clinical outcome of MCF is almost invariably fatal although mild cases of sheep-associated MCF were described and studies into multiple outbreaks by means of serological and PCR screening confirmed that such cases do occur. Recovery or mild infection has occasionally occurred in disease caused by AIHV-1, but the frequency with which this occurs is not clear at present. From personal experience and discussions with various roleplayers, this has been revealed to be a contentious issue.

**PATHOLOGY**

As is the case in clinical signs, the macroscopic and microscopic lesions caused by both AIHV-1 and OvHV-2 are essentially similar in all susceptible animal species. Animals which have suffered from chronic, protracted disease may be emaciated and dehydrated. The mucous membranes of the upper and lower respiratory tract are usually congested. In some cases they may become oedematous with small haemorrhages, multiple foci of epithelial necrosis, erosions and ulcerations, mucopurulent inflammation and pseudomembrane formation. The nasal passages, particularly between the turbinates, may be partially blocked by exudates. A patchy bronchopneumonia may be present in animals that suffered from more protracted disease.

The mucosa of the mouth may be hyperaemic. Petechiae and ecchymoses, foci of epithelial necrosis, erosions and, in some cases, diphtheritic exudates may be observed. Similar lesions may be found in the oesophagus, particularly its more cranial parts, the forestomachs and abomasum. In the latter organs, erosions and/or ulcers are frequently present - particularly on the margins of the mucosal folds. The small and large intestines may exhibit a cattarhal to haemorrhagic enteritis with their contents, particularly in the more acute cases, watery and blood-stained. Similar erosions or ulcerations to those in the abomasum may be present along the ridges of the mucosal rugae of the ileo-caecal valve, caecum, colon and rectum.

Most of the lymph and haemolymph nodes usually become enlarged due to the lymphoid hyperplasia, oedema and sometimes congestion. The spleen may, or may not, be enlarged and, on cut surfaces, white pulp is generally prominent. The liver is usually slightly enlarged and exhibits diffuse greyish-yellow colour and mottling. The gall bladder may show lesions of oedema, petechiae, ecchymoses in its walls and few, small mucosal erosions. Characteristic lesions are usually seen in the kidneys as varying numbers of greyish –white foci which are 1 to 5mm in diameter. These are seen the cortices and they may bulge above the renal surface. In some cases, the presence of small, scattered renal haemorrhages may be observed. The mucosa and wall of the urinary bladder may frequently be oedematous with petechiae and ecchymoses in the mucosa and serosa and, occasionally, erosions or ulcers on the mucosal surface. Haematuria may sometimes be present. Increased amounts of
cerebrospinal fluid are formed and the meninges may appear more moist than usual and the subarachnoid spaces may be slightly cloudy.

**DIAGNOSIS**

A diagnosis has, for many years, been mostly based on the demonstration of the characteristic histological changes of vasculitis, hyperplasia of lymphoid tissue and accumulations of lymphoid cells in non-lymphoid organs but particularly in the brain, kidneys and liver. Contact with sheep or wildebeest has been considered as further circumstantial evidence in support of a diagnosis. Prolonged incubation periods after transmission by aerosol or by fomites may make it difficult to establish contact in some reported outbreaks.

Russell (2009) recently reviewed the available diagnostic tests and quoted that The World Organisation for Animal Health (OIE) recognises histopathology as the definitive diagnostic test. Others tests reviewed included: indirect immunofluorescence assays to detect antibodies specific for MCF virus antigens and PCR assays that detect MCF virus DNA sequences.

A competitive inhibition (CI) - ELISA test and a direct ELISA has been developed that offer a simple and inexpensive alternative to other serological tests. From a practical point of view, serological screening is of limited value and rarely used as diagnostic tool. In contrast, PCR is commonly used nowadays as it allows for confirmation of the presence of, and differentiation between the MCF viruses in infected animals and the most suitable (and most commonly collected) sample would be EDTA blood and, less commonly, fresh tissue samples. It is also is useful for phylogenetic and epidemiological studies in both natural and MCF-susceptible hosts.

Russell (2009) refers to the development of conventional and real-time (quantitative) PCR assays for the detection of both OvHV-2 and AlHV-1 viral DNA. Conventional assays based on a nested PCR approach makes them about 10-fold more sensitive than quantitative PCR. Nested PCR assays, with their higher sensitivity, may make them a more attractive option to be used if viral load is low or for use in samples such as paraffin-embedded tissue, which are more difficult to test. Real-time PCR assays have the potential to define viral loads in a range of tissues from both natural and MCF-susceptible hosts when used in combination with an appropriate host gene assay. Russell (2009) also quotes and describes the use of both serological and PCR assays in more than one epidemiological study which makes this article a worthwhile read.

**CONTROL**

The only strategy which seems to be effective is to segregate animals and limit possible contact between MCF-susceptible species and the natural hosts of the viruses. As aerosol transmission does seem to occur, the distance by which both sheep and wildebeest need to be separated from susceptible species should be as far as possible, with a minimum distance of 100 metres suggested.

Sheep-associated MCF is more difficult to control due to the unpredictable way in which outbreaks may occur. Sheep and cattle are commonly mixed without clinical disease being seen. However, in the occasional catastrophic outbreaks observed, the particular sheep flock involved may continue to cause substantial losses over several years and it may be appropriate to eradicate such flocks by means of slaughter.
Results obtained from studies by Russell et al (2012) revealed that an attenuated AlHV-1 vaccine in a licensed adjuvant protected cattle from fatal, intranasal challenge with pathogenic AlHV-1 for at least three or six months. Furthermore, it was found that animals that were protected had significantly higher initial anti-viral antibody titres compared to animals that succumbed to disease. Antibody titres in these animals remained relatively stable after challenge. In contrast, titres in animals vaccinated with MCF increased significantly prior to the onset of clinical signs of MCF.

The development of an effective vaccine would probably be one of the fields in which further future studies will be focused due to the obvious advantages of such a vaccine.

REFERENCES

CPD QUESTIONAIRRE

Please select the most correct answer to the following questions:

1. Malignant catarrhal fever is caused by:
   a) AIHV-1
   b) OvHV-2
   c) AIHV-2
   d) OvHV-1
   e) both a and b

2. AIHV-1 associated clinical MCF has been diagnosed:
   a) solely on the African continent
   b) on occasion in zoological gardens where wildebeest are kept
   c) only in areas where black wildebeest occur naturally
   d) as natural infection in free living eland and sitatungas
   e) as natural infection in blue wildebeest all over the African continent

3. The pathogenesis of disease hinges mostly on
   a) a direct cytopathogenic effect of the virus
   b) high loads of virus in affected tissues
   c) opsonization of the virus
   d) the development of a plasma cell dyscrasia
   e) systemic immune dysregulation

4. Clinical MCF in cattle is:
   a) invariably fatal
   b) subclinical and sometimes fatal
   c) associated with subcutaneous lumps
   d) invariably associated with diarrhoea
   e) often a latent infection

5. Outbreaks of MCF are associated with
   a) high morbidity and low mortality rates
b) low morbidity and low case fatality rates

c) low morbidity and high case fatality rates

d) high morbidity and high fatality rates

e) none of the above

6. Practical control of MCF includes
   a) the use of available effective vaccines
   b) isolation of susceptible species
   c) slaughter out of carrier animals
   d) biosecurity managing contact between carriers and susceptible species
   e) controlling insect vectors

7. In South Africa most cases of MCF occur in
   a) KZN and Eastern Cape
   b) Gauteng province
   c) Limpopo and North West provinces
   d) in zoological cases
   e) Western Province

8. Peaks of wildebeest associated MCF infection occur
   a) only after periods of high rainfall
   b) after wildebeest calves are born
   c) after wildebeest calves are weaned
   d) a and b
   e) b and c

9. Sheep associated MCF in South Africa may
   a) occur frequently after contact with sheep
   b) occur after stress related immune suppression of calves
   c) occur more often after contact with Wildebeest
   d) occur sporadically even after no obvious contact with sheep
   e) none of the above

10. Carriers of MCF include
    a) blue wildebeest and sheep
    b) black wildebeest and sheep
    c) wildebeest and sheep
    d) most game species
    e) all ruminants

CORRECT ANSWERS

1- e
2- b
3- e
4- a
5- c
6- d
7- c
8- e
9- d
10-с