INTRODUCTION
Bovine malignant catarrhal fever (MCF), or more commonly known as snotsiekte in South Africa, is a viral disease of cattle and other susceptible ungulates, following an infection with viruses of the genus Macavirus, subfamily Gammaherpesvirinae. The two viruses of greatest clinical importance are alcelaphine herpesvirus 1 (AlHV-1) and ovine herpesvirus 2 (OvHV-2). Cattle are susceptible to infection with both AlHV-1 (wildebeest-associated MCF or WA-MCF) and OvHV-2 (sheep-associated MCF or SA-MCF).

Blue and black wildebeest (Connochaetes taurinus and Connochaetes gnou), respectively, are the natural hosts for AlHV-1. Sheep (Ovies aries) are the natural hosts for OvHV-2. Most cases of clinical snotsiekte in South Africa result from infection with AlHV-1. This article will, however, focus specifically on OvHV-2 - the sheep-associated form of snotsiekte.

THE VIRUS
Currently there are 10 known members of the MCFV group of viruses but it is expected that more will be identified in the future. Five of these viruses are known to be pathogenic namely OvHV-2, alcelaphine herpesvirus 1 (AlHV-1), alcelaphine herpesvirus 2 (AlHV-2), caprine herpesvirus 2 (CpHV-2) and an MCFV in white-tailed deer (Odocoileus virginianus). Gemsbok-MCFV, Muskox-MCFV, and Aoudad-MCFV are not associated with MCF and hippotragine herpesvirus 1 (HipHV-1) causes experimental MCF in rabbits (naturally transmitted disease has not been reported). Researchers have been able to sequence the genomes of OvHV-2 and AlHV-1. However, whilst permissive cell culture systems are available for AlHV-1, AlHV-2, and HipHV-1 up to now it has not been possible to culture OvHV-2, which has hampered research into OvHV-2.

EPIDEMIOLOGY
Globally SA-MCF is recognized as an economically important disease and seen in cattle, deer, bison and a wide array of captive wild animals. In their review O’Toole et al (2014) stated that although of minor importance in domestic cattle, MCF is of greater economic importance to the North American bison, captive and free-ranging cervid species, zoological collections, and domesticated species such as Bali cattle (Bos javanicus) and domestic water buffalo (Bubalus bubalis).
It has long been recognized that different species vary in their susceptibility to pathogenic members of the MCFV group. Banteng (B. javanicus) and some cervid species, such as Pere David’s deer (Elaphurus davidianus) and whitetailed deer are considered highly susceptible.

Water buffalo (B. bubalis) and many other cervid species are thought to be of intermediate susceptibility. By comparison domestic cattle particularly of Bos taurus, shows low susceptibility as shown by transmission studies. These differences were demonstrated in disparate mortality rates when bison and domestic cattle were exposed at the same time to OvHV-2. It is generally accepted the infectious virus are not shed by clinically affected susceptible hosts.

On the African continent SA-MCF has always been overshadowed by WA-MCF in clinical and economic importance as seen in the numbers of outbreaks of clinical disease recorded in cattle.

Pfizer et al (2015) reported that several cases of MCF in African buffaloes were confirmed in South Africa during the last few decades. In seven of these cases that were investigated viral nucleic acid was detected in blood or tissues in six animals that exhibited clinical signs compatible with MCF. Four were confirmed to be positive for infection with OvHV-2, and two were positive for A1HV-1.

Doboro et al (2016) quoted that only 10% of suspect MCF cases in cattle tested OvHV-2 positive whilst 90% tested OvHV-2 negative by means of PCR (at the Agricultural Research Council - Onderstepoort Veterinary Institute) in SA. Researchers reported an OvHV-2 prevalence of 77% in 86 sheep originating from different regions of South Africa.

Such a high prevalence in the natural reservoirs of OvHV-2 emanating from many different regions of SA underscores the potential significance of the disease. It also highlighted the need to determine the genetic diversity of OvHV-2 strains circulating in SA.

The inability to grow the virus in vitro in cell cultures, led to difficulties in studying the molecular epidemiology, diagnosis and pathogenesis of OvHV-2. As a consequence the epidemiology of SA-MCF in SA is also not yet fully elucidated.

Doboro et al (2016) attempted to characterize the OvHV-2 strains circulating in SA. They employed four selected genes encoding glycoproteins and tegument proteins - this included the genes, Ov 7, Ov 8 ex2, ORF 27 and ORF 73. All were selected by PCR and DNA sequencing. The findings of their studies into the nucleotide and amino acid multiple sequence revealed:

- The presence of two genotypes for ORF 27 and ORF 73.
- The presence three genotypes for Ov 7 and Ov 8 ex2.
- These were randomly distributed throughout the regions.
- Nucleotide sequence analysis of Ov 7 and ORF 27 revealed variations which distinguished these SA genotypes from those of the reference OvHV-2 strains.

OVHV-2 INFECTION

O’Toole et al (2014) reported that in studies following on experimental OvHV-2 infection in susceptible species that 2 peaks of OvHV-2 gene expression was a consistent finding:

- a preclinical peak involving the respiratory tract
- a second peak in multiple organ systems coinciding with clinical disease.

It was postulated that latent and lytic gene expression may coexist in tissues during the clinical stages in affected animals.
OVHV-2 INFECTION IN DOMESTIC SHEEP

In their review O’Toole et al (2014) quotes that all lambs, under natural conditions, were virtually born free of infection only becoming infected after 2 months of age. Viral shedding as assessed by quantitative real-time PCR indicated that adolescent lambs (6–9 months old) shed virus more frequently and intensively in comparison to adult animals. At the age of one year lifelong infection was established in most sheep and uninfected sheep remained susceptible to infection in adulthood.

Aerosol challenge seems the most important route of infection and the lower respiratory tract is the primary target for virus. Intravenous or intraperitoneal routes did not establish infection upon challenge. Transplacental infection seems to occur in sheep (in contrast to wildebeest infected with AlHV-1) and infection via colostrum or milk appeared to be a rare event.

Outbreaks of MCF in susceptible species is thought to be more likely when they are exposed to large groups of sheep and more so if those groups are predominantly of the 6 to 9 months old age group. New-born lambs are susceptible to aerosol infection, but this seems a rare occurrence due to a combination of limited viral shedding by ewes, combined with inefficient infection. OVHV-2 has been detected in the semen and reproductive glands of rams but it remains to be established whether or not this is of epidemiological significance.

A very interesting observation is that a percentage of lambs challenged via the aerogenous route aerosol may develop subclinical bronchointerstitial pneumonia. Histological lesions of degeneration, loss and hyperplasia of terminal bronchiolar cells, and intra-alveolar fibrin exudation may be seen within a week of infection persisting for at least 2 weeks after the challenge.

Viral replication occurs in the lungs within a week of infection but lytic infection does not occur in other tissues during this period. Unlike AlHV-1 infected wildebeest calves, in which an MCF-like disease has not been reported, lambs which were exposed to high doses of OVHV-2 may develop an MCF-like syndrome.

Clinically affected lambs are febrile developing mucopurulent nasal discharge. Lesions seem to remain restricted to respiratory and upper digestive tracts. It may be debatable if a similar syndrome occurs in naturally infected flocks. Foetal lambs challenged intravenously with infected leukocytes developed an MCF-like syndrome. This was characterized by lesions of lymphoproliferation and panarteritis. The presence of lesions of systemic vasculitis has been recognized sporadically in commercial flocks, prompting researchers to query the occurrence of ovine MCF. In these outbreaks, it was observed that the distribution of the vascular lesions is systemic, in contrast to sheep challenged intranasally - in which arteitis is restricted to the lungs.

However, the lack of a reliable immunological technique to demonstrate OVHV-2 antigens within lesions in formalin-fixed material and the fact that domesticated sheep are infected with OVHV-2 during their first year of life possibly still cast doubt on the detection of the virus in sheep with MCF-like lesions, as convincing evidence for a causative role.

OVHV-2 INFECTION IN OTHER SPECIES

O’Toole et al also reviewed OVHV-2 infection in other species:

MCF affects Bison, both the plains and wood subspecies of Bison bison, and the wisent (European bison; Bison bonasus). They discuss the disease in their review article in detail (a very worthwhile read) but to discuss this in detail would be beyond the scope of this article.

A few interesting observations made by them are a) the occurrence of OVHV-2–induced MCF transmissible over long distances (up to 5 km) b) the predominant form of MCF in bison is acute clinical disease with chronic and subclinical forms more rare and c) some clinically healthy bison have detectable OVHV-2 DNA in their peripheral blood, suggesting subclinical or latent infection and d) that the pathogenesis of MCF in bison, as in other species, remains largely unresolved.

In cattle OVHV-2 is transmitted predominantly by contact between infected and susceptible hosts, while the documentation of vertical transmission is rare. Similar to the situation in bison O’Toole et al also discusses the disease in this species in depth but this would also be too much to discuss in detail here.

It is quoted that outbreaks involve direct or indirect exposure to sheep and a small percentage of acutely affected cattle recover with chronic residual lesions in medium-caliber arteries and cornea.

As in bison, there is evidence that subclinical and/ or latent infection occurs in cattle as well, experimentally induced aerosol transmission of cattle is possible, preclinical events at the molecular and morphological level remain poorly defined and the significance of finding low levels of OVHV-2 in peripheral leukocytes of healthy cattle is unclear. Headley et al (2015) recently reported on the pathological and molecular findings associated with transplacental transmission of OVHV-2 in two Girolanda cows.
By means of a polymerase chain reaction viral DNA was detected in the brain of the pregnant cow and her foetus, as well as from the kidney of the pregnant cow.

Other small ruminant species which can be productively infected with OvHV-2 includes the bighorn sheep (Ovis canadensis) and mouflon (Ovis orientalis). The latter, which is considered an ancestor of domestic sheep, appears to be particularly productive sources of OvHV-2.

Domestic pigs (Sus scrofa) are sporadically infected with OvHV-2 and this has been recognized in pigs in European countries since 1901. Affected pigs may develop MCF and similar to cattle may present with a head and eye form. However, gross lesions are usually minimal in acutely affected animals. In these outbreaks the proportion of affected pigs has been reported to be small and it has been associated with unusually close contact with sheep.

Mild and chronic forms of an MCF-like disease has also been described during such outbreaks. Experimentally induced MCF has been achieved through aerosol challenge with OvHV-2 but this seem to require relatively high concentrations of virus.

Domestic Goats (Capra aegagrus hircus) can be infected with OvHV-2 and in most goats, infection is asymptomatic. However, MCF-like disease has been seen in some domestic goats in conjunction with the presence of lesions generalized arteritis and detectable OvHV-2 infection. However, in these cases it is not clear if the MCF-like syndrome reflects an unusually high challenge with OvHV-2; or if it is just a peculiar response to infection; or if presence of OvHV-2 DNA is incidental and in fact unrelated to the presence of lesions.

OvHV-2 is responsible for outbreaks of MCF in cervids (deer) but disease caused by CpHV-2, AhV-1, and MCPV-white-tailed deer (WTD) has also been reported. Sheep-associated MCF in deer is characterised by acute disease with death reported in 1 to 3 days accompanied by clinical signs of depression, separation from herdmates, reluctance to move and neurological signs such as convulsions.

Macroscopic lesions include congestion and perichonial haemorrhage in multiple tissues, including skeletal muscle, and most prominently in the gastrointestinal tract. Lymphadenopathy has also been observed.

Levels of susceptibility may vary between the cervid species - white-tailed deer and Pe`re David’s deer (E. davidianus) are highly susceptible, Moose (Alces alces) and elk (Cervus canadensis) moderately susceptible and Fallow deer (Dama dama) are relatively resistant.

Reports of MCF due to OvHV-2 in Water (Swamp) Buffalo (B. bubalis) are sparse but they are likely to be highly susceptible developing clinical signs and lesions are similar to those in cattle and although recovery may occur this is likely to be rare.

Due to limitations of high costs, and other factors, laboratory rabbits (Oryctolagus cuniculus) have been used for aerosol studies with OvHV-2 as they develop lesions very similar to those in naturally affected ruminant species.

**FUTURE RESEARCH**

MCF is not invariably fatal and as was observed since the late 1920s some dead-end host species recover from clinical disease. Researchers have also shown renewed interest in developing a vaccine following on the very discouraging results obtained during attempts in the 1970s and 1980s. Many aspects of MCF still need further investigation and research.

Doboro et al (2016) discusses the importance of nucleotide and amino acid sequence variations in viruses as it advances understanding genetic diversity, may provide information into virulence and pathogenesis of a particular virus, guidelines for development of diagnostic tools and effective antiviral drugs and vaccines.

In their study, the genotypes that were identified could not be directly linked to the virulence of OvHV-2 (Insufficient data available), but findings from their study have suggested that the OvHV-2 strains consisting of genotypes 1 and 2 of the Ov 8 ex2 and ORF 27 genes, respectively, are virulent, at least to cattle.

Doboro et al also states that genetic diversity may be influenced by geographical location because of environmental conditions such as climate, temperature or weather. Such genetic variations linked to geographic origin may possibly be significant in the manifestation of the disease. This has been been observed in other viruses such a fibropapilloma associated marine turtle herpesvirus (FPTHV) equine gammaherpesvirus 2 (EHV-2) and 5 (EHV 5) strains. The sequence analysis of Ov 7 and ORF 27 in their study revealed variations that distinguished SA and the reference OvHV-2 strains from each other (the latter originating from the United Kingdom and United State of America) but conceded that more research needs to be done to elucidate this.

Control measures against MCF includes the prevention of co-grazing of carriers (sheep or wildebeest) and susceptible animals (cattle or buffaloes) and knowledge of the different genotypes obtained from
SA strains and their distribution in the different provinces of SA may be of great significance. However, the numbers of samples analysed in their study was limited and varied for the different provinces and therefore no conclusive findings could be drawn in this regard.

Further fields of study identified by Doboro et al are the role B cell epitopes in vaccine development and serodiagnostic tests. B-cell epitopes identified for Ov 7, Ov 8 ex2 and ORF 27 genes seemed conserved which may offer certain advantages for serodiagnostic test development and vaccine design. Other proteins may also be of importance in improving sero-diagnostic tests and subunit vaccines. As example the glycoproteins encoded by Ov 7 and Ov 8 ex2 genes of OvHV-2 seem to be responsible for attachment of the virus to host receptor cells, and the protein encoded by ORF 27 seems to be responsible for transmission of the virus from one cell to another.

O’Toole et al also alludes to the importance of the ability to identify genetic resistance factors, to better define “safe distances” between carrier and susceptible host species, to develop efficient diagnostic tools, to clarify the pathogenic mechanisms by which immune dysregulation is triggered and to further investigate the possible role of lifelong latent or subclinical infection in MCF-susceptible species. The role of the latter is still unclear but likely to occur in domestic cattle and in some wildlife species, including bison, moose, reindeer (Rangifer tarandus), roe deer (C. capreolus), and red deer (Cervus elaphus). It is speculated that sporadic occurrences of MCF in herds - in which no recent contact with domestic sheep could be established - may be due to reactivation of infection or possible prolonged incubation.

References